Available online at
www.heca-analitika.com/malacca_pharmaceutics

Malacca Pharmaceutics

Vol. 4, No. 1, 2026

A Systematic Review on the Transformation of Bone Waste into Valuable Dental Biomaterials

Viona Diansari [1,2], Rinaldi Idroes [3,*], Sunarso Sunarso [4], and Sri Fitriyani [2]

[1] Graduate School of Mathematics and Applied Sciences, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia; viona_diansari@usk.ac.id (V.D.)
[2] Department of Dental Material, Faculty of Dentistry, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia; srifitriyani@usk.ac.id (S.F.)
[3] Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia; rinaldi.idroes@usk.ac.id (R.I.)
[4] Department of Dental Material, Faculty of Dentistry, Universitas Indonesia, Jakarta 10430, Indonesia; sunarso02@ui.ac.id (S.S)

* Correspondence: rinaldi.idroes@usk.ac.id

Article History

Received 23 December 2025
Revised 20 February 2026
Accepted 3 March 2026
Available Online 9 March 2026

Keywords:
Hydroxyapatite
Maxillofacial
Dentistry
Sustainable biomaterials
Bone waste

Abstract

Bone waste is a sustainable, calcium-rich resource for the production of hydroxyapatite (HA), a biomaterial widely used in dental and bone tissue engineering. This systematic review evaluates recent advances in the extraction, transformation, and biological performance of HA derived from bone waste. A total of 20 records were initially identified, of which 11 full-text articles met the eligibility criteria and were included in the qualitative synthesis. The reviewed studies demonstrate that bone waste can be effectively converted into HA through several routes, including thermal-based extraction (calcination, annealing, and sintering at 600–1000°C), alkaline hydrolysis, and hydrothermal or microwave-assisted methods, enabling the production of micro- and nano-sized HA with high purity. Post-extraction functionalization, such as ion doping (Mg^{2+}, Na^{+}, Co^{2+}), drug loading, and composite formation, further enhances osteogenic, antimicrobial, and mechanical properties. Physicochemical characterization using XRD and FTIR consistently confirmed the formation of non-stoichiometric, ion-substituted HA with Ca/P ratios ranging from 1.6 to 1.9, closely resembling biogenic apatite. The presence of multiscale porosity (25–65%) and nano-scale features promotes protein adsorption, ion exchange, and cellular interactions. In vitro studies confirmed cytocompatibility, while ALP activity and mineralization assays demonstrated strong osteogenic potential. Overall, bone waste–derived HA offers biomimetic, functional, and environmentally sustainable alternatives for dental and maxillofacial applications.

1. Introduction

The global meat industry has long been a cornerstone of global food security for a continually growing population. However, behind this massive production lies a vast and often overlooked environmental burden: bone waste. Annually, this industry generates approximately 130 million tons of bone waste from slaughterhouses worldwide [1]. This nearly inconceivable figure represents a modern paradox where industrial progress begets an acute waste problem. Currently, the utilization of this bone waste remains severely limited and suboptimal. Only about 30% of the total waste is converted into low-value by-products [2], such as gelatin for the food industry [3] or bone meal for animal feed [4]. The vast majority of the remainder ends up as unused material. This waste is ultimately landfilled, incinerated, or disposed of in ways that trigger secondary issues,

ranging from greenhouse gas emissions such as methane to potential soil and water contamination, to the inefficient use of increasingly scarce space [5]. This situation clearly depicts an unsustainable linear economic model in which resources are taken, used, and then discarded, creating a mounting ecological burden.

Amidst the pressures of global environmental and climate crises, a new paradigm has emerged offering a wiser path forward: the circular economy [5]. This paradigm, that 'waste is merely a resource in the wrong place', is not mere rhetoric but a chemical reality for bone waste [6]. Bone, in its essence, is not inert trash. It is a complex biological material rich in high-value compounds [7]. Its unique composition makes it a discarded treasure trove.

Structurally, mammalian bone primarily consists of two main components: an inorganic matrix and an organic matrix [8]. The inorganic component, comprising 60 to 70 percent of dry bone weight, is dominated by the mineral HA [9]. This is the very same mineral that forms the primary constituent of human tooth enamel and bone [10], providing exceptional hardness and biological compatibility. Meanwhile, collagen, the organic component, forms a tough, elastic fibril network that serves as the scaffold for hydroxyapatite deposition [11]. It is this perfect combination of mineral strength and organic flexibility that makes bone a highly inspiring natural composite structure in biomaterials engineering. Importantly, this structure is not exclusive to mammalian bone; it is also present in the skeletal remains of various vertebrates, including fish, pigs, and poultry. Consequently, diverse bone waste streams, from bovine femurs to fish scales and chicken bones, represent equally viable and compositionally rich precursors for HA extraction [12–14].

The valorization of bone waste into biomedical materials directly embodies several United Nations Sustainable Development Goals (SDGs). It reflects SDG 9 (industry, innovation and infrastructure) by transforming industrial by-products into high-value biomaterials, and SDG 12 (responsible consumption and production) through waste reduction, recycling, and reuse [15]. The resulting dental and maxillofacial applications further contribute to SDG 3 (good health and well-being) [16]. Within dentistry, the 'Green Dentistry' movement reflects this same paradigm shift, addressing both the carbon footprint of clinical practices and the life cycle of dental materials [17, 18]. There is growing demand for biomaterials that are clinically effective and environmentally responsible; those derived from waste streams, such as bone, directly fulfill this dual mandate [19].

Given the immense potential locked within bone waste and its alignment with global trends, it is natural that numerous studies over recent decades have sought to extract and purify hydroxyapatite from a wide array of bone waste sources. These include not only bovine bone, commonly termed Bovine Hydroxyapatite (BHA), but also fish bone and scale, porcine bone, and poultry bone waste [12, 14, 20, 21]. Various methods have been explored, ranging from simple thermal calcination to more complex chemical deorganification techniques using alkaline solutions or organic solvents [22]. Each method aims to remove the organic components (collagen, fat) while preserving or optimizing the desired crystalline structure of hydroxyapatite. As a result, BHA from this waste has been trialed in various dental and maxillofacial bone graft materials, implant coatings, and tissue engineering scaffolds, all of which require osteoconductivity, biocompatibility, and mechanical integration with host bone [23].

However, this body of knowledge remains highly fragmented. Each study typically focuses on a single bone source, a specific extraction technique, or isolated in vitro testing. Each study often focuses on a specific aspect, such as optimizing a single extraction method [20, 24], characterizing particular physico-chemical properties [25, 26], or conducting isolated in vitro biological tests [27]. Consequently, no systematic review has yet provided a structured, cross-source comparative synthesis of extraction methods, physicochemical outcomes, and biological performance across different bone wastes.

Several previous reviews have addressed hydroxyapatite derived from natural sources. A study by [12] provided a narrative overview of extraction methods for biogenic hydroxyapatite, while [28] reviewed hydroxyapatite from various natural wastes, including eggshells, animal bones, and shells. A study also surveyed HA synthesis routes more broadly [22], and most recently, [29] conducted a scoping review of natural hydroxyapatite sources encompassing bone, coral, and eggshell. However, no systematic review to date has employed a formal, PRISMA-guided methodology with explicit, predefined research questions specifically focused on transforming animal-derived bone waste into hydroxyapatite for dental, maxillofacial, and related bone tissue engineering applications. Furthermore, existing reviews treat bone as one of many precursor sources rather than the central subject, and none have attempted a structured, cross-source comparative synthesis of extraction methods, physicochemical characteristics, and biological performance across different bone types. The present review addresses these gaps by providing the

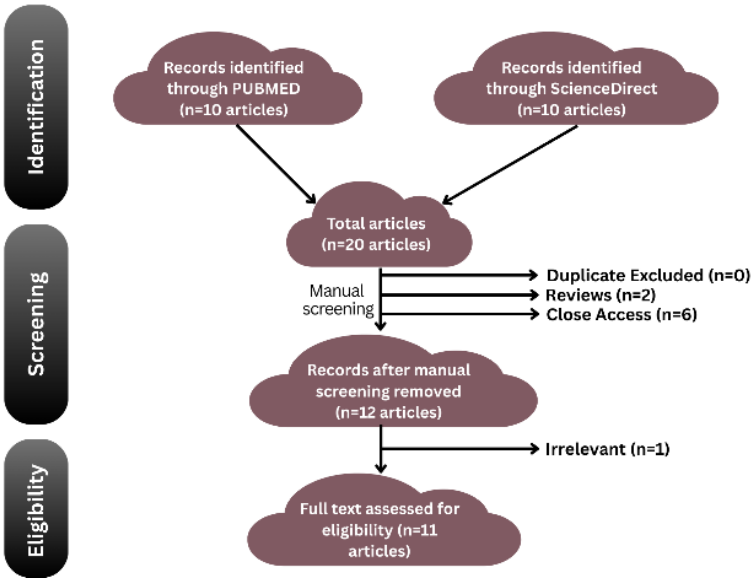

Figure 1. PRISMA flowchart of included studies.

first methodologically transparent, systematically conducted, and comparatively oriented synthesis of bone waste-derived HA, with explicit integration of the sustainability paradigm that frames this valorization process.

Therefore, this systematic review was guided by two predefined research questions: first, what extraction and transformation methods have been employed to convert bone waste into hydroxyapatite (HA), and what the resulting biomaterials exhibit physicochemical characteristics; and second, what in vitro biological performance, particularly in terms of cytocompatibility, osteogenic differentiation, and mineralization potential, has been demonstrated for bone waste derived HA, and how the sustainability dimension of this valorization process is articulated in the existing literature.

2. Materials and Methods

2.1. Study Design

The review process followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) standards (including predefined eligibility criteria, systematic literature searching, study selection, and data extraction procedures) to guarantee adherence to systematic review procedures. However, the protocol was not registered in an international prospective register such as PROSPERO.

2.2. Search Strategy and Keywords

The literature search was completed on January 10, 2026, in PubMed and ScienceDirect. A combination of free-text keywords and Boolean operators was used to capture variations in terminology related to bone-waste–derived hydroxyapatite. The terms search included *("bone waste" OR "slaughterhouse waste" OR "animal by-product") AND (hydroxyapatite OR "bone graft") AND (dental OR biomedical).* Boolean operators "AND" and "OR" were used to combine core concepts and their synonyms. In PubMed, relevant Medical Subject Headings (MeSH), such as Hydroxyapatite and Bone Transplantation, were explored; however, due to the interdisciplinary nature of this topic spanning materials science, biomaterials, and biomedical engineering, free-text searching was prioritized to ensure inclusion of studies that may not be consistently indexed under controlled vocabularies. Titles and abstracts were initially reviewed to exclude publications that were incompatible with the study's objectives. Articles published between 2018 and 2026 were considered to capture recent advances in bone waste valorization technologies, nanostructured hydroxyapatite synthesis, ion-substitution strategies, and dental biomaterial applications.

2.3. Eligibility Criteria

The inclusion criteria were: (1) original articles written in English; (2) studies involving animal-derived bone waste processing studies and their transformation into HA-based biomaterials; and (3) exclusion studies involving inaccessible full-text articles, grey literature, meta-analyses, and review articles. The obtained PRISMA illustrating the study's selection process is shown in Figure 1.

2.4. Study Selection Process (PRISMA Flow Diagram)

There is a total of 20 records (Figure 1). Following the initial identification stage, all retrieved records were manually screened. During this process, no duplicate

Table 1. Risk of bias summary for the included studies. (Note: ✓ = clear/adequate, X = not reported, △ = partial).

Ref.	Clear Objective	Bone Source Description	Reproducible Extraction Method	Adequate Characterization	Biological Evaluation	Outcomes Clearly Reported	Overall Quality
[30]	✓	✓	✓	✓	△	✓	Moderate
[31]	✓	✓	✓	✓	X	✓	Moderate
[20]	✓	✓	✓	✓	X	✓	Moderate
[32]	✓	✓	✓	✓	X	✓	Moderate
[33]	✓	✓	✓	✓	✓	✓	High
[34]	✓	✓	✓	✓	✓	✓	High
[14]	✓	✓	✓	△	X	✓	Low-moderate
[13]	✓	✓	✓	✓	✓	✓	High
[21]	✓	✓	✓	✓	△	✓	Moderate
[35]	✓	✓	✓	✓	X	✓	Moderate
[36]	✓	✓	✓	✓	✓	✓	High

articles were identified; however, two review articles and five studies with closed access were excluded. As a result, 12 records remained after the screening stage. Subsequently, these articles were further evaluated for relevance, leading to the exclusion of one irrelevant study. Ultimately, 11 full-text articles met the eligibility criteria and were included in the qualitative synthesis of this systematic review.

2.5. Data Extraction Process

Data extraction was conducted by the primary author and subsequently cross-checked by a co-author to ensure accuracy and consistency. Any ambiguities were resolved through discussion. The extracted data included: (i) bone waste source and origin, (ii) extraction methods, (iii) processing parameters (e.g., temperature, time, chemical agents), (iv) physicochemical characterization techniques, (v) key structural and compositional properties of hydroxyapatite (e.g., phase purity, Ca/P ratio, particle size, morphology, porosity), and (vi) reported biological performance where applicable. Following independent extraction, the reviewers compared their data entries.

2.6. Quality Assessment

The methodological quality of the included studies was assessed using an adapted Joanna Briggs Institute (JBI) critical appraisal framework tailored for experimental biomaterials research. The assessment focused on the clarity of objectives, transparency of the bone source, reproducibility of extraction methods, adequacy of physicochemical characterization, and, where applicable, the presence of biological evaluation.

3. Results and Discussion

3.1. Risk of Bias Assessment

The appraisal focused on the following domains: (i) clarity of research objectives, (ii) explicit description of bone waste source and pretreatment, (iii) reproducibility of extraction and transformation methods, (iv) adequacy of physicochemical characterization techniques (e.g., XRD, FTIR, SEM/EDX), (v) inclusion of biological evaluation where relevant to the study scope, and (vi) clarity of reported outcomes.

Each study was independently evaluated and categorized as high, moderate, or low methodological quality based on the overall completeness and transparency of these criteria. Studies lacking biological testing were not automatically downgraded, as several investigations were primarily focused on material synthesis and physicochemical optimization rather than biological performance. The results of the methodological quality assessment are summarized in Table 1.

3.2. Extraction and Transformation Methods of Bone Waste into Hydroxyapatite

Synthetic HA has been produced by the hydrothermal method, solid-state processes, the sol-gel process, emulsion and micro-emulsion methods, and, most commonly, chemical precipitation. In the meantime, natural hydroxyapatite was produced via calcination, hydrothermal synthesis, alkaline hydrolysis, precipitation, and combinations of these methods [12].

The schematic illustration in Figure 2 summarizes the valorization pathways of diverse bone-derived wastes into HA, highlighting the conversion of animal and marine by-products, including bovine, pig, chicken, and fish bones, as well as mussel shells, into value-added biomaterials. These waste streams are rich in calcium and phosphorus, making them ideal precursors for HA, a bioactive ceramic that closely resembles the mineral phase of natural bone and teeth. Several studies demonstrate that such wastes can be efficiently transformed into HA with tailored physicochemical properties while also addressing environmental concerns associated with biowaste disposal [14, 20, 31, 32].

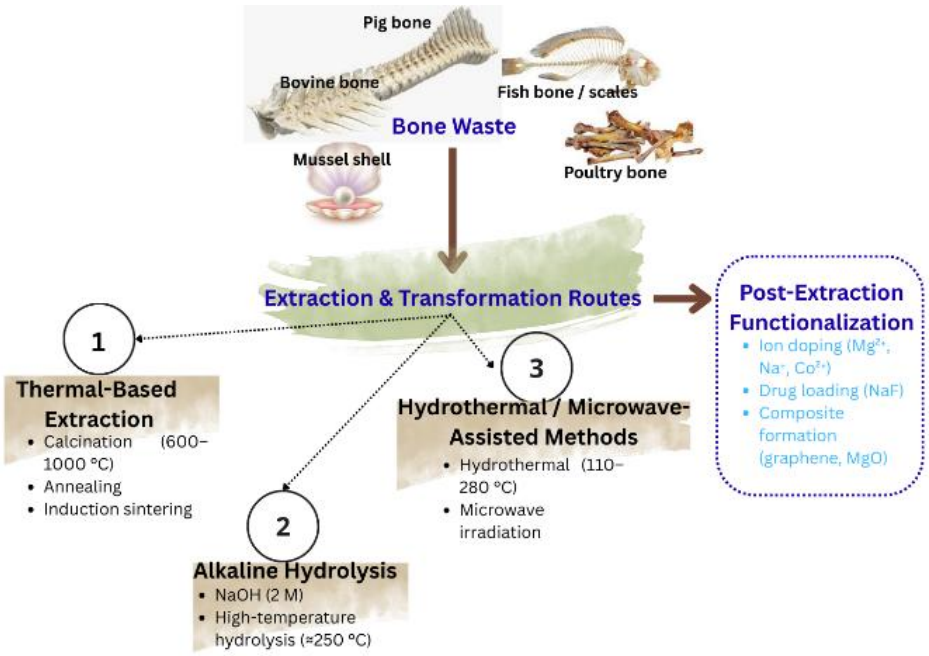

Figure 2. Pathways for transforming various bone wastes into hydroxyapatite.

The first major route depicted is thermal-based extraction, which primarily involves calcination, annealing, and sintering processes at temperatures ranging from 600 to 1000°C. This approach effectively removes organic components and yields crystalline HA with retained trace ions such as Mg, Na, and K, which are beneficial for biological performance. For instance, bovine, pig, sheep, and pigeon bone wastes have been successfully converted into highly crystalline HA through controlled calcination and sintering, resulting in materials with improved mechanical strength and bioactivity suitable for bone and dental applications [21, 30, 31, 37]. Thermal processing is particularly advantageous for producing structurally stable HA intended for load-bearing and dental bone filler applications. Calcination is the recommended approach for extraction and synthesis, producing highly crystalline hydroxyapatite powder, either alone or in tandem with other procedures [12].

The second and third pathways involve alkaline hydrolysis and hydrothermal or microwave-assisted methods, which are particularly effective for producing nanostructured, high-purity HA. Alkaline hydrolysis using concentrated NaOH at elevated temperatures (~250°C) enables the extraction of nano-hydroxyapatite with controlled particle size and excellent biocompatibility from fish bone waste [13, 20, 33]. Meanwhile, hydrothermal and microwave-assisted techniques facilitate the formation of carbonated and ion-substituted HA at relatively lower temperatures (110–280°C), often yielding nanocrystalline products that closely mimic biological apatite. These methods have also enabled innovative resource recovery strategies, such as combining calcium from mussel shells and phosphate from bovine bones to synthesize carbonate-rich apatite via microwave irradiation [32, 34, 35].

Finally, the illustration also emphasizes post-extraction functionalization, which transforms extracted HA into multifunctional biomaterials through ion doping, drug loading, and composite formation. Incorporation of biologically relevant ions (e.g., Mg^{2+}, Na^{+}, Co^{2+}) or functional additives such as graphene and MgO has been shown to enhance osteogenic potential, antimicrobial activity, and mechanical performance of bone-derived HA [30, 35, 36]. Additionally, drug-loaded HA systems, such as NaF-loaded porous HA substrates, have been developed for controlled release and dental tissue regeneration applications [33]. Collectively, these transformation routes demonstrate that bone waste-derived HA can be engineered into advanced, application-specific biomaterials, particularly for dental and bone tissue engineering purposes.

Across the 11 included studies, thermal calcination and hydrothermal approaches were the most frequently employed synthesis strategies. Calcination temperatures ranged from 600°C to 1000°C, generally yielding high-crystallinity HA (>84–90%). Hydrothermal and microwave-assisted methods enabled controlled particle morphology and the formation of carbonated apatite. Mechanochemical processing, such as ball milling combined with induction sintering, improved densification and mechanical strength, while ionic doping

strategies allowed functional enhancement without compromising structural integrity. Collectively, these findings indicate that controlled thermal treatment and post-processing modification are key determinants of crystallinity, densification, and functional performance in biogenic HA production.

3.3. Characteristics of HA from Bone Waste

3.3.1. Physicochemical Characteristics

The physicochemical characteristics of hydroxyapatite (HA) derived from bone waste constitute the fundamental basis for its performance as a dental biomaterial. Parameters such as phase composition, crystallinity, and chemical stability directly influence mechanical integrity, biological response, and long-term clinical functionality. Across the reviewed studies, physicochemical characterization, particularly X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR), is consistently employed to evaluate the quality and suitability of bone waste–derived HA for dental and bone-related applications.

3.3.1.1. Phase Composition and Crystallinity

X-ray diffraction analysis was reported in all studies summarized in this review (Table 2), highlighting its central role in confirming phase purity and crystallographic structure of HA synthesized from bone waste. Most studies demonstrated the successful formation of hexagonal HA [21, 34], commonly indexed to standard JCPDS or ICDD reference patterns, indicating effective removal of organic components and preservation of the apatite lattice during thermal or chemical processing.

A structural distinction observed in several studies concerns the formation of stoichiometric HA versus carbonated or ion-substituted HA that more closely resembles biological apatite. Carbonate substitution, predominantly B-type (CO_3^{2-} replacing PO_4^{3-}), was identified through combined XRD peak broadening and FTIR signatures in multiple reports [13, 21, 32, 35]. Such carbonated structures reflect the natural composition of bone and dental tissues. They are widely associated with improved biological affinity, although they may exhibit slightly lower crystallinity than fully stoichiometric HA.

Crystallinity index (CI) values, when reported, revealed a strong dependence on processing conditions and compositional modification. Several studies indicated high crystallinity following optimized calcination or sintering procedures, with values approaching or exceeding 90% in some cases [31, 35, 36]. Doping strategies, including Mg, Co, or graphene incorporation, were shown to influence lattice ordering, in certain instances increasing measured crystallinity [36]. Conversely, hydrothermal ion substitution or secondary chemical treatments introduced modest lattice distortion in some systems [35], suggesting a balance between structural order and compositional modification.

Thermal treatment plays a decisive role in determining HA phase composition and crystallinity. Higher calcination or sintering temperatures generally promote crystal growth and lattice ordering [21, 38]. This effect is consistently observed in studies involving bovine, pig, and pigeon bone wastes, whereas increasing temperature improved crystallinity and phase purity. However, elevated temperatures may also reduce carbonate content, thereby decreasing the biomimetic character of the material l [39]. These findings collectively emphasize the importance of temperature optimization in preserving both structural stability and biological relevance.

Mechanical activation techniques, particularly high-energy ball milling, were reported to affect crystallinity and microstructural uniformity. While high-energy ball milling enhances particle refinement and dopant distribution, it may also introduce lattice strain and defects, resulting in peak broadening in XRD patterns [36]. When combined with controlled sintering, these approaches improved densification and compressive strength without complete loss of phase stability [30].

Ionic substitution with biologically relevant ions, including Mg^{2+}, Na^{+}, and Co^{2+} was shown to modify lattice parameters and, in some cases, slightly reduce crystallinity due to structural distortion [35, 40]. In studies where biological evaluation was performed, such substitutions were associated with enhanced cell viability, osteogenic differentiation, or antimicrobial activity [33, 34, 36]. These findings indicate that controlled deviations from ideal stoichiometry may contribute to functional bioactivity.

Overall, the collective evidence supports a structure–property relationship in bone waste–derived HA materials. Studies involving higher sintering temperatures and improved lattice ordering frequently reported enhanced mechanical performance [30]. In contrast, carbonate incorporation or ionic substitution was associated with improved biological interaction in investigations that included cellular assays [32, 33]. This observed balance between structural stability and biological functionality underscores the need for tailored processing strategies, particularly for dental biomaterial

Table 2. Characterization of hydroxyapatite from various biological waste sources for dental applications.

Ref.	Sample	Method / Preparation	Characterization	Results
[30]	Sheep femur–derived hydroxyapatite (KHO); MgO-reinforced composites (KHM1, KHM5, KHM10); graphene hybrid composites (KHM1GRF0.1, 0.5, 1)	Ball milling–assisted powder metallurgy combined with induction sintering	XRD (phase/crystal structure), SEM (microstructure), Archimedes method (density & porosity), compressive strength testing	Optimal density achieved at 1200°C for 1.5 min. MgO and graphene reinforcement improved densification and compressive strength. SEM showed denser microstructure; XRD confirmed hydroxyapatite phase stability.
[31]	Aceh bovine femur bone	Cleaning by boiling → acetone soaking (2 h) → thermal calcination (700–1000°C, 3 h) to obtain bovine hydroxyapatite (BHA)	FTIR, XRD, SEM–EDX, Particle Size Analyzer (PSA)	XRD showed high crystallinity (>84%) at 900–1000°C. PSA and SEM revealed submicron spherical particles with uniform distribution. FTIR confirmed PO_4^{3-}, CO_3^{2-}, and OH^- functional groups. SEM–EDX demonstrated homogeneous Ca and P distribution with Ca/P ratio of 1.7–2.3, indicating suitability for dental bioapplications.
[20]	Bovine cortical bone	Cleaning and defatting → alkaline hydrolysis using NaOH → washing to neutral pH → drying → grinding to obtain hydroxyapatite powder	XRD, FTIR, SEM, EDX	XRD confirmed formation of crystalline hydroxyapatite. FTIR identified characteristic PO_4^{3-} and OH^- groups. SEM showed porous agglomerated particles. EDX revealed Ca and P with Ca/P ratio close to stoichiometric HA (~1.67), indicating successful conversion into biogenic hydroxyapatite.
[32]	Green mussel shell (Ca source) and bovine bone waste (P source)	Cleaning and pretreatment → preparation of Ca and P precursors → microwave-assisted hydrothermal synthesis to form carbonated apatite	XRD, FTIR, SEM, EDX	XRD confirmed formation of carbonated apatite phase. FTIR identified PO_4^{3-}, CO_3^{2-}, and OH^- groups indicating B-type carbonated apatite. SEM revealed agglomerated apatite particles. EDX showed Ca and P with appropriate Ca/P ratio, demonstrating successful conversion into dental-relevant apatite biomaterials.
[33]	Carp bones; porous nano-hydroxyapatite substrate loaded with sodium fluoride (NaF); dental pulp stem cells (DPSCs)	Fabrication of porous nano-hydroxyapatite scaffold → NaF loading → cell seeding with DPSCs	XRD, FTIR, SEM, MTT assay, alkaline phosphatase (ALP) activity, Alizarin Red S staining	XRD confirmed nano-hydroxyapatite crystalline phase. FTIR identified PO_4^{3-} and OH^- groups. SEM showed interconnected porous morphology suitable for cell attachment. NaF-loaded nHA significantly enhanced DPSC proliferation, ALP activity, and mineralized nodule formation, indicating promoted osteogenic differentiation.
[34]	Fish (*Lethrinus lentjan*) scale	Fish scales washed, dried, dispersed in Milli-Q water, and hydrothermally treated at 280°C for 3 h → filtration → dialysis and freeze-drying	UV–Vis–NIR, fluorescence spectroscopy, XRD, HRTEM, EDX, FTIR, MTT assay, AO/EB nuclear morphology, confocal microscopy, ALP activity, Alizarin Red S staining	FTIR confirmed PO_4^{3-} and OH^- groups in HA NPs and N/O-functionalized surfaces in CDs. XRD showed hexagonal HA (JCPDS 00-009-0432). HRTEM revealed rod-shaped HA NPs (8–12 nm diameter, 50–100 nm length) and spherical particles (15–50 nm). EDX confirmed Ca/P ratio ~2.33. HA NPs and CDs showed >90% cell viability and enhanced osteogenic differentiation.
[14]	Broiler chicken bone waste and eggshells	Cleaning → ethanol soaking → drying → calcination (800°C) → dissolution in HNO_3 → wet precipitation with $(NH_4)_2HPO_4$ (pH > 9) → aging → drying → recalcination	AAS (Ca), UV–Vis spectrophotometry (P), Ca:P ratio analysis	Chicken bone-derived material showed higher Ca (18.9 ± 0.386%) and P (13.906 ± 0.320%) than eggshell-derived material. Ca:P ratio of chicken bone (1:1.359) exceeded eggshell (1:1.158), indicating superior precursor potential for hydroxyapatite synthesis.
[13]	*Sardinella longiceps* fish bone	Boiling → alkaline–organic pretreatment (2% NaOH + acetone) → drying → grinding	XRD, FTIR, SEM, EDX, particle size analysis, in vitro cell assays	SEM showed agglomerated rod-like crystals and dense nano-sized particles. FTIR confirmed phosphate and carbonate

Ref.	Sample	Method / Preparation	Characterization	Results
		→ alkaline hydrolysis → phosphatization using H_3PO_4 → drying → sieving to obtain nano-hydroxyapatite		groups. XRD matched JCPDS 00-009-0432. AFM showed nanoscale particles (~19.65 nm). MTT and ARS assays indicated enhanced osteoblast proliferation and mineralization.
[21]	Pig cortical bone waste	Cleaning → deproteinization (NaOH + HCl) → drying → grinding → calcination (600–1000°C) → porous scaffold fabrication using ammonium bicarbonate → sintering	XRD, FTIR, TGA, SEM, EDX, TEM, SAED, density & porosity measurements	Single-phase hexagonal HA obtained with increasing crystallinity at higher temperatures. Rod-like nanoparticles observed. Ca/P ratio increased to ~1.88. Porous scaffolds (25–65% porosity) with interconnected pores (10–15 μm) suitable for bone tissue engineering.
[35]	Bovine bone wastes	Calcination (900°C, 8 h) → hydrothermal treatment with diammonium hydrogen phosphate (110°C, 12–18 h)	XRD, FTIR, SEM	~90 wt.% crystalline HA produced. Hydrothermal treatment yielded Mg- and Na-ion–substituted carbonated HA with Ca/P ratios of 1.6–1.9. SEM showed nanocrystals (60–80 nm) forming non-uniform agglomerates.
[36]	Natural HA, Co-HA, Mg-HA, and Co/Mg-HA from bovine bone	Bone burning (400–820°C) → solid-state doping via high-energy ball milling (HEBM)	TEM, SEM, SAXS/WAXS, FTIR, XPS, XANES, UV–Vis DRS, PL, VSM, biological assays	Doping increased crystallinity (up to 99.7%) and reduced bandgap to 1.94 eV. Co-doping induced ferromagnetism and significantly enhanced antimicrobial activity while maintaining high biocompatibility.

applications that require both mechanical integrity and biointegration.

3.3.1.2. Functional Groups and Ionic Substitution

Chemical functional group analysis is a critical component in evaluating HA derived from bone waste, as it reflects both the preservation of the apatite lattice and the extent to which the material resembles biological hard tissues [41]. Based on the studies summarized in Table 2, Fourier Transform Infrared Spectroscopy (FTIR) was consistently employed to identify the characteristic functional groups of bone waste-derived HA, including phosphate (PO_4^{3-}), hydroxyl (OH^-), and carbonate (CO_3^{2-}) ions. Across all studies, FTIR spectra confirmed the presence of the fundamental apatite structure, indicated by strong absorption bands corresponding to PO_4^{3-} vibrational modes. These bands validate the successful transformation of bone waste into HA following calcination, hydrothermal treatment, or combined processing routes [42]. In addition, OH^- stretching and libration bands were detected, although their intensity varied among studies, suggesting differences in crystallinity, thermal treatment temperature, and post-processing conditions.

Notably, several studies reported distinct carbonate-related absorption bands, indicating the formation of carbonated hydroxyapatite, which is characteristic of naturally derived HA rather than stoichiometric synthetic HA. Specifically, [32], [21], and [35] identified carbonate substitutions predominantly associated with B-type carbonated HA, where CO_3^{2-} ions substitute for PO_4^{3-} sites within the apatite lattice. This substitution pattern closely resembles that of biological apatite found in human enamel and dentin.

The presence of carbonate groups highlights a fundamental distinction between bone waste-derived HA and stoichiometric HA [43]. Across multiple investigations, carbonated HA was consistently characterized by lattice distortion and slight reductions in crystallinity compared to fully stoichiometric phases, reflecting the incorporation of CO_3^{2-} within the apatite structure. Such substitutions are known to increase solubility and surface reactivity, properties considered advantageous for dental applications that require dynamic interaction with surrounding tissues [44]. Collectively, these findings indicate that carbonate incorporation in bone waste–derived HA is not merely a compositional variation but a biomimetic feature that aligns the material more closely with naturally occurring enamel and dentin.

Beyond carbonate incorporation, several studies in the table also reported ionic substitutions involving divalent and monovalent cations, such as Mg^{2+}, Na^+, and Co^{2+}. These substitutions originate from the natural mineral composition of bone or from intentional doping during synthesis. For example, Mg^{2+} and Na^+ substitutions were directly identified in carbonated HA derived from bovine bone waste [35]. Magnesium substitution, for instance, is commonly associated with biological apatite and is known to influence crystal size and surface reactivity, as evidenced by its role in improving densification and altering electronic properties in reinforced composites

and doped HA [30, 36]. Sodium substitution contributes to charge balance within the lattice [45]. Furthermore, the intentional incorporation of Co^{2+} via doping strategies exemplifies functional modification, in which it was used to induce ferromagnetism and significantly enhance the biomaterial's antimicrobial activity [36].

From a dental biomaterial perspective, these chemically substituted apatite structures are highly relevant. Natural enamel and dentin consist of non-stoichiometric [46], ion-substituted apatite rather than pure HA. Across studies where biological evaluation was performed, bone waste–derived HA containing CO_3^{2-}, Mg^{2+}, and Na^+ substitutions demonstrated favourable cellular responses and biofunctional properties [29, 33]. This chemical resemblance to native hard tissues is expected to support improved interfacial bonding, bioactivity, and potential remineralization performance when applied in restorative, coating, or regenerative dental applications.

In summary, FTIR analysis across the reviewed studies demonstrates that bone waste–derived HA consistently exhibits the essential functional groups of apatite while retaining natural ionic substitutions. These chemical features reinforce the suitability of bone waste–derived HA as a promising dental biomaterial, particularly for applications requiring close structural and chemical resemblance to native enamel and dentin.

3.3.1.3. Elemental Composition and Ca/P Ratio

Elemental composition analysis provides essential insight into the chemical stoichiometry and biological performance of HA derived from bone waste. Based on the studies summarized in Table 2, techniques such as SEM–EDX, Atomic Absorption Spectroscopy (AAS), and UV–Vis phosphorus analysis were employed to quantify calcium (Ca) and phosphorus (P) content and to determine the Ca/P molar ratio of the synthesized HA.

Across the reviewed studies, bone waste–derived HA consistently exhibited Ca/P ratios ranging from approximately 1.6 to 1.9, which aligns well with the compositional characteristics of biogenic apatite rather than stoichiometric HA (Ca/P = 1.67). This variation reflects the inherent heterogeneity of natural bone sources as well as differences in thermal treatment, purification steps, and post-synthesis processing [31, 35].

Comparative analysis among different bone sources reveals observable trends. HA derived from bovine bone generally shows Ca/P ratios close to or slightly above the stoichiometric value, reflecting its dense mineral structure and relatively stable apatite phase [20]. In contrast, fish bone-derived HA often exhibits slightly lower or more variable Ca/P ratios, which may be attributed to higher organic content and lower mineral density in aquatic species [13, 34]. Poultry and pigeon bone-derived HA display intermediate Ca/P values, influenced by their distinct biological mineralization patterns and skeletal microstructures [14, 37].

Importantly, deviations from the ideal stoichiometric Ca/P ratio should not be interpreted as a disadvantage. On the contrary, such deviations are characteristic of natural bone and tooth mineral and are often associated with enhanced biological performance. A Ca/P ratio lower or higher than 1.67 can indicate the presence of carbonate substitution, ionic vacancies, or trace element incorporation, all of which contribute to increased lattice disorder and surface reactivity [35, 36].

From a functional standpoint, variations in the Ca/P ratio have direct implications for solubility and bioresorbability. HA with slightly lower Ca/P ratios tends to exhibit higher solubility, thereby facilitating ion release and accelerating biological integration. Conversely, higher Ca/P ratios may enhance structural stability while still maintaining sufficient biological responsiveness. This balance is particularly relevant for dental applications, where controlled degradation and long-term stability are both critical [33, 37].

In the context of dental graft compatibility, bone waste–derived HA with biogenic Ca/P ratios closely resembles the mineral composition of human enamel and dentin. Such chemical similarity supports favorable cellular responses, improved osteoconductivity, and effective integration with surrounding dental tissues [31, 33]. Consequently, the elemental composition and Ca/P ratio of HA derived from diverse bone waste sources underscore its suitability as a dental biomaterial, offering compositional advantages over purely synthetic, stoichiometric HA [13, 21].

The physicochemical characteristics reported across studies demonstrate notable convergence. Most bone waste-derived HA exhibited Ca/P ratios between 1.6 and 2.3, closely approximating the stoichiometric value (~1.67) and mirroring natural bone mineral composition. Nanoscale morphologies, including 8–12 nm rod-like particles and ~20 nm nano-crystals, along with carbonated apatite structures, were consistently observed. While increased sintering temperature enhanced crystallinity and compressive strength, it reduced porosity, highlighting a trade-off between densification and biological interaction. These recurring structural patterns strongly correlate with the enhanced osteogenic outcomes reported in Table 2.

Importantly, the variability observed in Ca/P ratios (1.6–2.3), optimal thermal conditions, and porosity values

across studies should not be interpreted merely as inconsistency, but rather as the result of identifiable sources of heterogeneity. First, intrinsic biological variability among bone sources contributes significantly to compositional differences. For instance, fish scale–derived HA exhibited a Ca/P ratio of ~2.33 [34], pig cortical bone–derived HA reached ~1.88 after calcination [21], while bovine bone–derived materials demonstrated values ranging from near-stoichiometric (~1.67) [20] to 1.6–1.9 following hydrothermal treatment [35]. These variations reflect species-specific mineralization patterns, marine versus terrestrial origin, and potential trace ion substitution (Mg^{2+}, Na^{+}), rather than simple experimental inconsistency.

Second, processing parameters critically influence structural outcomes. Temperature-dependent shifts are evident across studies. For example, increasing sintering temperatures enhanced densification and mechanical strength in sheep femur–derived HA composites at 1200°C [30]. Similarly, calcination at 900–1000°C yielded highly crystalline bovine HA (>84%) [31], whereas lower-temperature treatments preserved carbonate substitution in carbonated apatite systems [32]. These findings indicate that thermal history governs crystallinity, densification, carbonate retention, and microstructural evolution, resulting in structure–property trade-offs rather than contradictions.

Third, analytical methodology contributes to reported variability. Ca/P ratios determined by SEM–EDX [21, 31]. AAS and UV–Vis spectrophotometry [14], or combined spectroscopic approaches, differ in sampling depth, calibration standards, and surface sensitivity. Likewise, porosity values obtained via Archimedes' principle [30], density–porosity measurements in scaffold systems [21], or SEM-based morphological estimation are not directly interchangeable. Therefore, part of the apparent heterogeneity arises from methodological differences rather than intrinsic material inconsistency.

Collectively, the reported variability underscores the tunability of bone-waste–derived hydroxyapatite. Rather than indicating poor reproducibility, these differences demonstrate that compositional stoichiometry, crystallinity, and porosity can be modulated through controlled precursor selection and processing design to meet specific dental regenerative requirements.

3.3.2. Morphological and Structural Features

3.3.2.1. Particle Size and Morphology

Particle size and morphology are critical parameters that govern the interfacial behavior [47], biological interaction, and functional performance of HA derived from bone waste [48]. Based on the studies summarized in Table 2, morphological characterization was predominantly conducted using SEM, TEM, AFM, and particle size analysis (PSA), revealing that bone waste-derived HA is typically obtained in the nano-scale regime, albeit with considerable variation depending on the source material and processing route.

Nano-sized HA derived from bone waste has been consistently reported across a broad nanoscale range, typically spanning approximately 8 to 250 nm, reflecting differences in source material and processing conditions. Studies indicate that controlled thermal treatment and post-synthesis size refinement can yield ultra-fine crystallites in the lower nanoscale domain (8–20 nm), closely resembling the dimensions of biological apatite in enamel and dentin [13, 34]. Conversely, synthesis approaches involving thermal calcination, sintering, or mechanical processing often lead to crystal growth, particle coarsening, and agglomerated microstructures, as evidenced by submicron or nanocrystal aggregates observed in bovine, fish, and composite-derived hydroxyapatite systems [13, 21, 31, 35]. Collectively, these findings demonstrate that particle size in bone waste–derived HA is not intrinsic to the biological source alone but is strongly governed by processing parameters and post-treatment strategies.

The distinction between nano-HA and micro-HA is particularly significant in dental biomaterial applications. Nano-HA offers a substantially higher specific surface area, enabling enhanced protein adsorption, ion exchange, and interfacial contact with dental hard tissues [49]. In contrast, micro-HA particles, while mechanically more stable, exhibit reduced surface reactivity and limited biological interaction [50], making them less effective for applications requiring rapid remineralization or strong adhesion.

A recurring morphological feature across multiple studies is particle agglomeration, which remains a common challenge in bone waste-derived nano-HA. Agglomeration arises from high surface energy and strong interparticle interactions inherent to nanoparticles, especially following high-temperature calcination or sintering [51]. SEM and TEM images frequently show clusters of loosely bound nanoparticles rather than fully dispersed individual particles, as observed in studies by [20]. SEM showed porous agglomerated particles, and in another study by [13], SEM showed agglomerated rod-like crystals and dense nano-sized particles. While agglomeration may reduce effective surface area, it does not negate the nanoscale nature of the primary particles. It can be partially mitigated through post-processing techniques such as ball milling,

ultrasonication, or surface modification, as demonstrated in the synthesis protocols of [37] using ball milling and [36] with the high-energy ball milling.

From a functional perspective, particle size and morphology directly influence adhesion and remineralization behavior. Nano-sized HA particles can penetrate micro-defects and interprismatic spaces in enamel and dentin, promoting intimate interfacial bonding. Their high surface reactivity also facilitates the release of calcium and phosphate ions, which are essential for nucleation and growth of new apatite layers during remineralization processes. Consequently, the nanoscale morphology of bone waste–derived HA provides a structural advantage for dental applications, particularly in preventive and regenerative dentistry, as evidenced by the enhanced osteoblast proliferation and mineralization reported for nano-HA from fish bone [13], and the successful osteogenic differentiation of dental pulp stem cells (DPSCs) on porous nano-HA scaffolds [33]. Overall, the morphological and structural features observed across the reviewed studies confirm that bone waste-derived HA can be engineered at the nanoscale to closely mimic the size and morphology of natural dental apatite. This morphological compatibility strengthens its potential as a sustainable and high-performance dental biomaterial.

3.3.2.2 Porosity and Microstructure

Porosity and microstructural architecture play a decisive role in determining the biological performance of HA derived from bone waste [52], particularly for applications in dental bone regeneration. Based on the studies summarized in Table 2, porosity characteristics were primarily evaluated using SEM observations, Archimedes' principle, and mercury intrusion porosimetry, providing both qualitative and quantitative insights into pore structure and distribution.

A relatively wide porosity range of approximately 25–65% has been reported for bone waste–derived HA, reflecting variations in precursor source, calcination temperature, compaction technique, and sintering conditions. Porosity values within this range are consistently associated with interconnected pore structures and micro-scale pore dimensions (10–15 μm), characteristics that are critical for bone tissue infiltration and scaffold functionality [21], these findings indicate that porosity in bone waste–derived HA is highly tuneable and largely governed by processing parameters rather than being solely determined by the biological origin of the raw material.

Sintering temperature emerges as a dominant parameter governing pore evolution and densification in bone waste–derived HA. Materials synthesized at lower sintering temperatures or processed without excessive densification generally retain higher porosity, whereas increasing the sintering temperature promotes grain growth, pore shrinkage, and partial pore closure, thereby reducing overall porosity. Evidence from biogenic hydroxyapatite studies indicates that increasing sintering or calcination temperature promotes microstructural densification and crystal growth, thereby enhancing mechanical performance and reducing porosity [21, 30, 31]. Likewise, near-optimal density conditions have been achieved at 1200°C with short holding times of approximately 1.5 minutes, further underscoring the inverse relationship between temperature-driven densification and pore retention [30]. Collectively, these findings confirm that porosity reduction is a temperature-mediated phenomenon that must be carefully controlled to balance mechanical integrity and biological functionality.

SEM micrographs consistently reveal interconnected pore networks, with pore sizes commonly observed in the 10–15 μm range. Such interconnected porosity is a critical microstructural feature, as it facilitates fluid transport, nutrient diffusion, and cellular migration within the material. Mercury porosimetry analysis reported by [21] further confirms the presence of open, connected pores, supporting SEM-based morphological observations and providing quantitative pore-size distribution data.

From a structural standpoint, the coexistence of micro- and mesopores contributes to a hierarchical architecture that closely resembles natural cancellous bone [53]. While macropores enhance cell infiltration and vascularization, smaller pores increase surface area and promote protein adsorption and ion exchange [52]. This multiscale porosity is particularly relevant for dental bone regeneration, where rapid integration with surrounding alveolar bone is essential.

In dental applications, an optimal balance between porosity and mechanical integrity is required. Porosity levels within the reported range enable sufficient space for new bone ingrowth while maintaining adequate structural stability for load-bearing conditions in the oral environment. Moreover, interconnected pores in the 10–15 μm range support osteoconductivity and accelerate remodeling, making bone-waste-derived HA suitable for dental grafts, ridge augmentation, and periodontal regeneration [21].

Consequently, the porosity and microstructural architecture observed in bone waste-derived HA demonstrate its strong potential as a dental regenerative biomaterial. The ability to control pore characteristics through processing parameters further enhances its

applicability, allowing customization for specific clinical needs in dental bone regeneration [33].

The wide porosity range reported (25–65%) in scaffold systems [21], compared with the denser microstructures achieved through high-temperature sintering and reinforcement strategies [30], further illustrates processing-dependent structural control. Such variability is particularly relevant in dental applications, where higher porosity may favor cell infiltration and osteogenesis. In comparison, lower porosity and higher densification may be preferable for load-bearing graft substitutes.

Nevertheless, the relatively broad porosity range (25–65%) reported across studies warrants critical consideration. This variability can be attributed to several interacting factors. First, differences in scaffold fabrication strategy, such as the incorporation of pore-forming agents (e.g., ammonium bicarbonate in pig bone scaffolds) [21], powder metallurgy combined with induction sintering [30], or conventional calcination without templating directly influence initial pore formation and retention. Second, compaction pressure and holding time during sintering significantly alter pore shrinkage dynamics, as demonstrated by the enhanced densification observed at 1200°C [30]. Third, reported porosity values depend on the characterization technique employed; measurements obtained via Archimedes' principle, mercury intrusion porosimetry, or density-based calculations are not strictly interchangeable and may yield systematically different quantitative results. Therefore, the heterogeneity in porosity values reflects processing- and measurement-dependent variability rather than fundamental inconsistencies in bone waste–derived hydroxyapatite systems. Importantly, this tunability enables structural optimization according to specific dental requirements, ranging from highly porous regenerative scaffolds to mechanically reinforced graft substitutes.

3.3.3. Influence of Processing Parameters on HA Characteristics

3.3.3.1. Mechanical Properties Relevant to Dental Applications

Thermal processing through calcination and sintering represents a critical step in transforming raw bone waste into phase-pure HA suitable for dental applications. As summarized in Table 2, all reviewed studies employed calcination temperatures ranging from approximately 600 to 1000°C to eliminate organic components such as collagen, lipids, and residual proteins inherent to animal bone sources [14, 21, 31]. SEM observations reported by [33] and [32] consistently indicate that controlled thermal treatment is key to microstructural development, with higher sintering temperatures promoting denser morphologies.

Beyond organic removal, increasing calcination and sintering temperatures significantly enhanced phase purity and crystallinity, as evidenced by the narrowing of XRD peaks and the dominance of HA characteristic reflections. For instance, [31] reported crystallinity exceeding 84% at 900–1000°C, while [36] achieved crystallinity up to 99.7% through high-temperature processing. However, this improvement in crystallinity was accompanied by grain coarsening. Several studies in Table 2 report that Ca/P ratios drift closer to stoichiometric values after high-temperature sintering, thereby improving mechanical stability but potentially reducing surface reactivity relative to slightly calcium-deficient biogenic HA. The process of high-temperature sintering tends to drive the Ca/P ratio of the resulting hydroxyapatite closer to the ideal stoichiometric value of 1.67, as demonstrated by a Ca/P ratio of 1.7 at 1050°C and values reported as close to 1.67 in other studies [20, 37].

Chemical modification further differentiates bone waste-derived HA from conventional synthetic HA by enhancing its functional and biological performance. As shown in Table 2, several studies reported B-type carbonate substitution, where carbonate ions (CO_3^{2-}) partially replaced phosphate groups in the HA lattice. This substitution, confirmed by FTIR analysis in studies [31, 32], closely mimics the composition of natural dental and bone apatite and is associated with increased solubility and improved biological responsiveness. In addition to carbonate substitution, ionic incorporation of Mg^{2+}, Na^{+}, and Co^{2+} has been consistently reported in bone waste–derived HA (Table 2). Hydrothermal processing routes have been shown to facilitate the formation of Mg- and Na-substituted carbonated HA. At the same time, solid-state doping strategies enable the controlled incorporation of transition and alkaline-earth metal ions into the HA lattice [35, 36]. Such ionic substitutions modify lattice parameters and influence densification behavior, mechanical performance, and biological response. Magnesium incorporation, in particular, is associated with improved densification and enhanced compressive strength, especially when introduced through MgO-based reinforcement systems [30]. Sodium substitution contributes to lattice stabilization and charge balance, whereas cobalt incorporation has been linked to enhanced antimicrobial activity, an advantageous property for dental implant and graft applications [36]. Collectively, these findings indicate that controlled ionic substitution provides a versatile strategy

for tailoring both the structural and functional properties of bone-waste–derived HA for advanced dental biomaterials.

From a mechanical standpoint, sintering temperatures above 900°C are consistently associated with marked increases in hardness and compressive strength (Table 2), bringing the resulting values closer to those reported for cancellous and alveolar bone. An increase in temperature toward 1200°C promotes near-optimal density conditions, although such thermal intensification is frequently accompanied by a reduction in porosity [30]. This recurring trade-off between mechanical strengthening and porosity retention highlights a central structure–property balance in bone waste–derived HA, where temperature-mediated densification must be carefully optimized to preserve bioactivity while ensuring sufficient structural integrity for dental graft applications.

Mechanical performance is a decisive factor in determining the clinical applicability of HA for dental and maxillofacial applications, where materials must withstand masticatory forces, maintain dimensional stability, and support load transfer during bone remodeling. In dental environments, HA-based materials are subjected to complex stress conditions, including compressive, shear, and cyclic loading, particularly in alveolar bone regions following tooth extraction or implant placement. While synthetic stoichiometric HA is widely recognized for its excellent biocompatibility [34, 36], its intrinsic brittleness and limited mechanical strength have constrained its use in load-bearing dental applications [21, 30]. Consequently, improving the mechanical properties of HA without compromising biological performance remains a critical challenge in dental biomaterials research [30, 35, 36].

Unlike general reviews that primarily discuss HA from a compositional or biological perspective, this section focuses on structure-property relationships in bone-waste-derived HA. Three interlinked processing parameters predominantly govern the mechanical enhancement of hydroxyapatite derived from bone waste. First, sintering temperature exhibits a direct and positive correlation with key mechanical properties; studies demonstrate that higher temperatures within the range of 1050-1200°C significantly improve compressive strength and material hardness, as evidenced by enhanced densification and compressive strength at elevated sintering temperatures, with optimal consolidation observed near 1200°C [30]. Second, strategic reinforcement through the incorporation of secondary phases, such as MgO and graphene, has been proven to further enhance densification and compressive strength via composite effects, effectively addressing HA's intrinsic brittleness [30]. Underpinning both strategies is the central role of densification behavior; the consistent pursuit of optimal density across studies is fundamentally linked to reduced porosity and the consequent improvement in mechanical integrity, forming a critical bridge between processing conditions and final structural performance [30, 37].

Finally, composite reinforcement strategies, particularly the incorporation of MgO and graphene, have been shown to enhance densification and compressive strength while maintaining the structural integrity of hydroxyapatite [30]. These findings demonstrate that composite engineering can mitigate the intrinsic mechanical limitations of pure HA, supporting the potential of bone-waste-derived hydroxyapatite as a sustainable candidate for advanced dental biomaterials.

By examining these relationships, this review emphasizes correlating measurable mechanical characteristics, compressive strength, and hardness with the functional requirements of alveolar and cancellous bone. This approach provides a clinically relevant framework for evaluating the suitability of bone-derived HA in dental regeneration, moving beyond mere biocompatibility to assess structural competence.

3.3.3.2. Size Reduction and Structural Refinement

Particle size refinement plays a decisive role in tailoring the performance of bone waste-derived HA for dental biomaterials. As detailed in Table 2, various studies employed ball milling and hydrothermal treatments to reduce HA particle sizes from the micrometer range to nanoscale dimensions. Across the reviewed literature, nanoscale hydroxyapatite particles have been reported within a broad range of approximately 8–100 nm, with ultra-fine crystallites in the lower range (8–12 nm) typically associated with controlled hydrothermal processing. In contrast, larger nanoscale aggregates are frequently linked to post-treatment conditions and particle agglomeration [13, 34]. Collectively, these findings indicate that particle size in bone-waste–derived HA is strongly governed by synthesis and refinement strategies rather than being inherently determined by the biological precursor.

The distinctions evident in Table 2 facilitate a direct comparison between nano-HA and micro-HA derived from bone waste. Nano-HA, with particle sizes of 8–12 nm [35] and ~20 nm [13], offers enhanced surface properties critical for dental adhesion and remineralization. Conversely, the table highlights a recurring trade-off: the ball milling processes used to achieve or refine these sizes [30, 37] often lead to particle agglomeration, a phenomenon extensively documented in SEM results [13,

20]. This agglomeration diminishes the effective surface area, thereby offsetting some of the inherent benefits of the nanoscale morphology.

Hydrothermal treatment emerged as a highly effective low-temperature strategy to refine crystal morphology while simultaneously limiting particle agglomeration. Studies employing microwave-assisted hydrothermal synthesis, such as that by [31], successfully produced agglomerated apatite particles from mussel shell and bovine bone precursors. More strikingly, the hydrothermal method used by [34] at 280°C resulted in well-defined, rod-shaped HA nanoparticles with diameters of 8–12 nm, demonstrating superior control over nanocrystal geometry. Similarly, [35] utilized hydrothermal treatment to convert calcined bovine bone into nanocrystals measuring 60–80 nm. These refined, uniform rod- or plate-like HA nanostructures generally exhibit improved dispersion compared to the heavily agglomerated powders often resulting from high-energy mechanical milling alone. The precise microstructural control afforded by hydrothermal synthesis is particularly advantageous for dental applications, where uniform particle size and morphology are critical for achieving intimate interfacial contact between the biomaterial and tooth or bone surfaces-a prerequisite for effective long-term stability and tissue regeneration.

3.4. Biocompatibility and Biological Performance

Biological performance is a fundamental requirement for the successful clinical translation of HA in dental and craniofacial applications [29]. Beyond physicochemical and mechanical suitability, HA-based biomaterials must support cellular viability, attachment, and metabolic activity to ensure effective bone regeneration and long-term tissue integration [54, 55]. For dental applications in particular, materials are expected to interact favorably with osteogenic and odontogenic cells under physiological conditions [56], without inducing cytotoxic or inflammatory responses. Consequently, in vitro biocompatibility assessments are mandatory evaluation criteria in dental and biomaterials research. In this study, evidence supporting enhanced osteogenic activity and biocompatibility assay was consistently reported in studies rated as high methodological quality. In contrast, studies with limited biological assessment primarily focused on physicochemical and morphological outcomes.

3.4.1. In Vitro Biocompatibility

Based on the studies summarized in Table 3, in vitro biocompatibility of bone waste-derived hydroxyapatite (HA) has been extensively evaluated using standard cytotoxicity and cell viability assays, including MTT, Live/Dead staining, and Alamar Blue tests, across various cell lines relevant to dental and bone tissue engineering.

Across the reviewed studies, bone waste–derived nano-hydroxyapatite consistently demonstrated high cytocompatibility and enhanced cellular proliferation compared to conventional synthetic HA. Using MTT and related viability assays, osteoblast and stem cell models commonly exhibited high viability, typically exceeding 80%, indicating low cytotoxicity and favorable cellular interactions [34, 36]. In certain cases, proliferation rates surpassed baseline controls, with reported values reaching approximately 141.3 ± 3.1% at optimal concentrations around 100 µg/mL in MG-63 osteoblast-like cells [13], underscoring the bioactive potential of biogenic nano-HA.

In dental-specific cellular environments, porous nano-HA scaffolds derived from bone waste supported enhanced proliferation of dental pulp stem cells (DPSCs), particularly when combined with fluoride incorporation, as assessed through MTT-based evaluation [33]. These findings collectively suggest that bone-derived HA not only maintains cytocompatibility across multiple relevant cell lines, including hMSCs, MG-63 osteoblast-like cells, and DPSCs, but also promotes cellular activity conducive to dentin–pulp regeneration and alveolar bone repair.

Comprehensive biological assessments, including MTS, Alamar Blue, and Live/Dead assays, further confirm that both pure and ion-doped bone-derived HA systems maintain cell viability above 80% with minimal hemolytic activity [36]. Importantly, ionic modifications such as Mg and Co incorporation did not compromise cytocompatibility, indicating that functional enhancements through doping can be achieved without sacrificing biological safety. Collectively, the in vitro evidence summarized in Table 3 demonstrates a consistent trend of favorable cellular response, reinforcing the suitability of bone waste–derived HA for dental and maxillofacial tissue engineering applications. Collectively, as evidenced by the studies listed in Table 3, bone waste-derived HA, regardless of animal source (bovine, fish, poultry, or pigeon), consistently demonstrates high in vitro biocompatibility, often outperforming synthetic HA. This trend underscores the biological advantage of naturally derived HA, likely attributed to its non-stoichiometric composition and trace ionic substitutions inherited from biological precursors.

3.4.2. Osteogenic and Mineralization Potential

Beyond cytocompatibility, several studies in Table 3 explicitly evaluated the osteogenic differentiation and mineralization capability of bone waste-derived HA

Table 3. In vitro biocompatibility and osteogenic performance of bone waste–derived hydroxyapatite.

Ref.	Sample / HA Source	Cell Type	Biocompatibility Assay	Osteogenic / Mineralization Assay	Key Findings
[33]	Porous nano-HA from carp bone loaded with NaF	Dental pulp stem cells (DPSCs)	MTT assay	ALP activity, Alizarin Red S (ARS) staining	NaF-loaded porous nHA significantly enhanced DPSC viability, ALP activity, and mineralized nodule formation, indicating promoted osteogenic differentiation.
[34]	HA nanoparticles from Lethrinus lentjan fish scales	Human mesenchymal stem cells (hMSCs)	MTT assay, AO/EB nuclear staining	ALP activity, ARS staining	HA nanoparticles showed >90% cell viability up to 200 μg/mL and significantly enhanced ALP activity and calcium nodule formation, confirming excellent cytocompatibility and osteogenic potential.
[13]	Nano-hydroxyapatite from Sardinella longiceps fish bone	Human osteoblast-like cells (MG-63)	MTT assay	ARS staining	n-HAP promoted osteoblast proliferation (up to 141.3 ± 3.1% at 100 μg/mL) and enhanced calcium deposition at optimal concentrations, indicating strong mineralization potential.
[36]	Natural HA, Mg-HA, Co-HA, Co/Mg-HA from bovine bone	Mammalian cells (various in vitro assays)	MTS, Alamar Blue, Live/Dead, hemolysis tests	–	All HA samples maintained high biocompatibility (>80% cell viability) with low hemolysis, confirming that ionic doping did not compromise cytocompatibility.

through alkaline phosphatase (ALP) activity and Alizarin Red S (ARS) staining. Across the reviewed studies, bone waste-derived nano-hydroxyapatite consistently enhances early osteogenic differentiation and matrix mineralization, as evidenced by increased alkaline phosphatase (ALP) activity and intensified Alizarin Red S (ARS) staining. Independent reports on HA derived from pigeon, carp bone, fish scale, and marine fish bone collectively demonstrate elevated ALP expression in osteoblasts, DPSCs, and mesenchymal stem cells compared to synthetic or untreated controls [13, 33, 34]. The repeated observation of well-defined mineralized nodules and dose-dependent calcium deposition across different cell models confirms that biogenic nano-HA actively promotes osteoblastic maturation rather than merely supporting cell viability.

The enhanced osteogenic performance observed across studies can be attributed directly to the intrinsic physicochemical characteristics of bone-waste-derived HA. Key features include nanoscale dimensions, partial carbonate substitution, and Ca/P ratios approaching those of natural bone mineral. These properties increase surface reactivity, improve protein adsorption, and facilitate ion exchange at the cell–material interface, thereby promoting osteoblast differentiation and extracellular matrix mineralization. By closely mimicking the mineral phase of dentin and alveolar bone, biogenic HA provides a biomimetic microenvironment that supports DPSCs' proliferation and osteoblast maturation, reinforcing its suitability for dental bone grafting and regeneration at the dentin–enamel interface.

Overall, explicit evidence from ALP and mineralization assays across multiple studies confirms that bone waste-derived HA is not only biocompatible but also biologically active, exhibiting strong osteogenic and mineralization potential, which is essential for dental and maxillofacial tissue engineering.

Importantly, these biological findings have direct implications for specific dental clinical scenarios. In alveolar bone regeneration and socket preservation procedures following tooth extraction, biomaterials must promote early osteoblast differentiation and stable mineralized matrix formation to support subsequent implant placement. The consistent enhancement of ALP activity and mineral deposition observed across studies [13, 33] suggests that bone waste-derived HA may effectively facilitate early-stage bone regeneration in such contexts.

In periodontal defect repair, where guided tissue regeneration requires osteoconductive materials that support cellular attachment, the reported nanoscale morphology and interconnected porosity [21, 30] provide favorable microenvironments for osteoblast interaction. Furthermore, the inclusion of dental pulp stem cells (DPSCs) in several in vitro investigations [33] directly

supports potential applications in regenerative endodontics and dentin–pulp complex reconstruction.

Beyond its demonstrated biological performance, the reviewed evidence underscores the broader sustainability implications of bone-waste-derived HA production. The successful conversion of bovine, poultry, fish, pig, and marine shell wastes into high-crystallinity, bioactive hydroxyapatite demonstrates a viable waste-valorization pathway within a circular bioeconomy framework. Rather than relying solely on synthetic calcium precursors, these approaches transform underutilized biowaste streams into clinically relevant biomaterials. The consistent physicochemical quality and osteogenic performance reported across diverse waste sources indicate that sustainability objectives can be achieved without compromising material functionality. Therefore, bone waste-derived HA represents not only a biologically effective dental biomaterial but also a strategically sustainable alternative for future regenerative applications.

From a sustainability perspective, the included studies collectively demonstrate the successful valorization of diverse biological wastes, including bovine bone, poultry waste, fish scales, mussel shells, and pig cortical bone, into biomedical-grade hydroxyapatite. The consistent achievement of high crystallinity and biological functionality from waste-derived sources underscores the feasibility of integrating HA production within a circular bioeconomy framework. This dual benefit of waste reduction and biomaterial generation positions bone waste-derived HA as a sustainable alternative to conventional synthetic HA production routes.

Collectively, these studies demonstrate the favorable cytocompatibility and osteogenic potential of bone-waste–derived HA systems across various in vitro models. Despite encouraging biological findings, it is important to recognize that the majority of the reviewed studies are limited to in vitro assays, primarily short-term cytocompatibility and differentiation evaluations. Long-term functional outcomes, gene expression profiling, and standardized comparative controls are often lacking. Furthermore, in vivo investigations remain scarce, and clinical studies are currently absent. Therefore, the current level of evidence should be considered preliminary and preclinical.

3.5. Limitations of This Review

This systematic review has several limitations that should be acknowledged. Although bone waste–derived HA has been synthesized using diverse extraction and transformation routes, the number of eligible studies remains relatively limited, restricting the ability to perform quantitative comparison among different synthesis methods. Most included studies were heterogeneous in terms of bone source (bovine, fish, pig, etc), processing conditions, and post-extraction treatments, which complicates direct cross-study comparison. The majority of included studies focused on in vitro assessments, with limited in vivo or long-term biological evaluation. This restricts the ability to extrapolate laboratory findings to clinical dental and maxillofacial applications.

Although several studies report excellent cytocompatibility and osteogenic performance of bone waste–derived hydroxyapatite, direct comparative evaluations against synthetic hydroxyapatite remain limited. Most studies assessed bone-derived HA independently without parallel synthetic controls. Therefore, while current evidence suggests promising biological advantages, definitive conclusions regarding superiority require systematic, standardized comparative investigations conducted under identical processing and biological testing protocols.

The most significant limitation of this review is the lack of access to major multidisciplinary databases such as Scopus and Web of Science due to institutional subscription constraints. While we employed rigorous supplementary search methods, such as citation snowballing and searches in open-access aggregators, some relevant studies may have been missed. Therefore, the findings should be interpreted with this potential database bias in mind. The absence of formal protocol registration may also be considered a limitation of this systematic review, although the methodology was predefined and consistently applied throughout the study. These constraints may introduce reporting bias related to database coverage and publication accessibility.

4. Conclusions and Implications for Future Research

This systematic review demonstrates that bone waste is a viable and sustainable source of HA for biomedical and dental applications. Thermal, alkaline, and hydrothermal-based extraction methods effectively produce HA with physicochemical characteristics closely resembling biogenic apatite, including non-stoichiometric Ca/P ratios, ion substitution, and multiscale porosity. Despite variability in precursor source and processing parameters, these methods enable tunable control over crystallinity, composition, and microstructure.

The resulting material characteristics, particularly ion substitution and hierarchical porosity, contribute to enhanced surface reactivity, biocompatibility, and osteogenic potential. Both natural and ion-doped bone-

derived HA systems consistently showed favorable in vitro biological performance, indicating potential suitability for dental and maxillofacial tissue regeneration, pending comprehensive in vivo and clinical validation. However, the limited number of in vivo studies and the absence of standardized comparative investigations with synthetic HA highlight the need for further translational validation. At present, bone-derived HA remains at a preclinical stage of development.

From a sustainability perspective, the valorization of bone waste into high-value HA biomaterials represents a practical implementation of circular economy principles. By converting underutilized animal by-products into functional biomedical materials, this approach reduces environmental burdens associated with waste disposal and decreases reliance on conventional synthetic precursors. Such resource-efficient strategies align with Sustainable Development Goal 12 (Responsible Consumption and Production) and SDG 3 (Good Health and Well-being). Nevertheless, future studies should incorporate life-cycle assessment and quantitative environmental impact analysis to substantiate these sustainability claims and strengthen the environmental dimension of bone-derived HA development.

To facilitate clinical and translational advancement, several priority actions should be considered. First, the development of standardized extraction, purification, and characterization protocols is essential to reduce methodological heterogeneity and enable cross-study comparability. Second, well-designed head-to-head comparative studies between bone-derived and synthetic HA under identical processing and biological testing conditions are necessary to establish reliable performance benchmarks. Third, expanded in vivo investigations and long-term safety assessments should be prioritized to determine clinical readiness in dental and maxillofacial applications. Finally, the integration of life-cycle assessment (LCA) and quantitative sustainability metrics will be critical for validating environmental claims and informing regulatory and policy decision-making.

Overall, bone waste-derived HA emerges as a promising biomimetic and environmentally responsible material platform, provided that future research addresses standardization, in vivo validation, and sustainability benchmarking.

Author Contributions: Conceptualization, V.D. and R.I.; methodology, V.D.; software, V.D.; validation, R.I., S.S., and S.F.; formal analysis, V.D.; investigation, V.D.; resources, S.F.; data curation, S.S.; writing—original draft preparation, V.D.; writing—review and editing, V.D.; visualization, R.I.; supervision, R.I, S.S., and S.F.; project administration, R.I.; funding acquisition, R.I. All authors have read and agreed to the published version of the manuscript.

Funding: This study does not receive external funding.

Ethical Clearance: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments: The authors would like to thank Universitas Syiah Kuala for the support in this research.

Conflicts of Interest: All the authors declare no conflicts of interest.

References

1. Macavei, M. G., Gheorghe, V.-C., Ionescu, G., Volceanov, A., Pătrașcu, R., Mărculescu, C., and Magdziarz, A. (2024). Thermochemical Conversion of Animal-Derived Waste: A Mini-Review with a Focus on Chicken Bone Waste, *Processes*, 358. doi:10.3390/pr12020358.
2. Álvarez-Castillo, E., Guerrero, P., de la Caba, K., Bengoechea, C., and Guerrero, A. (2023). Biorefinery Concept in the Meat Industry: From Slaughterhouse Biowastes to Superaborbent Materials, *Chemical Engineering Journal*, Vol. 471, 144564. doi:10.1016/j.cej.2023.144564.
3. Rigueto, C. V. T., Rosseto, M., Alessandretti, I., de Oliveira, R., Wohlmuth, D. A. R., Ferreira Menezes, J., Loss, R. A., Dettmer, A., and Pizzutti, I. R. (2022). Gelatin Films from Wastes: A Review of Production, Characterization, and Application Trends in Food Preservation and Agriculture, *Food Research International*, Vol. 162, 112114. doi:10.1016/j.foodres.2022.112114.
4. Mozhiarasi, V., and Natarajan, T. S. (2025). Slaughterhouse and Poultry Wastes: Management Practices, Feedstocks for Renewable Energy Production, and Recovery of Value Added Products, *Biomass Conversion and Biorefinery*, Vol. 15, No. 2, 1705–1728. doi:10.1007/s13399-022-02352-0.
5. Ashilenje, D., Ashour, F., Barz, M., Belandria, V., Borello, A., Bostyn, S., Boushaki, T., Branciari, R., Bwapwa, J. K., Cerza, E., Dell'Orto, A., Bari, H. El, Govender, M., Habchi, S., Karouach, F., Khashaba, N. H., Kouisni, L., Marconi, O., Marrocchi, A., Saber, M., Sarh, B., Trois, C., Willenbacher, M., and Wohlgemuth, V. (2026). A Literature Review of Slaughterhouse Waste Valorisation: Techniques, Environmental, and Economic Implications, *Resources, Conservation and Recycling*, Vol. 224, 108571. doi:10.1016/j.resconrec.2025.108571.
6. State, R. N., Ionescu, G., Volceanov, A., Coman, A. E., Magdziarz, A., and Marculescu, C. (2025). Combined Valorization of Bone Waste as Feedstock and Support Material for Ex-Situ Catalytic Pyrolysis, *Journal of Environmental Chemical Engineering*, Vol. 13, No. 5, 119091. doi:10.1016/j.jece.2025.119091.
7. Hart, A., Ebiundu, K., Peretomode, E., Onyeaka, H., Nwabor, O. F., and Obileke, K. (2022). Value-Added Materials Recovered from Waste Bone Biomass: Technologies and Applications, *RSC Advances*, Vol. 12, No. 34, 22302–22330. doi:10.1039/D2RA03557J.
8. Hong, M.-H., Lee, J. H., Jung, H. S., Shin, H., and Shin, H. (2026). Biomineralization of Bone Tissue: Calcium Phosphate-Based Inorganics in Collagen Fibrillar Organic Matrices, *Biomaterials Research*, Vol. 26, No. 1, 42. doi:10.1186/s40824-022-00288-0.
9. Zhu, X., Wang, C., Bai, H., Zhang, J., Wang, Z., Li, Z., Zhao, X., Wang, J., and Liu, H. (2023). Functionalization of Biomimetic Mineralized Collagen for Bone Tissue Engineering, *Materials Today Bio*, Vol. 20, 100660. doi:10.1016/j.mtbio.2023.100660.

10. Gao, M., Wang, Z., Xiao, W., Miao, L., Yang, Z., Liang, W., Ao, T., and Chen, W. (2024). Capacitive Deionization toward Fluoride Elimination: Selective Advantage, State of the Art, and Future Perspectives, *Desalination*, Vol. 577, 117392. doi:10.1016/j.desal.2024.117392.

11. Schwarcz, H. P., Binkley, D. M., Luo, L., and Grandfield, K. (2020). A Search for Apatite Crystals in the Gap Zone of Collagen Fibrils in Bone Using Dark-Field Illumination, *Bone*, Vol. 135, 115304. doi:10.1016/j.bone.2020.115304.

12. Firdaus Hussin, M. S., Abdullah, H. Z., Idris, M. I., and Abdul Wahap, M. A. (2022). Extraction of Natural Hydroxyapatite for Biomedical Applications—A Review, *Heliyon*, Vol. 8, No. 8. doi:10.1016/j.heliyon.2022.e10356.

13. Surya, P., Nithin, A., Sundaramanickam, A., and Sathish, M. (2021). Synthesis and Characterization of Nano-Hydroxyapatite from Sardinella Longiceps Fish Bone and Its Effects on Human Osteoblast Bone Cells, *Journal of the Mechanical Behavior of Biomedical Materials*, Vol. 119, 104501. doi:10.1016/j.jmbbm.2021.104501.

14. Adji, D., Sutrisno, B., Prastiwi, A., Anggoro, D., and Wuryastuti, H. (2025). Sustainable Synthesis of Hydroxyapatite from Poultry Waste for Veterinary Applications: A Calcination Approach, *Open Veterinary Journal*, Vol. 15, No. 4, 1695–1701. doi:10.5455/OVJ.2025.v15.i4.21.

15. Mlonka-Mędrala, A., Sobek, S., Wądrzyk, M., Werle, S., Ionescu, G., Mărculescu, C., and Magdziarz, A. (2025). Energy and Material Recovery from Bone Waste: Steam Gasification for Biochar and Syngas Production in a Circular Economy Framework, *Energy*, Vol. 325, 136179. doi:10.1016/j.energy.2025.136179.

16. Guerra, M., Morgado, M., Leira, Y., Leitão, T., Botelho, J., and Mendes, J. J. (2025). Integrating Sustainability in Dentistry: A Pathway towards Achieving the UN 2030 Agenda, *Frontiers in Oral Health*, Vol. Volume 6-. doi:10.3389/froh.2025.1549020.

17. Mulimani, P. (2017). Green Dentistry: The Art and Science of Sustainable Practice, *British Dental Journal*, Vol. 222, No. 12, 954–961. doi:10.1038/sj.bdj.2017.546.

18. Di Spirito, F., Giordano, F., Di Palo, M. P., De Benedetto, G., Aulisio, L., and Boccia, G. (2025). Sustainable Dental and Periodontal Practice: A Narrative Review on the 4R-Framework—Reduce, Reuse, Rethink, Recycle—And Waste Management Rationalization, *Dentistry Journal*, 392. doi:10.3390/dj13090392.

19. Vyas, J., Raytthatha, N., Singh, S., Prajapati, B. G., Mohite, P., and Munde, S. (2024). Sustainable Sources of Raw Materials as Substituting Biomaterials for Additive Manufacturing of Dental Implants: A Review, *Periodontal and Implant Research*, Vol. 8, No. 1, 3. doi:10.1007/s41894-024-00130-x.

20. Gnanasekaran, R., Yuvaraj, D., Muthu, C. M. M., Ashwin, R., Kaarthikeyan, K., Kumar, V. V., Ramalingam, R. J., Al-Lohedan, H., and Reddy, K. (2024). Extraction and Characterization of Biocompatible Hydroxyapatite (Hap) from Red Big Eye Fish Bone: Potential for Biomedical Applications and Reducing Biowastes, *Sustainable Chemistry for the Environment*, Vol. 7, 100142. doi:10.1016/j.scenv.2024.100142.

21. Ofudje, E. A., Rajendran, A., Adeogun, A. I., Idowu, M. A., Kareem, S. O., and Pattanayak, D. K. (2018). Synthesis of Organic Derived Hydroxyapatite Scaffold from Pig Bone Waste for Tissue Engineering Applications, *Advanced Powder Technology*, Vol. 29, No. 1, 1–8. doi:10.1016/j.apt.2017.09.008.

22. Kumar, R., and Mohanty, S. (2022). Hydroxyapatite: A Versatile Bioceramic for Tissue Engineering Application, *Journal of Inorganic and Organometallic Polymers and Materials*, Vol. 32, No. 12, 4461–4477. doi:10.1007/s10904-022-02454-2.

23. Dewi, N., Rahmah, R. A., Wardhana, A. S., Puspitasari, D., Wasiaturrahmah, Y., and Gustiono, D. (2024). Remineralizing Potential of Natural Hydroxyapatite from Snakehead (Channa Striata) Fish Bone on Remineralization of Primary Teeth Enamel: An In Vitro Study, *European Journal of General Dentistry*, Vol. 14, No. 1, 11–19. doi:10.1055/s-0044-1791706.

24. Osuchukwu, O. A., Salihi, A., Abdullahi, I., and Obada, D. O. (2022). Taguchi Grey Relational Optimization of Sol–Gel Derived Hydroxyapatite from a Novel Mix of Two Natural Biowastes for Biomedical Applications, *Scientific Reports*, Vol. 12, No. 1, 17968. doi:10.1038/s41598-022-22888-5.

25. Alsharif, S. A., Badran, M. I., Moustafa, M. H., Meshref, R. A., and Mohamed, E. I. (2023). Hydrothermal Extraction and Physicochemical Characterization of Biogenic Hydroxyapatite Nanoparticles from Buffalo Waste Bones for in Vivo Xenograft in Experimental Rats, *Scientific Reports*, Vol. 13, No. 1, 17490. doi:10.1038/s41598-023-43989-9.

26. Bih, N. L., Mahamat, A. A., Chinweze, C., Ayeni, O., Bidossèssi, H. J., Onwualu, P. A., and Boakye, E. E. (2022). The Effect of Bone Ash on the Physio-Chemical and Mechanical Properties of Clay Ceramic Bricks, *Buildings*, 336. doi:10.3390/buildings12030336.

27. Horta, M. K. dos S., Westin, C., Rocha, D. N. da, de Campos, J. B., Souza, R. F. M. de, Aguilar, M. S., and Moura, F. J. (2023). Hydroxyapatite from Biowaste for Biomedical Applications: Obtainment, Characterization and in Vitro Assays, *Materials Research*, Vol. 26. doi:10.1590/1980-5373-MR-2022-0466.

28. Afriani, F., Siswoyo, Amelia, R., Hudatwi, M., Zaitun, and Tiandho, Y. (2020). Hydroxyapatite from Natural Sources: Methods and Its Characteristics, *IOP Conference Series: Earth and Environmental Science*, Vol. 599, No. 1, 12055. doi:10.1088/1755-1315/599/1/012055.

29. Suresh, N., Sweety, V. K., Suresh, N., Suraj, A. R., Waltimo, T., and Anil, S. (2026). NATURAL Sources of Hydroxyapatite for Biomedical Applications, *Ceramics International*, Vol. 52, No. 2, 1383–1391. doi:10.1016/j.ceramint.2025.12.019.

30. Usta, E. N., Demirkol, N., Turan, B. C., Gürbüz, M., Göller, G., Barbaro, K., Sagrafoli, D., Fosca, M., and Rau, J. V. (2025). Characterization of Eco-Friendly Fabricated and Induction-Sintered Hydroxyapatite-Based Hybrid Composites, *Materials*, 5359. doi:10.3390/ma18235359.

31. Diansari, V., Idroes, R., Sunarso, S., and Fitriyani, S. (2025). Extraction and Characterization of Aceh Bovine Bone-Derived Hydroxyapatite for Applications in Dentistry., *European Journal of Dentistry*. doi:10.1055/s-0045-1802946.

32. Irfa'i, M. A., Muryanto, S., Prihanto, A., Pusparizkita, Y. M., Ismail, R., Jamari, J., Bayuseno, A. P., and Show, P. L. (2024). Microwave-Assisted Hydrothermal Synthesis of Carbonated Apatite with Calcium and Phosphate Resources Derived from Green Mussel Shell and Bovine Bone Wastes, *Environmental Advances*, Vol. 17, 100582. doi:10.1016/j.envadv.2024.100582.

33. Arab, S., Bahraminasab, M., Asgharzade, S., Doostmohammadi, A., Zadeh, Z. K., and Nooshabadi, V. T. (2024). On the Osteogenic Differentiation of Dental Pulp Stem Cells by a Fabricated Porous Nano-Hydroxyapatite Substrate Loaded with Sodium Fluoride, *BMC Oral Health*, Vol. 24, No. 1, 1218. doi:10.1186/s12903-024-04987-z.

34. Athinarayanan, J., Periasamy, V. S., and Alshatwi, A. A. (2020). Simultaneous Fabrication of Carbon Nanodots and Hydroxyapatite Nanoparticles from Fish Scale for Biomedical Applications, *Materials Science and Engineering: C*, Vol. 117, 111313. doi:10.1016/j.msec.2020.111313.

35. Pusparizkita, Y. M., Schmahl, W. W., Satria, G. D. A., Ismail, R., Jamari, J., and Bayuseno, A. P. (2026). Synthesizing Calcium Phosphate Powder from Bovine Bone Wastes Using Calcination and Hydrothermal Techniques to Evaluate Physicochemical Properties and Mineralogy Speciation, *Sustainable Chemistry for Climate Action*, Vol. 8, 100165. doi:10.1016/j.scca.2025.100165.

36. Maleki-Ghaleh, H., Kamiński, B., Moradpur-Tari, E., Raza, S., Khanmohammadi, M., Zbonikowski, R., Shakeri, M. S., Siadati, M. H., Akbari-Fakhrabadi, A., and Paczesny, J. (2024). Visible Light-Sensitive Sustainable Quantum Dot Crystals of Co/Mg Doped Natural Hydroxyapatite Possessing Antimicrobial Activity and

Biocompatibility, *Small*, Vol. 20, No. 52, 2405708. doi:10.1002/smll.202405708.

37. Sharifianjazi, F., Esmaeilkhanian, A., Moradi, M., Pakseresht, A., Asl, M. S., Karimi-Maleh, H., Jang, H. W., Shokouhimehr, M., and Varma, R. S. (2021). Biocompatibility and Mechanical Properties of Pigeon Bone Waste Extracted Natural Nano-Hydroxyapatite for Bone Tissue Engineering, *Materials Science and Engineering: B*, Vol. 264, 114950. doi:10.1016/j.mseb.2020.114950.

38. Kusumawardani, R., Noviyanti, A. R., Nurhadi, M., Umar, A. A., Irwansyah, F. S., and Permana, M. D. (2025). Effect of Sintering Conditions on the Structural and Morphological Characteristics of Hydroxyapatite Synthesized from Belida Fish (Chitala Lopis) Bone, *ASEAN Journal of Science and Engineering*, Vol. 5, No. 3. doi:10.17509/ajse.v5i3.88775.

39. Liu, Q., Matinlinna, J. P., Chen, Z., Ning, C., Ni, G., Pan, H., and Darvell, B. W. (2015). Effect of Thermal Treatment on Carbonated Hydroxyapatite: Morphology, Composition, Crystal Characteristics and Solubility, *Ceramics International*, Vol. 41, Nos. 5, Part A, 6149–6157. doi:10.1016/j.ceramint.2014.11.062.

40. Kurzyk, A., Szwed-Georgiou, A., Pagacz, J., Antosik, A., Tymowicz-Grzyb, P., Gerle, A., Szterner, P., Włodarczyk, M., Płociński, P., Urbaniak, M. M., Rudnicka, K., and Biernat, M. (2023). Calcination and Ion Substitution Improve Physicochemical and Biological Properties of Nanohydroxyapatite for Bone Tissue Engineering Applications, *Scientific Reports*, Vol. 13, No. 1, 15384. doi:10.1038/s41598-023-42271-2.

41. Malla, K. P., Regmi, S., Nepal, A., Bhattarai, S., Yadav, R. J., Sakurai, S., and Adhikari, R. (2020). Extraction and Characterization of Novel Natural Hydroxyapatite Bioceramic by Thermal Decomposition of Waste Ostrich Bone, *International Journal of Biomaterials*, Vol. 2020, No. 1, 1690178. doi:10.1155/2020/1690178.

42. Touareb, D., Latifi, S., Saoiabi, S., Habraji, L., Hammani, O., Azzaoui, K., Jodeh, S., Yaghi, S., Sabbahi, R., Hammouti, B., and Saoiabi, S. (2025). Influence of Calcination Temperature on Equine Bone Hydroxyapatite Structure and Lead Adsorption Efficiency, *Scientific Reports*, Vol. 15, No. 1, 33990. doi:10.1038/s41598-025-11961-4.

43. Rahyussalim, A. J., Supriadi, S., Marsetio, A. F., Pribadi, P. M., and Suharno, B. (2019). The Potential of Carbonate Apatite as an Alternative Bone Substitute Material, *Medical Journal of Indonesia*, Vol. 28, No. 1, 92–7. doi:10.13181/mji.v28i1.2681.

44. Vijayan, A., Vishnu, J., A, R., Shankar, B., and Sambhudevan, S. (2025). A Review on Hydroxyapatite Fabrication: From Powders to Additive Manufactured Scaffolds, *Biomaterials Science*, Vol. 13, No. 4, 913–945. doi:10.1039/D4BM00972J.

45. Correa-Piña, B. A., Gomez-Vazquez, O. M., and Rodriguez-García, M. E. (2026). Structural and Thermal Effects of Controlled Sodium Substitution in Nanocrystalline Carbonate Hydroxyapatite Synthesized by Precipitation with Spray-Based Reactant Delivery, *Materials Chemistry and Physics*, Vol. 349, 131836. doi:10.1016/j.matchemphys.2025.131836.

46. Koontongkaew, S., Utispan, K., Worawongvasu, R., Chawhuaveang, D. D., and Yu, O. Y. (2024). Enamel and Its Interaction with the Oral Environment, L. C. Ardelean; L.-C. Rusu (Eds.), , IntechOpen, London. doi:10.5772/intechopen.114839.

47. Torres, F. C. L., Sousa, E. M. B. De, and Cipreste, M. F. (2022). A Brief Review on Hydroxyapatite Nanoparticles Interactions with Biological Constituents, *Journal of Biomaterials and Nanobiotechnology*, Vol. 13, 12–44. doi:10.4236/jbnb.2022.131002.

48. Lebre, F., Sridharan, R., Sawkins, M. J., Kelly, D. J., O'Brien, F. J., and Lavelle, E. C. (2017). The Shape and Size of Hydroxyapatite Particles Dictate Inflammatory Responses Following Implantation, *Scientific Reports*, Vol. 7, No. 1, 2922. doi:10.1038/s41598-017-03086-0.

49. Abere, D. V., Ojo, S. A., Oyatogun, M. G., Paredes-epinosa, M. B., Niluxsshun, M. C. D., and Hakami, A. (2022). Mechanical and Morphological Characterization of Nano-Hydroxyapatite (NHA) for Bone Regeneration : A Mini Review, *Biomedical Engineering Advances*, Vol. 4, No. October, 100056. doi:10.1016/j.bea.2022.100056.

50. Kareem, M. M., and Tanner, K. E. (2020). Optimising Micro-Hydroxyapatite Reinforced Poly(Lactide Acid) Electrospun Scaffolds for Bone Tissue Engineering, *Journal of Materials Science: Materials in Medicine*, Vol. 31, No. 4, 38. doi:10.1007/s10856-020-06376-8.

51. Brum, I. da silva, Elias, C. N., Ciambarella, B. T., Fonseca, G. A. M. D. da, Frigo, L., Carvalho, M. A., and de Carvalho, J. J. (2024). Physicochemical Characterization of a Nano-Hydroxyapatite/β-Tricalcium Phosphate (n-HA/β-TCP) and Type 1 Collagen Composite: An *In Vitro* Study, *Preprints*, Preprints. doi:10.20944/preprints202412.2444.v1.

52. Yoshikawa, H., Tamai, N., Murase, T., and Myoui, A. (2008). Interconnected Porous Hydroxyapatite Ceramics for Bone Tissue Engineering, *Journal of The Royal Society Interface*, Vol. 6, No. suppl_3, S341–S348. doi:10.1098/rsif.2008.0425.focus.

53. Xie, P., Du, J., Li, Y., Wu, J., He, H., Jiang, X., and Liu, C. (2019). Robust Hierarchical Porous MBG Scaffolds with Promoted Biomineralization Ability, *Colloids and Surfaces B: Biointerfaces*, Vol. 178, 22–31. doi:10.1016/j.colsurfb.2019.02.042.

54. Shan, E., Chamorro, C., Ferrández-Montero, A., Martin-Rodriguez, R. M., Ferrari, B., Sanchez-Herencia, A. J., Virto, L., Marín, M. J., Figuero, E., and Sanz, M. (2025). In Vitro Biological Properties Assessment of 3D-Printed Hydroxyapatite–Polylactic Acid Scaffolds Intended for Bone Regeneration, *Journal of Functional Biomaterials*, 218. doi:10.3390/jfb16060218.

55. Pinteala, T., Sirbu, P.-D., Anghel, N., Rosca, I., Voicu, G., Calin, M., and Spiridon, I. (2024). Integrating Hydroxyapatite and Bovine Bone Mineral into Cellulose–Collagen Matrices for Enhanced Osteogenesis, *Materials Advances*, Vol. 5, No. 24, 9573–9585. doi:10.1039/D4MA00456F.

56. Huang, X., Li, Z., Liu, A., Liu, X., Guo, H., Wu, M., Yang, X., Han, B., and Xuan, K. (2021). Microenvironment Influences Odontogenic Mesenchymal Stem Cells Mediated Dental Pulp Regeneration, *Frontiers in Physiology*, Vol. 12. doi:10.3389/fphys.2021.656588.