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Review

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Ceramic Hydroxyapatite Derived from Fish Scales in Biomedical Applications: a Systematic Review

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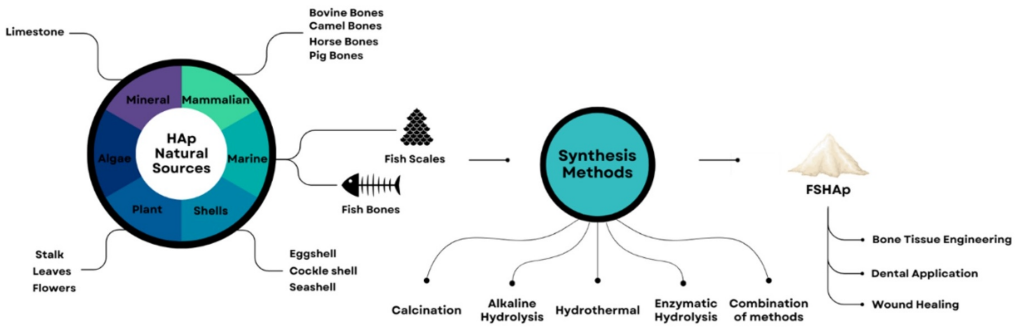
Graphical Abstract



Conversion of fish scale bio-waste into hydroxyapatite (HAp)

Abstract

The production of fish waste is increasing dramatically, as more than 70% of captured fish require processing before being sold. These enormous amounts of fish by-products are wasted, generating an undesirable environmental impact and a loss of value-added products. Recently, there has been a growing interest in utilizing fish scale waste as a low-cost source to produce natural nano-hydroxyapatite (HAp) ceramic growth and sustainability. On 1 April 2023, an electronic search was performed across four major databases (ScienceDirect, ProQuest, Scopus, and PubMed). Among the 1824 articles identified, only 27 met this review's inclusion criteria. These studies extracted HAp from fish scales and conducted in vitro or in vivo assessments. The in vitro studies demonstrated the non-cytotoxic nature of HAp derived from fish scales and its superior cell viability compared to control or synthetic HAp. Furthermore, histopathological evaluations revealed favorable bioavailability and biological responses. These findings indicate that HAp obtained from natural sources offers an environmentally friendly, sustainable, and cost-effective approach for manufacturing these materials, which are fully utilized for high-commercial purposes. Such materials are promising for biomedical applications, contributing positively to the economy, environment, and overall community well-being.

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Keywords: biomaterial; biomedical application; calcium phosphate; ceramics; fish scale; fish waste; hydroxyapatite

Purpose, Rationale, and Limitations

The escalating production of fish waste, particularly fish scales, poses significant environmental and economic challenges. Over 70% of captured fish require processing, resulting in substantial by-product waste that often goes unutilized, leading to environmental degradation and economic losses. The growing interest in adopting sustainable practices has spotlighted the potential of these by-products, particularly fish scales, as a resource for generating value-added products like natural nano-hydroxyapatite (HAp). This systematic review aims to explore using fish scales, a low-cost and natural source, to produce ceramic HAp intended for biomedical applications. It highlights its non-cytotoxic nature, superior cell viability, and favorable bioavailability. This review aims to establish a comprehensive understanding of the advancements and potential of fish scale-derived HAp (FSHAp) in biomedical applications, thereby contributing to economic growth, sustainability, and community well-being. However, the review is limited by including studies primarily in vitro or in vivo, with a noticeable absence of clinical trials that could substantiate the practical applications of FSHAp in human healthcare.

Summary of relevant literature

According to the Food and Agriculture Organization (FAO) [1], there has been a significant increase in the consumption of aquatic foods, excluding algae, in recent decades, going from 19 million tons in 1950 to an all-time record of about 179 million tons in 2018, with an annual growth rate of 3.3% (Figure 1). The fishery and aquacultural production have increased drastically, in line with the expanding world's population and rapid urbanization and industrialization. As a result of high fish demand, the volume of fish waste has increased dramatically worldwide. A large amount of underutilized waste is generated in the fish processing industry and fish markets, including viscera (12–18%), heads (9–12%), skins and fins (1–3%), scales (5%), bones (9–15%), and muscle-trimmings (15–20%) [2]. Discarding most fish wastes of low commercial value is a common practice, resulting in environmental pollution and the loss of value-added by-products. Fish processing is a critical necessity for big fish companies, both for reducing transport costs of unused parts and increasing product stability and quality. Companies remove parts, such as the viscera, which might have enzymes and bacteria that pose a risk during processing or storage [3]. Approximately 20 to 80% of solid fish waste must be discarded [4]. These vast quantities of fish by-products are discarded, leading to an adverse environmental impact. Hydroxyapatite (HAp), chemically recognized as $Ca_{10}(PO_4)_6(OH)_2$, is a mineral form of calcium apatite ceramics, a group of phosphate-containing minerals [8]. It is classified within the domain of bioresorbable ceramics. These materials are characterized by their capacity for gradual degradation and subsequent absorption by the body, ultimately facilitating their replacement by native tissue [9].

Among different aquacultural wastes, fish scales have gained much interest in recent years. Fish scales have limited industrial values and are usually thrown away as aquacultural waste. Nevertheless, recent studies revealed that fishery discards might potentially serve as an alternative source of bioactive compounds, such as hydroxyapatite, collagen, gelatin, lipids, enzymes, hydrolysates, and peptides, exhibiting significant promise for diverse applications in nutraceuticals and functional foods [5].

In fact, using marine species, including fish products, for medical purposes is common, and they are considered promising sources of beneficial animal-derived medicines [6]. The composition of fish scales predominantly consists of approximately 41%-45% organic constituents (including collagen, fat, lecithin, sclerotin, vitamins, etc.) and 38%-46% inorganic components (such as calcium-deficient hydroxyapatite, calcium phosphate, etc.), along with trace elements like zinc and iron [7].

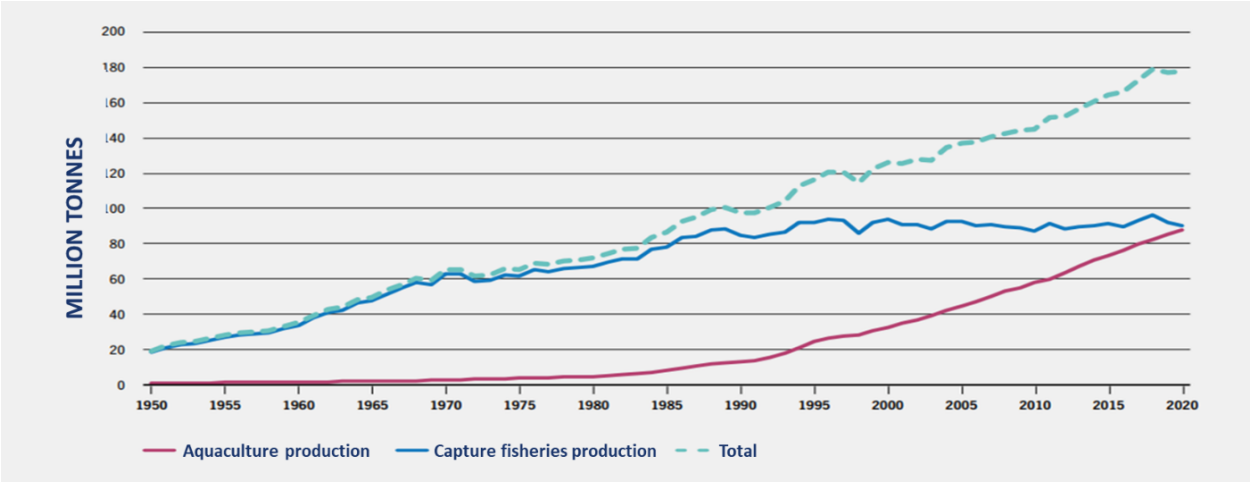

Figure 1. World capture fisheries and aquaculture production.

HAp crystallizes in a hexagonal system, forming a complex lattice structure comprised of calcium ions, phosphate groups, and hydroxide ions. Such a structure endows HAp with high stability and enables it to replicate the mineral components of human bones and teeth closely. The lattice of HAp is versatile and capable of incorporating various ions, which facilitates the modification of its physical and chemical properties. Among the various calcium phosphate (CaP) salts listed in Table 1, hydroxyapatite stands out because it chemically and structurally mimics the phosphate complexes found in bones and teeth, with a calcium-to-phosphorus (Ca/P) ratio of 1.5 to 1.7 [10]. In biomedicine, HAp is recognized for its biocompatibility, osteo-conductivity, and bioactivity, notably its capacity to form direct bonds with living tissues [11]. It is the predominant bio-mineral in bones and teeth, with the inorganic phase of bone largely consisting of calcium phosphates, primarily hydroxyapatite [12]. Ap exhibits thermodynamic stability in body fluids when crystallized and shares a compositional likeness with bone minerals, facilitating its integration into bone structures without eliciting inflammatory responses or local or systemic toxicity [13]. This compatibility underscores its significant potential in different biomedical applications and for bone tissue engineering (BTE) in particular [14], [15].

Typically, natural hydroxyapatite is derived from biological sources or by utilizing waste materials, such as mammalian bone (e.g., bovine [26], [27], camel [28], horse [29], and pig [30]), aquatic or marine sources (e.g., fish bone [31] and fish scale[32]), shell sources (e.g., cockle [33], eggshell [34], and seashell [35]), and plants and algae [36], [37] and also from mineral sources (e.g., limestone) [38]. Shells consist of calcium carbonate, which can undergo a chemical transformation into HAp by reacting with an appropriate source of phosphorus. On the other hand, animal bones, fish bones, and scales serve as excellent sources of naturally occurring hydroxyapatite due to their inherent composition. Hence, the extraction process alone is sufficient to obtain hydroxyapatite from these biological sources, eliminating the need for additional chemical reactions. The unique physical properties of fish scales, characterized by higher collagen content and greater flexibility than bone, facilitate a more straightforward and easier extraction of HAp [39]. It is noteworthy that hydroxyapatite, irrespective of its source, contains certain impurities such as hydroxyl ions (OH^-), phosphite ions (PO_3^{3-}), fluoride ions (F^-), and chloride ions (Cl^-). The presence of chloride and phosphate ions can alter the configuration of HAp, whereas hydroxyl and fluoride ions are known to enhance its conformational robustness [40]. This aspect of HAp's composition is crucial in understanding its interaction with biological systems and its potential modifications for specific applications. Various techniques can be employed to extract HAp from fish scales. Among these, the widely used method is the calcination process, which entails subjecting the scales to varying temperatures of up to 1400°C in a furnace [32].

Table 1. Different Salts of Calcium Phosphate (CaP)

Material	Symbol	Chemical Formula	Ca/P Ratio	Application	Reference
Monocalcium phosphate monohydrate	MCPM	$Ca(H_2PO_4)_2 \cdot H_2O$	0.5	Used in fertilizers and as a leavening agent in the food industry	[16]
Dicalcium phosphate dihydrate	DCPD	$CaHPO_4 \cdot 2H_2O$	1.0	Commonly used as a dietary supplement and in toothpaste formulations	[17]
Octacalcium phosphate	OCP	$Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O$	1.33	Applied in bone regeneration and as a precursor to hydroxyapatite in biomedical applications	[18]
α–Tricalcium phosphate	α-TCP	$\alpha\text{-}Ca_3(PO_4)_2$	1.5	Utilized in bone graft substitutes and orthopaedic applications	[19]
β–Tricalcium phosphate	β-TCP	$\beta\text{-}Ca_3(PO_4)_2$	1.5	Widely used in dental and orthopaedic bone grafting procedures	[20]
Amorphous calcium phosphate	ACP	$CaxHy(PO_4)z \cdot nH_2O$, n = 3–4.5, 15–20% H_2O	1.0–2.2	Employed in dental care products for remineralization of teeth	[21]
Hydroxyapatite	HA or HAp	$Ca_{10}(PO_4)_6(OH)_2$	1.67	Extensively used in bone grafting, dental implants, and as a coating for orthopaedic implants	[22]
Fluorapatite	FA or FAp	$Ca_{10}(PO_4)\,6F_2$	1.67	Used in dental care for the prevention of tooth decay and in bone graft materials	[23]
Oxyapatite	OA or OAp	$Ca_{10}(PO_4)\,6O$	1.67	Investigated for potential applications in high-temperature superconductors and luminescent materials	[24]
Tetracalcium phosphate	TTC	$Ca_{10}(PO_4)\,2O$	2.0	Used in dental cement and as a precursor for hydroxyapatite in biomedical applications	[25]

Alternative techniques like alkaline heat treatment have been employed to extract HAp from fish scales. This method uses an alkaline solution, typically sodium hydroxide (NaOH), to eliminate organic constituents in the scales [41]. The enzymatic hydrolysis technique involves the application of enzymes to break down the organic constituents present in fish scales. Specifically, to effectively hydrolyze collagen, a specific enzyme capable of targeting this protein component, such as protease, is utilized during the treatment process [42]. Ionic liquid pretreatment involves the application of ionic liquids possessing elevated hydrogen bond basicity to dissolve biopolymers like collagen or cellulose [43].

This literature review aims to assess the utilization of fish scales as a cost-effective natural source for producing HAp for biomedical applications. Emphasis is placed on evaluating studies

that have successfully extracted hydroxyapatite from fish scales and conducted subsequent biological evaluations of the extracted material in vitro and in vivo. Despite the extensive body of research on hydroxyapatite, there are currently no systematic reviews exploring the possibilities of various biomedical applications of fish scale-derived hydroxyapatite (FSHAp).

Methodology

The design of the present systematic review adhered to the guidelines provided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).[44]

Study Design

The research question for this study was formulated following the PICO (Population, Interventions, Comparisons, Outcomes) model:

(1) Studies that use biological evaluation, including invitro cytotoxicity (population)

(2) Studies evaluating HAp from fish scale sources as a biomaterial (interventions)

(3) Studies employed control interventions, such as untreated (blank) controls and HAp treatment comparisons

(4) Studies examined and reported the in vitro cytotoxicity test results to emphasize the biocompatibility of HAp derived from fish scales (outcomes)

Search Strategies

On 1 April 2023, a comprehensive systematic search was performed on four major databases, i.e., ScienceDirect, ProQuest, Scopus, and PubMed. The search strategy incorporated a combination of relevant keywords, such as ("hydroxyapatite" OR "bioceramic") AND ("fish scale" OR "fish waste*"), along with appropriate Boolean operators. The search was restricted to articles published in English, and the title, abstract, and keywords were encompassed in the search terms.

Inclusion and Exclusion Criteria

Biocompatibility publications that reported in vitro cytotoxicity or in vivo testing of HAp obtained from fish scales were incorporated into the review. Studies were excluded based on specific criteria:

The fish scale biomaterial used was not HAp (collagen, etc.)

The HAp is from natural sources other than fish scales (fish bones, eggshells, etc.).

No biological study

Non-primary studies, case reports, reviews, and book chapters

Data Extraction and Collection

All publications from the four databases were imported into a reference manager to eliminate duplicate records. Subsequently, these records underwent initial screening based on their titles and abstracts to identify studies that align with the scope of the review and meet the predefined inclusion criteria. After the title and abstract screening process, the authors retrieved, screened, and evaluated full-text articles. Studies that met any exclusion criteria during the full-text screening phase were omitted from the analysis. Descriptive summaries of the included studies were then compiled into tables and presented in the Supplemental files, containing information on the author(s), fish species, synthesis method, intervention, biological test, and critical findings respectively.

Data Synthesis and Statistical Analysis

Owing to the variations observed in study protocols, biomaterials utilized, methodologies employed for outcome assessment, and outcome measures, it was not feasible to conduct a meta-analysis. Consequently, only qualitative data from each study were amalgamated and presented in analytical tables.

Results

Study Selection

The initial search of four databases resulted in 1824 records, of which 236 were from Scopus, 56 from PubMed, 701 from ProQuest, and 831 from ScienceDirect. Duplicates, which comprised 176 records, were first removed. After excluding 1486 records based on title screening, 160 records underwent abstract screening, resulting in 113 exclusions.

Among the exclusions, 50 were not primary studies, 18 were out of scope, three involved HAp from sources other than fish scales, and 28 had no biological study. The remaining 48 studies underwent full-text screening, which helped to reject 21 more studies in which inclusion criteria were not met.

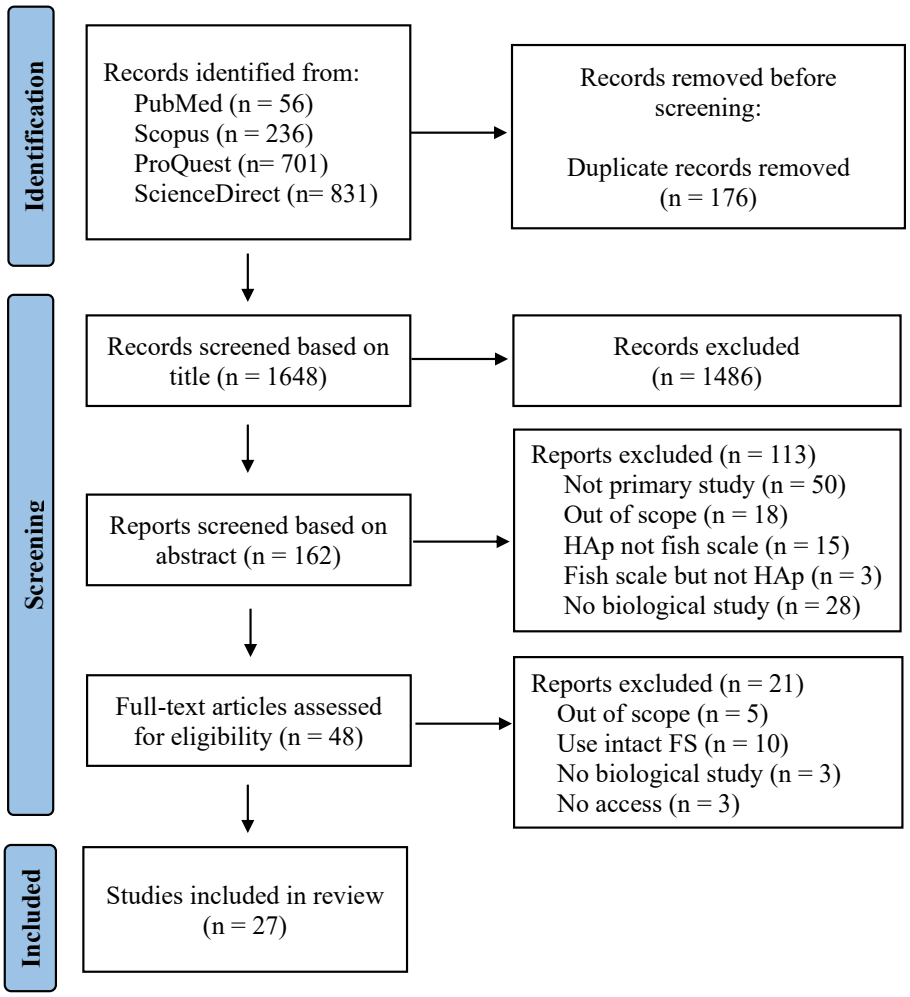

Figure 2. *Flowchart for the identification and selection of studies according to the PRISMA statement.*

The exclusion decision took place due to being out of scope (five studies), using the whole fish scale without extracting HAp (10 studies), having no biological study (three studies), and having a lack of access (three studies). Ultimately, twenty-seven articles were deemed eligible for inclusion in the review. The entire search and screening process was meticulously documented and presented in Figure 2.

Study Characteristics

This review includes 27 eligible articles that underwent thorough screening and assessment to obtain relevant data for comparison. Out of the 27 studies, 11 were conducted solely to evaluate the in vitro properties of fish scale-derived HAp, while four studies assessed both in vitro and in vivo characteristics. In addition, 13 studies examined the in vitro properties of fish scale-derived HAp in combination with other materials. The specific parameters of the studies were organized and presented in three tables *(Supplemental files)*: Table S1 outlines the 14 studies that examined in vitro properties of HAp bio-ceramics only, Table S2 presents the 13 studies that examined the in vitro properties of materials composed of a mixture of HAp and other materials, and Table S3 outlines the four studies that examined the in vivo properties of HAp bio-ceramics. Several in vitro tests and cells have been employed to study the impact of FSHAp; for example, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, with a primary focus on assessing cellular viability and identifying any potential toxic effects.

Study subjects

The research papers vary in their use of fish species and methodology for extracting HAp from fish scales. The variation in fish species examined across studies is influenced by the geographical locations where the research is conducted.

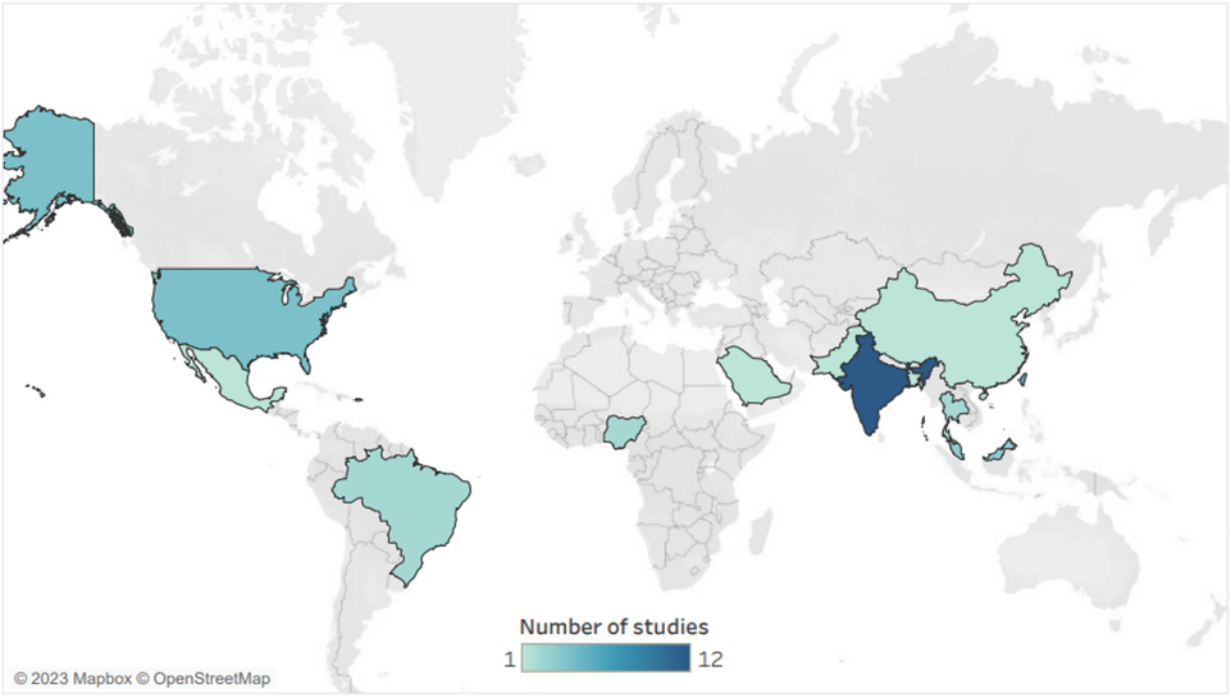

Figure 3. *Geographical distribution of the included studies.*

Among the published papers, India has the highest number of publications, totalling 12. With five papers, Taiwan followed closely, and the United States, with four papers. Brazil and Malaysia have an equal number of publications, each with three papers, while Nigeria and Thailand have two. Lastly, Bangladesh, China, Mexico, Pakistan, Saudi Arabia, and Singapore have each contributed one paper to the body of research on this topic (Figure 3). Various synthesis approaches are employed to develop natural HAp from fish scales, including calcination (up to 1200 °C), hydrothermal methods, alkaline hydrolysis, enzymatic hydrolysis, and combinations of these methods. New techniques like ionic liquid pretreatment and microwave irradiation have also been introduced. These different methodologies have produced distinct morphologies and sizes of FSHAp. Detailed information about the fish species used and the extraction techniques in these studies is provided in Tables S1 and S2.

Discussion

This study aimed to systematically review the potential applications of naturally extracted HAp from fish scales in different biomedical fields. The review encompassed both in vitro and in vivo assessments, providing insights into its suitability for diverse biomedical settings. It is worth mentioning that the characteristics of inorganic powders derived from fish scales (e.g., particle size, morphology, degree of crystallinity, porosity, etc.) are influenced by various factors, including the fishing region, fish species, extraction technique, and heat treatment temperature. Calcination methods serve the purpose of eliminating organic substances and eradicating any potentially present pathogens. However, it should be noted that the elevated temperatures used in this process can transform the HAp phases into β-tricalcium phosphate (β-TCP).

Nevertheless, compared to alternative approaches, calcination results in the production of HAp phases with greater crystallinity. This is supported by the observation that the calcium-to-phosphorus (Ca/P) ratios found in the extracted HAp samples lie within the range of 1.62–1.71,

demonstrating a close resemblance to the stoichiometric composition of HAp [32]. In contrast, the alkaline heat treatment, ionic liquid pretreatment, and enzymatic hydrolysis methods yield pure phase HAp but with reduced crystallinity compared to the calcination approach. In alkaline heat treatment, the NaOH solution induces hydrolysis of the organic matter within the scales, while the resulting inorganic residue is subsequently separated and purified through a filtration process [41]. As for enzymatic hydrolysis, it is to be noted that enzymes exhibit optimal activity at specific temperatures and pH conditions. Once all the collagen has been degraded through enzymatic action, the resulting mixture containing the enzyme is subjected to elevated temperatures to inactivate the enzymatic activity. Subsequently, the remaining HAp is isolated and collected through centrifugation [42]. In the ionic liquid pretreatment process, hydrogen bonding facilitates the interaction between collagen in fish scales and the ionic liquid, resulting in the dissolution of this organic component.

Meanwhile, HAp, the inorganic component, remains insoluble and precipitates. The subsequent separation of HAp is achieved through filtration and evaporation techniques [43]. Overall, the combination of ionic pretreatment and hydrolysis methods (enzymatic and alkaline hydrolysis) enables the extraction of pure HAp without converting or modifying its chemical structure into other calcium phosphate phases. Furthermore, most of these methods can produce nanosized HAp particles with diverse morphologies.

After extraction through various techniques, the characterization of the produced FSHAp is crucial for ensuring its quality and suitability for biomedical applications. FTIR analysis is essential for identifying the functional groups in HAp, revealing characteristic bands for P-O bending and stretching, carbonate, and amide linkages. These bands confirm the purity of FSHAp and its potential compatibility with biological tissues [32]. XRD analysis provides insights into the crystalline structure of HAp, a critical factor in bone grafting and dental applications due to its impact on mechanical strength and bioactivity. The sharp peaks observed in the XRD spectra of FSHAp, aligning with the Joint Committee on Powder Diffraction Standards data for pure HAp, indicate a high degree of crystallinity, essential for its structural integrity in load-bearing applications [46], [47]. Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) offer detailed visualization of FSHAp's morphology and particle size. These techniques reveal the surface topography and porosity of FSHAp, crucial factors for cell attachment and proliferation in tissue engineering. Recent advances in SEM and TEM have allowed for even more precise characterization of nanoscale features, enhancing our understanding of how FSHAp interacts at the cellular level. Mechanical testing provides valuable data on the hardness and modulus of elasticity of FSHAp. These properties are critical for ensuring FSHAp-based implants can withstand physiological loads without degrading. Compared to synthetic HAp, FSHAp often exhibits unique mechanical properties due to its natural origin, which may offer advantages in specific biomedical applications [46], [47], [70]. Various cell viability assays and in vitro cultures are employed to assess the biocompatibility of synthesized FSHAp. These tests are crucial for identifying potential toxic effects and establishing comprehensive safety profiles. They also provide insights into the proliferation and growth rates of cell populations, which is essential for predicting the in vivo behavior of FSHAp-based materials. According to ISO 10993-5 guidelines, a decrease in cell viability exceeding 30% indicates cytotoxicity [71]. This benchmark is vital for ensuring that FSHAp is safe for clinical use, making these tests an integral part of the material's characterization process.

MG-63 osteoblast-like cells have been extensively utilized as a valuable model for evaluating cell viability following the introduction of FSHAp material. Firstly, MG-63 cells exhibit characteristics similar to human osteoblasts, making them a relevant and reliable model for studying bone-related processes and responses to biomaterials. Secondly, these cells have a well-established culture system and are readily available, allowing for convenient and consistent experimentation. Among the existing literature, six studies have focused on this cell type. Notably, three of these studies [45], [48], [72] have demonstrated a significant increase in cell viability when compared to synthetic HAp or control groups.

This improvement in cell viability was attributed to trace elements such as Na and Mg and carbonate substitutions, which made the commercial HAp more similar to the mineral composition of natural bones.[73], [74] Furthermore, the different particle sizes between FSHAp (nanoscale) and synthetic HAp (microscale) could potentially influence cell viability [75]. Prior investigations have suggested that nanoscale particles enhance cell viability and function [76]. The remaining three studies [32], [46], [47] have reported satisfactory cell viability; however, it was still lower than that observed in the control group. This disparity could be attributed to irregularly shaped hydroxyapatite particles, which create obstacles to the proliferation of new cells. Moreover, cell proliferation is influenced by the surface roughness and surface area of the samples. In the case of FSHAp, the sintering process led to increased porosity and surface roughness. Overall, these factors did not have a negative impact on cell proliferation. Consistent findings across various cell types indicate a high percentage of cell viability when using FSHAp (Table S1). Additionally, it has been observed that hydroxyapatite derived from fish scales exhibits superior adherence and promotes cell proliferation compared to synthetic HAp. Due to its natural origin, the hydroxyapatite extracted from fish scales may contain more ions incorporated into its structure, such as carbonate ions [55]. This increased ion content enhances the material's dissolution and leads to a quicker bioactivity response than the synthetic sample. These findings demonstrate that fish scale-derived HAp is not cytotoxic in vitro. The successful clinical performance of biomedical implants largely relies on the interaction between the implants and the surrounding tissues at the interfacial level.

One of the primary challenges in developing scaffolds for biomedical applications is ensuring their high biocompatibility. To address this challenge, utilizing biological substances that closely resemble human components offers several advantages over synthetic materials. A promising approach involves the conversion of biological waste, such as fish scales, into valuable resources, specifically as a source of hydroxyapatite. As a result, numerous studies have explored the incorporation of fish scale-derived HAp in combination with other materials and polymers to create composites or scaffolds suitable for various biomedical applications, including BTE, dental applications, and wound healing. These fabricated scaffolds are subsequently assessed for cell viability using different in vitro cell models. The studies' findings in Table S2 align with the ISO 10993 standard, which specifies that a material is considered non-toxic if its cell viability exceeds 70%. These studies consistently demonstrated that FSHAp, both alone and in combination with other materials, exhibits excellent biocompatibility and is well-suited for biomedical applications. However, it is to be noted that although increasing the FSHAp enhances the bioactive properties of the scaffolds, it introduces a compromise in the mechanical characteristics of the scaffolds due to agglomeration [64]. Nonetheless, using extracted scale-based HAp fillers presents an opportunity for cost-effective ceramics. These investigations also indicate the possibility of creating novel biodegradable and biocompatible materials and recycling technologies to tackle forthcoming waste management challenges in an environmentally sustainable approach.

Four in vivo studies were carried out to acquire more detailed data about the presence of granulation tissue, inflammatory process, new bone formation, and biomaterial removal. The studies provided information about the species examined, the surgical procedures conducted, and the site of material implantation (see Table S3). Different animal models were then subjected to histopathological assessment to investigate the response of bone tissue after implantation of the material above. The objective was to evaluate the biocompatibility of these substituted bone implants. According to Prado et al. (2021) [51], the implantation of FSHAp resulted in the formation of granulation tissue in a structured and systematic manner, indicating a favorable biological response. Histomorphometric analysis further supported these observations, which revealed no statistically significant differences in the percentage of bone formation between the FSHAp and control groups. According to Mondal et al. (2016) [46], a microscopic examination of the FSHAp scaffold revealed distinct healing areas. These regions exhibited the formation of new cell linings, possibly indicating osteo-conduction.

Moreover, it was noted that the recovery rate and osteointegration ability of synthetic HAp were considerably lower when compared to FSHAp. Similar outcomes demonstrated that cell infiltration occurs in the healing regions. The recovery rate was also lower in the control and synthetic HAp groups compared to FSHAp [47]. Using chemical solution-based synthetic methods for preparing HAp may introduce a higher probability of contamination by various ions. This potential contamination could contribute to the compromised osteointegration property observed when synthetic HAp particles are implanted within the animal body system. In a study by Mondal et al. (2014) [49], the histological examination findings indicated the infiltration and integration of cells within the FSHAp scaffold, suggesting favorable bioactivity.

Furthermore, newly formed cell linings in certain regions may be attributed to osteoconduction. Overall, the observed new bone-cell formation in affected areas across various studies provides evidence of the favorable affinity and osteoconductive properties exhibited by the FSHAp biomaterial. However, additional clinical research is needed to establish and confirm the biocompatibility of FSHAp for its potential use in biomedical applications.

Conclusion

In conclusion, utilizing biowaste offers a dual advantage by enabling the extraction of valuable products such as HAp while simultaneously addressing solid waste management concerns within the fishery industry. This practice significantly contributes to a circular economy by reprocessing waste into high-value biomedical products, reducing the environmental burden and promoting resource efficiency. Most studies have provided substantial evidence supporting the excellent biocompatibility of FSHAp, demonstrating no cytotoxic effects in both in vitro and in vivo models. Additionally, natural HAp has displayed superior activity to synthetic HAp due to its resemblance to natural tissue constituents, suggesting a greener alternative that is more harmonious with biological systems. Histological and histomorphometric analyses conducted in these studies have further confirmed the efficacy of bone regeneration in animals treated with natural HAp compared to those with untreated defects. However, further research is still warranted to control the size and morphology of naturally produced HAp effectively. Advancements in this area could unlock diverse applications of FSHAp in the medical field, including tissue engineering and drug delivery, thereby enhancing the sustainability of healthcare systems.

Author Contributions: Chan KW conceptualized the research, designed the experiments, edited the manuscript, and acquired funding; Bakar ZA conceptualized the research and edited the manuscript; Ibrahim MA and Abdelmonem M- designed and carried out experiments, analyzed data and wrote the manuscript; Ismail N, Razis AFA, and Ch'ng SE- reviewed and commented on the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: Author Soo Ee Ch'ng was employed by the company CAIQ Biosecurity Sdn. Bhd. (Malaysia). The authors declare that this study received funding from DTS R&D Sdn. Bhd. (Malaysia). The funder was involved in the study regarding the development of value-added health products from fishery by-products. Other authors declare no conflict of interest. The funders had no role in writing the manuscript or deciding to publish the results.

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References

[1] The State of World Fisheries and Aquaculture 2022. FAO, 2022. doi: 10.4060/cc0461en.

[2] M. H. Pan et al., "Purification and characterization of a fish scale-degrading enzyme from a newly identified Vogesella sp.," J Agric Food Chem, vol. 58, no. 23, pp. 12541–12546, Dec. 2010, doi: 10.1021/jf1034042.

[3] A. Singh and S. Benjakul, "Proteolysis and Its Control Using Protease Inhibitors in Fish and Fish Products: A Review," Compr Rev Food Sci Food Saf, vol. 17, no. 2, pp. 496–509, Mar. 2018, doi: 10.1111/1541-4337.12337.

[4] D. Coppola, C. Lauritano, F. P. Esposito, G. Riccio, C. Rizzo, and D. de Pascale, "Fish Waste: From Problem to Valuable Resource," Marine Drugs, vol. 19, no. 2. MDPI, Feb. 01, 2021. doi: 10.3390/MD19020116.

[5] G. Caruso, R. Floris, C. Serangeli, and L. Di Paola, "Fishery Wastes as a Yet Undiscovered Treasure from the Sea: Biomolecules Sources, Extraction Methods and Valorization," Marine Drugs, vol. 18, no. 12. MDPI, Dec. 01, 2020. doi: 10.3390/md18120622.

[6] I. A. Prokopov et al., "Animal-derived medicinal products in Russia: Current nomenclature and specific aspects of quality control," Journal of Ethnopharmacology, vol. 240. Elsevier Ireland Ltd, Aug. 10, 2019. doi: 10.1016/j.jep.2019.111933.

[7] M. Holá, J. Kalvoda, H. Nováková, R. Škoda, and V. Kanický, "Possibilities of LA-ICP-MS technique for the spatial elemental analysis of the recent fish scales: Line scan vs. depth profiling," Appl Surf Sci, vol. 257, no. 6, pp. 1932–1940, Jan. 2011, doi: 10.1016/j.apsusc.2010.09.029.

[8] A. Szcześ, L. Hołysz, and E. Chibowski, "Synthesis of hydroxyapatite for biomedical applications," Advances in Colloid and Interface Science, vol. 249. Elsevier B.V., pp. 321–330, Nov. 01, 2017. doi: 10.1016/j.cis.2017.04.007.

[9] M. Vallet-Regí and E. Ruiz-Hernández, "Bioceramics: From bone regeneration to cancer nanomedicine," Advanced Materials, vol. 23, no. 44. pp. 5177–5218, Nov. 23, 2011. doi: 10.1002/adma.201101586.

[10] G. Borciani, T. Fischetti, G. Ciapetti, M. Montesissa, N. Baldini, and G. Graziani, "Marine biological waste as a source of hydroxyapatite for bone tissue engineering applications," Ceramics International, vol. 49, no. 2. Elsevier Ltd, pp. 1572–1584, Jan. 15, 2023. doi: 10.1016/j.ceramint.2022.10.341.

[11] S. Lara-Ochoa, W. Ortega-Lara, and C. E. Guerrero-Beltrán, "Hydroxyapatite nanoparticles in drug delivery: Physicochemistry and applications," Pharmaceutics, vol. 13, no. 10. MDPI, Oct. 01, 2021. doi: 10.3390/pharmaceutics13101642.

[12] D. S. Gomes, A. M. C. Santos, G. A. Neves, and R. R. Menezes, "A brief review on hydroxyapatite production and use in biomedicine," Ceramica, vol. 65, no. 374. Associacao Brasileira de Ceramica, pp. 282–302, 2019. doi: 10.1590/0366-69132019653742706.

[13] M. Sadat-Shojai, M. T. Khorasani, E. Dinpanah-Khoshdargi, and A. Jamshidi, "Synthesis methods for nanosized hydroxyapatite with diverse structures," Acta Biomaterialia, vol. 9, no. 8. Elsevier Ltd, pp. 7591–7621, 2013. doi: 10.1016/j.actbio.2013.04.012.

[14] R. Verma, S. R. Mishra, V. Gadore, and M. Ahmaruzzaman, "Hydroxyapatite-based composites: Excellent materials for environmental remediation and biomedical applications," Advances in Colloid and Interface Science, vol. 315. Elsevier B.V., May 01, 2023. doi: 10.1016/j.cis.2023.102890.

[15] F. Damiri et al., "Nano-hydroxyapatite (nHAp) scaffolds for bone regeneration: Preparation, characterization and biological applications," Journal of Drug Delivery Science and Technology, vol. 95. Editions de Sante, May 01, 2024. doi: 10.1016/j.jddst.2024.105601.

[16] A. Prihanto et al., “Aqueous crystallization of monocalcium phosphate monohydrate with green mussel shells (Verna piridis) for calcium sources,” J Environ Chem Eng, vol. 9, no. 6, Dec. 2021, doi: 10.1016/j.jece.2021.106913.
[17] N. Iqbal et al., “Dicalcium Phosphate Dihydrate Mineral Loaded Freeze-Dried Scaffolds for Potential Synthetic Bone Applications,” Materials, vol. 15, no. 18, Sep. 2022, doi: 10.3390/ma15186245.
[18] I. Kovrlija, J. Locs, and D. Loca, “Octacalcium phosphate: Innovative vehicle for the local biologically active substance delivery in bone regeneration,” Acta Biomaterialia, vol. 135. Acta Materialia Inc, pp. 27–47, Nov. 01, 2021. doi: 10.1016/j.actbio.2021.08.021.
[19] D. Moreno, F. Vargas, J. Ruiz, and M. E. López, “Solid-state synthesis of alpha tricalcium phosphate for cements used in biomedical applications,” Boletin de la Sociedad Espanola de Ceramica y Vidrio, vol. 59, no. 5, pp. 193–200, Sep. 2020, doi: 10.1016/j.bsecv.2019.11.004.
[20] H. Lu et al., “Current Application of Beta-Tricalcium Phosphate in Bone Repair and Its Mechanism to Regulate Osteogenesis,” Frontiers in Materials, vol. 8. Frontiers Media S.A., Jul. 15, 2021. doi: 10.3389/fmats.2021.698915.
[21] L. Degli Esposti and M. Iafisco, “Amorphous calcium phosphate, the lack of order is an abundance of possibilities,” Biomaterials and Biosystems, vol. 5, Mar. 2022, doi: 10.1016/j.bbiosy.2021.100037.
[22] I. Ielo, G. Calabrese, G. De Luca, and S. Conoci, “Recent Advances in Hydroxyapatite-Based Biocomposites for Bone Tissue Regeneration in Orthopedics,” International Journal of Molecular Sciences, vol. 23, no. 17. MDPI, Sep. 01, 2022. doi: 10.3390/ijms23179721.
[23] E. Santiago, V. Martin, B. Colaço, M. H. Fernandes, C. Santos, and P. S. Gomes, “Hydrothermal Synthesis of Fluorapatite Coatings over Titanium Implants for Enhanced Osseointegration—An In Vivo Study in the Rabbit,” J Funct Biomater, vol. 13, no. 4, Dec. 2022, doi: 10.3390/jfb13040241.
[24] N. V. Bulina, L. A. Avakyan, S. V. Makarova, I. B. Orehov, and V. S. Bystrov, “Structural Features of Oxyapatite,” Minerals, vol. 13, no. 1, Jan. 2023, doi: 10.3390/min13010102.
[25] T. Qin, X. Li, H. Long, S. Bin, and Y. Xu, “Bioactive tetracalcium phosphate scaffolds fabricated by selective laser sintering for bone regeneration applications,” Materials, vol. 13, no. 10, May 2020, doi: 10.3390/ma13102268.
[26] S. M. Londoño-Restrepo, C. F. Ramirez-Gutierrez, A. del Real, E. Rubio-Rosas, and M. E. Rodriguez-García, “Study of bovine hydroxyapatite obtained by calcination at low heating rates and cooled in furnace air,” J Mater Sci, vol. 51, no. 9, pp. 4431–4441, May 2016, doi: 10.1007/s10853-016-9755-4.
[27] R. X. Sun et al., “Physicochemical and biological properties of bovine-derived porous hydroxyapatite/collagen composite and its hydroxyapatite powders,” Ceram Int, vol. 43, no. 18, pp. 16792–16798, Dec. 2017, doi: 10.1016/j.ceramint.2017.09.075.
[28] H. L. Jaber, A. S. Hammood, and N. Parvin, “Synthesis and characterization of hydroxyapatite powder from natural Camelus bone,” Journal of the Australian Ceramic Society, vol. 54, no. 1, pp. 1–10, Mar. 2018, doi: 10.1007/s41779-017-0120-0.
[29] S. S. Rahavi, O. Ghaderi, A. Monshi, and M. H. Fathi, “A comparative study on physicochemical properties of hydroxyapatite powders derived from natural and synthetic sources,” Russian Journal of Non-Ferrous Metals, vol. 58, no. 3, pp. 276–286, May 2017, doi: 10.3103/S1067821217030178.
[30] E. A. Ofudje, A. Rajendran, A. I. Adeogun, M. A. Idowu, S. O. Kareem, and D. K. Pattanayak, “Synthesis of organic derived hydroxyapatite scaffold from pig bone waste for tissue engineering applications,” Advanced Powder Technology, vol. 29, no. 1, pp. 1–8, Jan. 2018, doi: 10.1016/j.apt.2017.09.008.
[31] A. Pal, S. Paul, A. R. Choudhury, V. K. Balla, M. Das, and A. Sinha, “Synthesis of hydroxyapatite from Lates calcarifer fish bone for biomedical applications,” Mater Lett, vol. 203, pp. 89–92, Sep. 2017, doi: 10.1016/j.matlet.2017.05.103.
[32] S. Paul et al., “Effect of trace elements on the sintering effect of fish scale derived hydroxyapatite and its bioactivity,” Ceram Int, vol. 43, no. 17, pp. 15678–15684, Dec. 2017, doi: 10.1016/j.ceramint.2017.08.127.
[33] R. Mustaffa, M. R. Mohd Yusof, and Y. Abdullah, “A Novelty of Synthetic Hydroxyapatite from Cockle Shell and Characterization,” Adv Mat Res, vol. 1087, pp. 429–433, Feb. 2015, doi: 10.4028/www.scientific.net/amr.1087.429.

[34] D. L. Goloshchapov et al., “Synthesis of nanocrystalline hydroxyapatite by precipitation using hen’s eggshell,” Ceram Int, vol. 39, no. 4, pp. 4539–4549, May 2013, doi: 10.1016/j.ceramint.2012.11.050.
[35] S. Santhosh and S. Balasivanandha Prabu, “Thermal stability of nano hydroxyapatite synthesized from seashells through wet chemical synthesis,” Mater Lett, vol. 97, pp. 121–124, 2013, doi: 10.1016/j.matlet.2013.01.081.
[36] D. Govindaraj and M. Rajan, “Synthesis and Spectral Characterization of Novel nano-Hydroxyapatite from Moringaoleifera Leaves,” in Materials Today: Proceedings, Elsevier Ltd, 2016, pp. 2394–2398. doi: 10.1016/j.matpr.2016.04.153.
[37] A. Teymouri, B. J. Stuart, and S. Kumar, “Hydroxyapatite and dittmarite precipitation from algae hydrolysate,” Algal Res, vol. 29, pp. 202–211, Jan. 2018, doi: 10.1016/j.algal.2017.11.030.
[38] J. Klinkaewnarong and S. Utara, “Ultrasonic-assisted conversion of limestone into needle-like hydroxyapatite nanoparticles,” Ultrason Sonochem, vol. 46, pp. 18–25, Sep. 2018, doi: 10.1016/j.ultsonch.2018.04.002.
[39] N. A. S. Mohd Pu’ad, P. Koshy, H. Z. Abdullah, M. I. Idris, and T. C. Lee, “Syntheses of hydroxyapatite from natural sources,” Heliyon, vol. 5, no. 5. Elsevier Ltd, May 01, 2019. doi: 10.1016/j.heliyon.2019.e01588.
[40] N. S. Resende, M. Nele, and V. M. M. Salim, “Effects of anion substitution on the acid properties of hydroxyapatite,” Thermochim Acta, vol. 451, no. 1–2, pp. 16–21, Dec. 2006, doi: 10.1016/j.tca.2006.08.012.
[41] W. Pon-On, P. Suntornsaratoon, N. Charoenphandhu, J. Thongbunchoo, N. Krishnamra, and I. M. Tang, “Hydroxyapatite from fish scale for potential use as bone scaffold or regenerative material,” Materials Science and Engineering C, vol. 62, pp. 183–189, May 2016, doi: 10.1016/j.msec.2016.01.051.
[42] Y. C. Huang, P. C. Hsiao, and H. J. Chai, “Hydroxyapatite extracted from fish scale: Effects on MG63 osteoblast-like cells,” Ceram Int, vol. 37, no. 6, pp. 1825–1831, Aug. 2011, doi: 10.1016/j.ceramint.2011.01.018.
[43] N. Muhammad et al., “Extraction of biocompatible hydroxyapatite from fish scales using novel approach of ionic liquid pretreatment,” Sep Purif Technol, vol. 161, pp. 129–135, Mar. 2016, doi: 10.1016/j.seppur.2016.01.047.
[44] D. Moher, A. Liberati, J. Tetzlaff, D. G. Altman, and P. Group, “Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement.” [Online]. Available: www.annals.org
[45] S. Sathiskumar et al., “Green synthesis of biocompatible nanostructured hydroxyapatite from Cirrhinus mrigala fish scale – A biowaste to biomaterial,” Ceram Int, vol. 45, no. 6, pp. 7804–7810, Apr. 2019, doi: 10.1016/j.ceramint.2019.01.086.
[46] S. Mondal, U. Pal, and A. Dey, “Natural origin hydroxyapatite scaffold as potential bone tissue engineering substitute,” Ceram Int, vol. 42, no. 16, pp. 18338–18346, Dec. 2016, doi: 10.1016/j.ceramint.2016.08.165.
[47] B. Mondal, S. Mondal, A. Mondal, and N. Mandal, “Fish scale derived hydroxyapatite scaffold for bone tissue engineering,” Mater Charact, vol. 121, pp. 112–124, Nov. 2016, doi: 10.1016/j.matchar.2016.09.034.
[48] G. Suresh Kumar, T. Sivakumar, S. Karthi, R. Govindan, and E. K. Girija, “Fish scale derived nanocrystalline hydroxyapatite: A potential candidate for orthopedic applications,” Journal of Bionanoscience, vol. 10, no. 2, pp. 140–144, Apr. 2016, doi: 10.1166/jbns.2016.1346.
[49] S. Mondal et al., “Physico-chemical characterization and biological response of Labeo rohita-derived hydroxyapatite scaffold,” Bioprocess Biosyst Eng, vol. 37, no. 7, pp. 1233–1240, 2014, doi: 10.1007/s00449-013-1095-z.
[50] S. Mondal et al., “Synthesis, characterization and in vitro cytotoxicity assessment of hydroxyapatite from different bioresources for tissue engineering application,” 2012.
[51] J. P. dos S. Prado et al., “In vitro and in vivo biological performance of hydroxyapatite from fish waste,” J Mater Sci Mater Med, vol. 32, no. 9, Sep. 2021, doi: 10.1007/s10856-021-06591-x.

[52] J. Athinarayanan, V. S. Periasamy, and A. A. Alshatwi, "Simultaneous fabrication of carbon nanodots and hydroxyapatite nanoparticles from fish scale for biomedical applications," Materials Science and Engineering C, vol. 117, Dec. 2020, doi: 10.1016/j.msec.2020.111313.

[53] N. N. Panda, K. Pramanik, and L. B. Sukla, "Extraction and characterization of biocompatible hydroxyapatite from freshwater fish scales for tissue engineering scaffold," Bioprocess Biosyst Eng, vol. 37, no. 3, pp. 433–440, 2014, doi: 10.1007/s00449-013-1009-0.

[54] M. K. dos S. Horta, F. J. Moura, M. S. Aguilar, C. B. Westin, D. Navarro da Rocha, and J. B. de Campos, "In vitro evaluation of natural hydroxyapatite from Osteoglossum bicirrhosum fish scales for biomedical application," Int J Appl Ceram Technol, vol. 18, no. 6, pp. 1930–1937, Nov. 2021, doi: 10.1111/ijac.13846.

[55] W. Pon-On, P. Suntornsaratoon, N. Charoenphandhu, J. Thongbunchoo, N. Krishnamra, and I. M. Tang, "Hydroxyapatite from fish scale for potential use as bone scaffold or regenerative material," Materials Science and Engineering C, vol. 62, pp. 183–189, May 2016, doi: 10.1016/j.msec.2016.01.051.

[56] N. Muhammad et al., "Extraction of biocompatible hydroxyapatite from fish scales using novel approach of ionic liquid pretreatment," Sep Purif Technol, vol. 161, pp. 129–135, Mar. 2016, doi: 10.1016/j.seppur.2016.01.047.

[57] F. A. Anene et al., "Biomechanical Properties and Corrosion Resistance of Plasma-Sprayed Fish Scale Hydroxyapatite (FsHA) and FsHA-Doped Yttria-Stabilized Zirconia Coatings on Ti–6Al–4V Alloy for Biomedical Applications," Coatings, vol. 13, no. 1, Jan. 2023, doi: 10.3390/coatings13010199.

[58] C. N. Aiza Jaafar, I. Zainol, M. I. Izyan Khairani, and T. T. Dele-Afolabi, "Physical and Mechanical Properties of Tilapia Scale Hydroxyapatite-Filled High-Density Polyethylene Composites," Polymers (Basel), vol. 14, no. 2, Jan. 2022, doi: 10.3390/polym14020251.

[59] S. Rajan, K. Marimuthu, C. B. Ayyanar, and M. E. Hoque, "Development and in-vitro characterization of HAP blended PVA/PEG bio-membrane," Journal of Materials Research and Technology, vol. 18, pp. 4956–4964, May 2022, doi: 10.1016/j.jmrt.2022.04.130.

[60] C. S. Wu, D. Y. Wu, and S. S. Wang, "Biodegradable Composite Nanofiber Containing Fish-Scale Extracts," ACS Appl Bio Mater, vol. 4, no. 1, pp. 462–469, Jan. 2021, doi: 10.1021/acsabm.0c00955.

[61] C. B. Ayyanar, K. Marimuthu, B. Gayathri, and Sankarrajan, "Characterization and in vitro cytotoxicity evaluation of fish scale and seashell derived nano-hydroxyapatite high-density polyethylene composite," Polymers and Polymer Composites, vol. 29, no. 9, pp. 1534–1542, Nov. 2021, doi: 10.1177/0967391120981551.

[62] A. A. Moorthy, N. Nanjappan, C. B. Ayyanar, and D. Dinesh, "in vitro Cytotoxicity examination of the seashell and fish scale substances embedded with high degree of compactness in polyethylene composite," Journal of Engineering Research (Kuwait), vol. 9, 2021, doi: 10.36909/jer.ICMMM.12431.

[63] Y. C. Huang and H. W. Chu, "Using hydroxyapatite from fish scales to prepare chitosan/gelatin/hydroxyapatite membrane: Exploring potential for bone tissue engineering," Journal of Marine Science and Technology (Taiwan), vol. 21, no. 6, pp. 716–722, 2013, doi: 10.6119/JMST-013-0320-1.

[64] D. Kodali, V. Hembrick-Holloman, D. R. Gunturu, T. Samuel, S. Jeelani, and V. K. Rangari, "Influence of Fish Scale-Based Hydroxyapatite on Forcespun Polycaprolactone Fiber Scaffolds," ACS Omega, vol. 7, no. 10, pp. 8323–8335, Mar. 2022, doi: 10.1021/acsomega.1c05593.

[65] J. K. Wang, Ç. Çimenoğlu, N. M. J. Cheam, X. Hu, and C. Y. Tay, "Sustainable aquaculture side-streams derived hybrid biocomposite for bone tissue engineering," Materials Science and Engineering C, vol. 126, Jul. 2021, doi: 10.1016/j.msec.2021.112104.

[66] C. S. Wu, S. S. Wang, D. Y. Wu, and W. L. Shih, "Novel composite 3D-printed filament made from fish scale-derived hydroxyapatite, eggshell and polylactic acid via a fused fabrication approach," Addit Manuf, vol. 46, Oct. 2021, doi: 10.1016/j.addma.2021.102169.

[67] B. Matiyal, H. Sharma, N. Kumar, and R. Kaur, "Microstructure and biocompatibility of biphasic ceramic (HA/β-TCP) from scales of freshwater fish common carp (Cyprinus carpio)," Mater Lett, vol. 304, Dec. 2021, doi: 10.1016/j.matlet.2021.130669.

[68] N. P. Wijedasa, S. M. Broas, R. E. Daso, and I. A. Banerjee, “Varying fish scale derived hydroxyapatite bound hybrid peptide nanofiber scaffolds for potential applications in periodontal tissue regeneration,” Materials Science and Engineering C, vol. 109, Apr. 2020, doi: 10.1016/j.msec.2019.110540.

[69] W. Pon-On, P. Suntornsaratoon, N. Charoenphandhu, J. Thongbunchoo, N. Krishnamra, and I. M. Tang, “Synthesis and investigations of mineral ions-loaded apatite from fish scale and PLA/chitosan composite for bone scaffolds,” Mater Lett, vol. 221, pp. 143–146, Jun. 2018, doi: 10.1016/j.matlet.2018.03.063.

[70] M. Kumar Yadav, R. Hiren Shukla, and K. G. Prashanth, “A comprehensive review on development of waste derived hydroxyapatite (HAp) for tissue engineering application,” Mater Today Proc, May 2023, doi: 10.1016/j.matpr.2023.04.669.

[71] International Organization for Standardization, “Biological evaluation of medical devices-part 5: tests for in vitro cytotoxicity.,” in ISO 10993–5. , Geneva, 2009, pp. 1–34.

[72] Y. C. Huang, P. C. Hsiao, and H. J. Chai, “Hydroxyapatite extracted from fish scale: Effects on MG63 osteoblast-like cells,” Ceram Int, vol. 37, no. 6, pp. 1825–1831, Aug. 2011, doi: 10.1016/j.ceramint.2011.01.018.

[73] A. Pal, S. Paul, A. R. Choudhury, V. K. Balla, M. Das, and A. Sinha, “Synthesis of hydroxyapatite from Lates calcarifer fish bone for biomedical applications,” Mater Lett, vol. 203, pp. 89–92, Sep. 2017, doi: 10.1016/j.matlet.2017.05.103.

[74] P. Shi, M. Liu, F. Fan, C. Yu, W. Lu, and M. Du, “Characterization of natural hydroxyapatite originated from fish bone and its biocompatibility with osteoblasts,” Materials Science and Engineering C, vol. 90, pp. 706–712, Sep. 2018, doi: 10.1016/j.msec.2018.04.026.

[75] M. Akram, R. Ahmed, I. Shakir, W. A. W. Ibrahim, and R. Hussain, “Extracting hydroxyapatite and its precursors from natural resources,” Journal of Materials Science, vol. 49, no. 4. pp. 1461–1475, Feb. 2014. doi: 10.1007/s10853-013-7864-x.

[76] Y. Yuan, C. Liu, J. Qian, J. Wang, and Y. Zhang, “Size-mediated cytotoxicity and apoptosis of hydroxyapatite nanoparticles in human hepatoma HepG2 cells,” Biomaterials, vol. 31, no. 4, pp. 730–740, 2010, doi: 10.1016/j.biomaterials.2009.09.088.