

# Natural Hydroxyapatite from Fish Bones and Scales for Biomedical Applications—A Review on Current Progress

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## Abstract

Hydroxyapatite has attracted a significant attention in biomedical applications due to its excellent biocompatibility, bioactivity, osteoconductivity, and non-toxic nature. Fish processing wastes, particularly fish bones and scales, have emerged as a sustainable and cost-effective natural source for hydroxyapatite extraction. The increasing global demand for environmentally friendly biomaterials has accelerated research into the utilization of fish-derived waste for bone tissue engineering. This review comprehensively discusses the extraction, characterization, and *in vitro* studies of hydroxyapatite from various fish species. Furthermore, the review highlights structural, morphological, thermal, and biological characteristics of fish-derived hydroxyapatite. Despite considerable progress, challenges associated with compositional variability, reproducibility, and large-scale commercialization remain significant barriers to clinical translation. Fish-derived hydroxyapatite demonstrates substantial potential as a sustainable biomaterial for future orthopedic and dental applications.

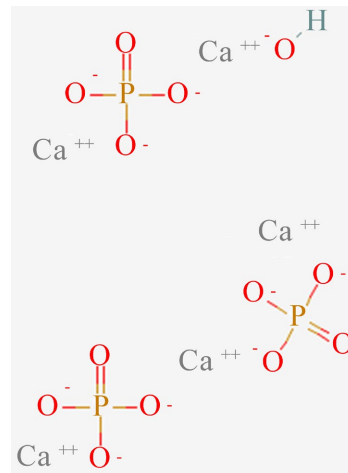
## Keywords

Material Science, Health Science, Biomedical Engineering, Regenerative Medicine

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## 1. Introduction

Natural bone is a hierarchically organized porous composite consisting of both organic and inorganic phases that function synergistically to achieve exceptional mechanical and biological performance. The organic matrix is predominantly composed of collagen and provides flexibility, toughness, and resistance to crack propagation. In contrast, the inorganic phase is mainly represented by hydroxyapatite (HA),  $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ , a crystalline calcium phosphate mineral that contributes stiffness and compressive strength to the skeletal structure (**Figure 1**) [1].



**Figure 1.** Hydroxyapatite [1].

While HA enhances load-bearing capacity and structural rigidity, it exhibits relatively limited resistance to torsional and shear stresses [2]. The combination of mineralized HA with the collagenous matrix therefore enables natural bone to balance strength and toughness effectively [3]. Owing to its chemical similarity to the mineral component of human hard tissue, HA has attracted considerable attention as a biomaterial for bone repair and regeneration, particularly due to its excellent biocompatibility, osteoconductivity, and ability to promote direct interactions with surrounding cells and biological tissues.

Bone tissue regeneration remains a central focus within regenerative medicine, driving extensive investigation into calcium-based biomaterials for the reconstruction, repair, and functional restoration of damaged skeletal structures. Among these materials, studies demonstrate their strong potential to support bone formation and integration with host tissue [4]-[7]. These foundational findings stimulated substantial research efforts aimed at optimizing HA synthesis, tailoring its physicochemical properties, and expanding its biomedical applications.

In recent years, increasing attention has been directed toward the extraction of hydroxyapatite from naturally derived resources. Beyond conventional synthetic sources, researchers have explored alternative biomaterials including mammalian bones, fish bones, and scales, marine shells, plant-based precursor, and mineral deposits. Natural-source HA has been reported to exhibit distinct physicochemi-

cal characteristics compared with fully synthetic counterparts, such as higher aspect ratios, increased porosity and surface area, and improved particle dispersibility. These features can enhance biological performance by promoting favorable cell-material interactions, improving osteoconductivity, and supporting cellular proliferation while reducing apoptosis rates [8]-[11]. Consequently, naturally derived HA has gained significant interest as a biomimetic material capable of more closely replicating the hierarchical structure and functionality of native bone.

Hydroxyapatite research is inherently interdisciplinary, integrating contributions from dentistry, materials science, chemistry, biochemistry, biology, and biomedical engineering. The review aims to provide a comprehensive overview of the evolution of hydroxyapatite research in fish bones and scales, with particular emphasis on its role as a biomimetic and bioactive material. The article synthesizes current advancements and critically evaluates state-of-the-art evidence, including findings from clinical investigations, *in vitro* and *in vivo* studies supporting the efficacy of hydroxyapatite.

## 2. Methodology

The literature was reviewed from the year 2021 within the scope of fish bones and fish scales for biomedical applications using the Scopus and Google Scholar databases. The keywords used in this review were “fish bones” or “fish scales” in “biomedical applications”. The scope of the study focused on the characterization of the physicochemical properties and the *in vitro* evaluation of natural hydroxyapatite from fish by-product.

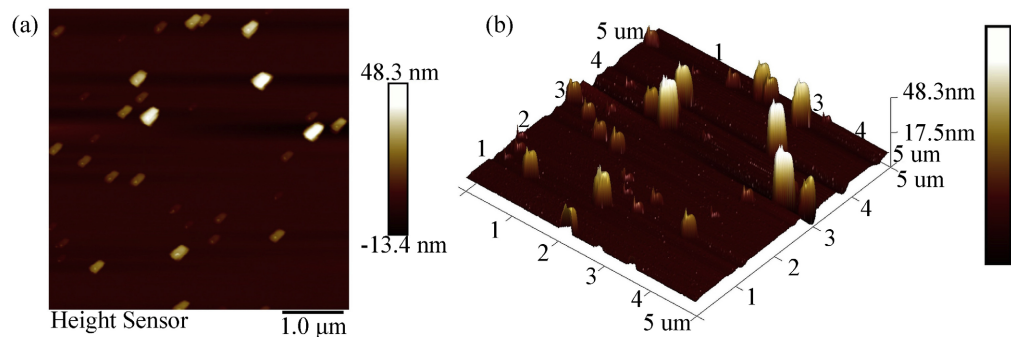
## 3. Fish Bones and Scales for Biomedical Applications

Fish bones and scales have emerged as promising biogenic resources for biomedical applications due to structural similarity to human hard tissues. These materials are primarily. Unlike synthetic counterparts, HA derived from fish waste often exhibits superior bioactivity, osteoconductivity, and biocompatibility, attributed to its naturally occurring ionic substitutions and hierarchical microstructure. In addition to their favourable biological properties, fish bones and scales represent an abundant by-product of the global fisheries and aquaculture industries, making them cost effective and environmentally responsible alternative to sources like bovine bone, which may pose risks related to disease transmission.

A study reported by Swamiappan *et al.* [12] that synthesized hydroxyapatite (HA) from *Cirrhinus molitorella* fish scale using ultrasonic assisted method produce Ca/P molar ratio of 1.67, similar to ideal stoichiometric ratio. Rod-like HA was produced with high specific surface area of 112 m<sup>2</sup>/g and mesoporous features. Using Atomic Force Microscopy (AFM) (Figure 2), the spectra show a dominant spiky surface texture, which can enhance cell interaction. The synthesized HA exhibited a compressive strength of approximately 114 MPa, suggesting adequate mechanical performance for potential bone substitute materials.

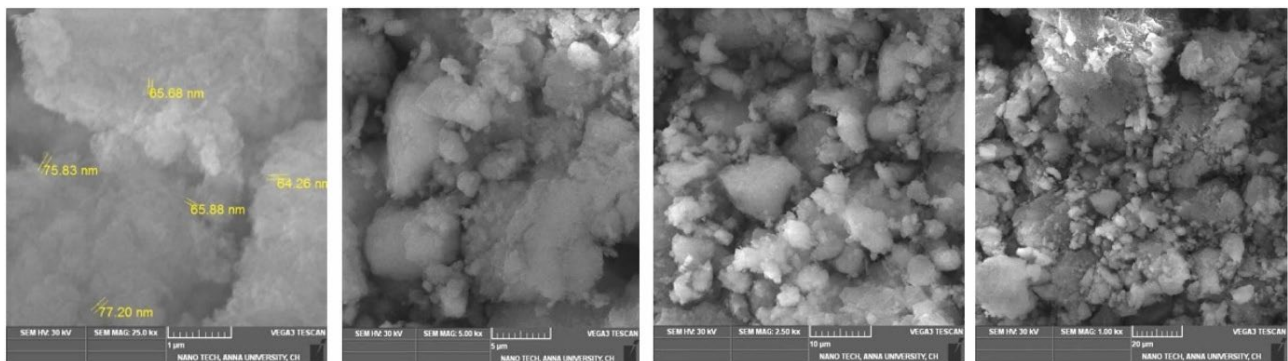
Horta *et al.* [13] investigated the properties of hydroxyapatite extracted from

*Osteoglossum bicirrhosum* fish scale using the combination of alkaline treatment and thermal calcination at the temperature of 600°C and 800°C. The natural HA derived shows non-toxic behaviour toward dental pulp stem cells. No adverse cellular effects were observed at the tested concentrations, demonstrating that the material is biocompatible for biomedical use. *In-vitro* bioactivity assays showed that the fish scale-derived HA formed a bone-like apatite layer more rapidly than synthetic HA when incubated in McCoy culture medium for 3 days.



**Figure 2.** AFM spectra of *Cirrhinus molitorella* fish scale [12].

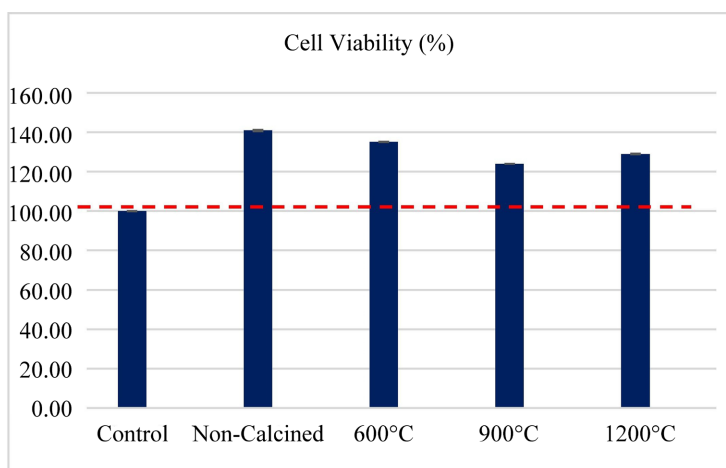
Gnanasekaran *et al.* [14] investigated the production of HA from bones of red big eye fish using alkaline hydrolysis and thermal calcination methods. The optimized alkaline hydrolysis treatment with sodium hydroxide produced high-purity hydroxyapatite by effectively removing organic components such as collagen from the bone matrix. Microscopic analysis through SEM showed that the synthesized HA particles possessed spherical morphology with nanoscale sizes ranging approximately 50 - 80 nm (Figure 3). In addition, *in vitro* biological evaluation using MG-63 osteoblast-like cells demonstrated that the extracted HA was non-cytotoxic and biocompatible with cell proliferation racing about 92 % relative to control.



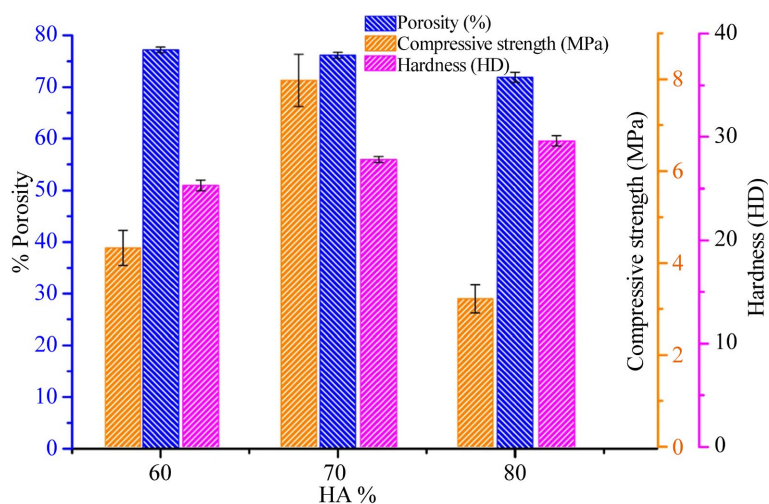
**Figure 3.** SEM images of HA from red big eye fish bones [14].

In another research, Hussin and colleagues [15] evaluated the hydroxyapatite extracted from *Fringescale sardinella* bones. Bioactivity evaluation in simulated

body fluid (SBF) showed the formation of a bone-like apatite layer on the HA surface which demonstrated good apatite-forming ability. This behaviour indicates that the material has osteoconductive potential which is essential for bone bonding and regeneration. Besides, cell viability studies indicate that the extracted HA exhibited low cytotoxicity and good biocompatibility in supporting cell survival and proliferation with cell viability of more than 100 % in comparison with positive control (**Figure 4**).



**Figure 4.** Cell viability of HA of *Fringescale sardinella* bones [15].

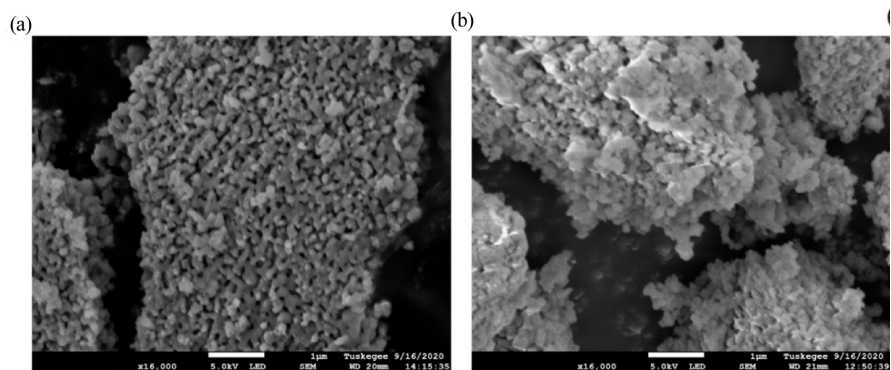


**Figure 5.** Compressive strength, porosity, and hardness of PMMA/HA scaffolds from *Catla catla* fish scale [16].

Deb *et al.* [16] in their study investigated the fabrication and performance of a composite scaffold incorporating hydroxyapatite derived from the scales of *Catla catla* scales. Mechanical testing demonstrated that the inclusion of HA significantly enhanced the compressive strength at 70% HA and reduced the hardness of the scaffold in comparison with the pure polymer matrix (**Figure 5**). The mechanical performance approached the range required for cancellous bone appli-

cations. Meanwhile, *in vitro* evaluation showed that the scaffold exhibited good cytocompatibility, supporting cell adhesion, proliferation, and viability.

Kodali *et al.* [17] found in their study that HA extracted from carpa and pink perch fish scales can be successfully incorporated into polycaprolactone (PCL) fibres using the forcespinning process. **Figure 6** shows the morphology of both calcined HA powder and nanomilled HA powder that have irregular size. Structural characterization confirmed that the HA particles were uniformly distributed within the fibrous matrix. The addition of fish scale derived HA influenced the fibre morphology by producing rougher fibre surfaces and slightly reduced fibre diameters, which increased the surface area. These morphological changes enhanced the scaffold's suitability for cell attachment and proliferation. The results also demonstrated that incorporating HA into forcespun PCL fibre scaffolds improved mechanical performance and cellular response, indicating strong potential for developing advanced biomimetic scaffolds for bone tissue engineering.



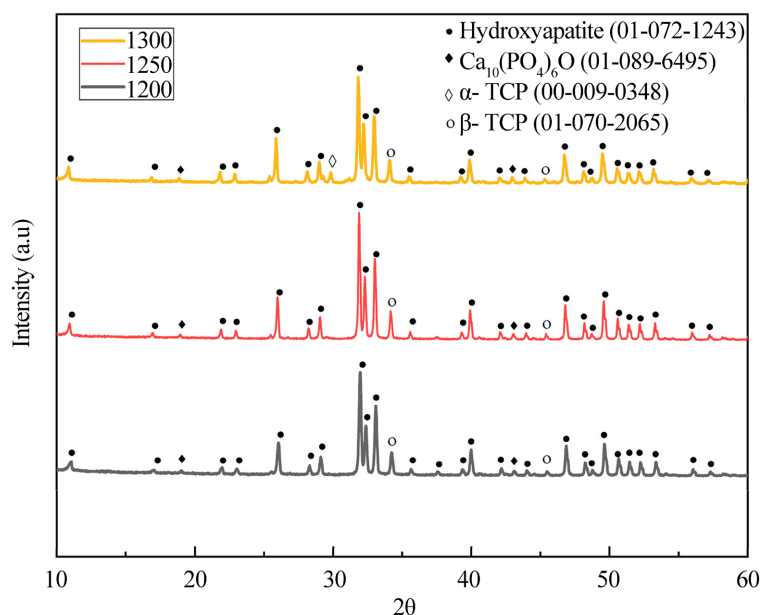
**Figure 6.** SEM images of (a) calcined HA powder and (b) nanomilled HA powder [17].

Parthasarathy and team [18] explored the potential of HA from *Scarus ghibban* scales for wound healing. Highly crystalline hydroxyapatite with nanoscale particle size was produced, resembling the mineral composition of natural bone. The synthesized material exhibited nanoscale morphology with porous and agglomerated structures, which provide a high surface area beneficial for biological interactions. Cell migration assays revealed that HA significantly enhanced cell migration and wound closure rates, suggesting that the material can promote tissue regeneration and accelerate wound healing process. This property is particularly valuable for regenerative medicine.

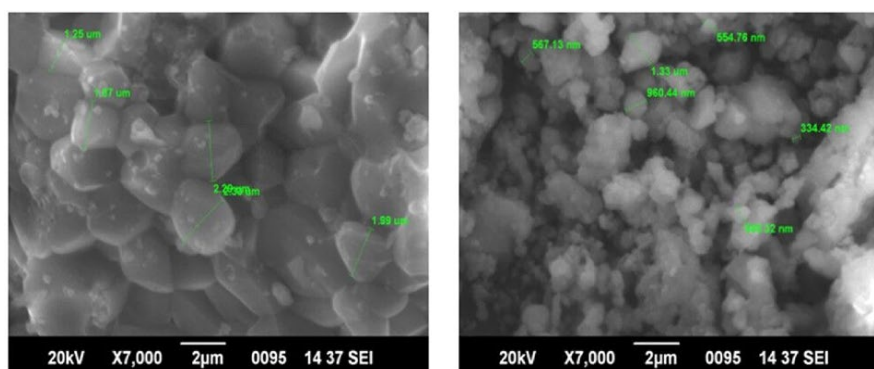
Kamal *et al.* [19] investigated the influence of sintering temperature on the properties of HA extracted from bones and skull of *Anabas testudineus*. The study showed that increasing sintering temperature significantly improved crystallinity (**Figure 7**) and grain growth of the HA. They also found that HA exhibited higher density, reduced porosity, and improved mechanical strength. These improvements are attributed to enhanced particle bonding and microstructural densification during the calcination process.

Jaffri *et al.* [20] evaluated the preparation and characterization of HA derived

from fish scale waste of Black Tilapia. Microscopic analysis revealed that the synthesized HA exhibited irregular and agglomerated microstructures, which are typical for biogenic HA obtained through calcination of natural sources. Analytical results also showed characteristic diffraction peaks and functional groups associated with phosphate and hydroxyl, indicating successful transformation of the fish scale mineral component into HA.



**Figure 7.** XRD spectrum of HA at 1200, 1250, and 1300°C [19].



**Figure 8.** SEM images of nano-HA of (a) Tuna and (b) Tilapia [22].

Mashrafi *et al.* [21] examined the extraction and characterization of HA from scales of *Tenualosa ilisha* and *Labeo rohita*. Thermal calcination was used to remove organic components and both fish species yielded pure HA. However, HA derived from *Tenualosa ilisha* exhibited higher crystallinity. HA derived from both fish scales showed agglomerated particles composed of nanoscale crystallites, in which *Tenualosa ilisha*-derived HA showed more uniform and compact particle morphology. The *Labeo rohita*-derived HA displayed relatively less uniform

particle distribution, indicating slight differences in mineral composition and growth behaviour.

Archana and colleagues have successfully extracted nano-HA from both tuna and tilapia bones [22]. Both species yielded pure HA with Ca/P ratio close to the stoichiometric value, indicating compositional similarity to natural bone mineral. Minor trace element inherent to fish bone were retained, which may enhance biological activity. From the electron microscopy, it was revealed that both source produced agglomerated nano-HA (Figure 8), resulting in high surface area. Both tuna and tilapia derived nano-HA exhibited low cytotoxicity and good biocompatibility while cell viability studies demonstrated that the materials supported cell adhesion and proliferation, indicating compatibility with mammalian cells.

Fatmawati *et al.* [23] explored the nano-calcium derived from fish waste of *Clarias gariepinus* (catfish) and *Channa striata* (snakehead fish). They found that both fish species produced calcium-rich materials dominated by HA and calcium phosphate phases. The extracted particles exhibited irregular and agglomerated structures, typical of biogenic calcium powders. The agglomeration is caused by strong interactions between nanoscale calcium phosphate particles.

Kim *et al.* [24] have successfully fabricated 3D-printed scaffold composed of PCL, marine collagen, and carbonated HA extracted from fish bones. The composite scaffold maintained structural stability and homogenous dispersion of HA particles within the polymer matrix. The presence of collagen enhanced the hydrophilicity and surface bioactivity of the scaffold. In addition, 3D printing method generated regular and interconnected macropores, enabling nutrient diffusion and cell migration. The incorporation of collagen and HA improved surface roughness, which favours cell attachment.

Eknapakul *et al.* in their study [25] investigated a cost effective approach of extracting HA from fish scale of Nile Tilapia. The synthesized HA exhibited irregular and agglomerated particle morphology and the particle size ranging of fine micro- to nanoscale crystallites (250 - 2500 nm). In terms of chemical composition, the primary elements detected were calcium, phosphorus, and oxygen.

In general, fish scale derived HA demonstrates higher crystallinity and phase purity compared to fish scale derived HA due to the mineral component in bones is more abundant and structurally organized, resulting in enhanced thermal stability. In contrast, fish bone derived HA often retains a greater amount of organic constituents and trace elements, which may contribute to improved biological performance.

**Table 1** highlights considerable variability in the pre-treatment and calcination parameters in processing fish bones and scales from different species. Boiling pre-treatment durations range from as short as 10 minutes to as long as 12 hours, indicating flexibility in inorganic matter removal depending on the raw material's density. Drying conditions are relatively consistent, typically conducted between 40 and 120°C, with durations spanning 1 to 24 hours, ensuring adequate moisture removal prior to thermal treatment. Calcination temperature exhibits a wide var-

iation ranging from as low as 200 °C to as high as 1100 °C, with higher temperature commonly applied to tilapia-derived bones to achieve increased crystallinity and phase purity of HA. Dwell times during calcination vary between 2 and 8 hours, with longer durations typically associated with lower calcination temperatures to ensure complete decomposition of organic components.

**Table 1.** Pretreatment and calcination parameters in processing fish bones and scales.

Species	Pretreatment (Boiling)	Drying	Heating Rate	Temperature	Dwelling Time	References
Salmon Bone	12 h	12 h at 60 °C	-	650 °C	5 h	[26]
Nile Tilapia Bone	1 h	1 h at 50 °C	-	900 °C - 1100 °C	-	[27]
Tilapia Bone	1 h	-	5 °C/min	900 °C	8 h	[28]
Snakehead Bone	3 h	12 h at 80 °C	5 °C/min	600 °C	5 h	[29]
Spanish Mackerel Bone	10 minutes	3 h at 100 °C	-	400, 600, & 800 °C	3 h	[30]
Grass Carp Scale	-	24 h at 100 °C	-	-	-	[31]
Carp Scale	-	3 h at 40 °C	-	-	-	[32]
Common Carp Bone	4 h	17 h at 120 °C	5 °C/min	200 - 1000 °C	2 h	[33]

**Table 2** presents the *in vitro* evaluations conducted on HA derived from various fish-based sources, highlighting the diversity of biological models employed to assess their biomedical potential. A broad spectrum of cell lines and biological systems has been used to evaluate the biocompatibility and bioactivity of these materials. Several studies employed osteoblast-related cell lines such as MG-63 and SaOS-2 to investigate cell viability, proliferation, and osteogenic response. For instance, HA derived from catla scales, silver carp bones, emperor bones, and snakehead fish was tested on MG-63 cells, indicating a strong emphasis on osteoblastic compatibility. Similarly, HA from sardine scales and *Labeo rohita* scales was evaluated using SaOS-2 human osteosarcoma cells, further confirming its suitability for bone-related applications.

In addition to osteoblast-like cells, preosteoblastic and stem cell models were also utilized to provide deeper insights into osteogenic differentiation potential. Whitemouth croaker bone-derived HA was assessed using MC3T3-E1 preosteoblast cells, while black tilapia scale and catla bone-derived HA were tested using human fetal osteoblasts and mesenchymal stem cells, respectively. Moreover, human bone marrow-derived mesenchymal stem cells were employed in the evaluation of HA from *Lates calcarifer* bone. Beyond cellular studies, several investigations incorporated simulated physiological environments and microbiological assays. Simulated body fluid (SBF) testing was conducted on HA derived from black tilapia scales and catla bones to evaluate apatite-forming ability and bioactivity. Additionally, antibacterial properties were examined using pathogens such as *Streptococcus mutans*, particularly for HA derived from black tilapia and snakehead bones. Agar well diffusion assays were also performed for *Labeo rohita* scale-

derived HA, indicating an interest in assessing antimicrobial performance alongside biocompatibility.

**Table 2.** Summary of *in vitro* evaluations.

Hydroxyapatite Source	Cell/Solution/Bacteria	References
Catla Scale	MG 63 Cell Line	[34]
Whitemouth Croaker Bone	Preosteoblast MC3T3-E1 Cell	[35]
<i>Labeo rohita</i> Scale	Saos-2 Human Osteosarcoma Cell	[36]
Silver Carp Bone	Osteoblast MG 63 Cell Line	[37]
Sardine Scale	SaOS-2, L929, and LIG Cells	[38]
Black Tilapia Scale	Simulated Body Fluid, Human Fetal Osteoblast Cell Line	[39]
<i>Lates calcarifer</i> Bone	Human Bone Marrow-Derived Mesenchymal Stem Cell	[40]
Emperor Bone	MG 63 Cell Line	[41]
Snakehead Fish	MG 63 Cell Line	[42]
Catla Bone	Simulated Body Fluid, Human Mesenchymal Stem Cell	[43]
Black Tilapia Bone	<i>Streptococcus mutans</i>	[44]
Snakehead Bone	<i>Streptococcus mutans</i>	[45]
<i>Labeo rohita</i> Scale	Agar Well Diffusion Assay	[46]

#### 4. Conclusion

Fish bones and scales offer a promising potential for the extraction of hydroxyapatite due to their sustainability, economically-wise, and availability compared to synthetic and mammalian-derived biomaterials. The abundance of fishery waste generated worldwide provides significant opportunities for converting biological resources into high-value biomaterials for orthopedic, dental, and bone tissue engineering applications. Various extraction approaches have demonstrated the capability to produce hydroxyapatite with desirable physicochemical and biological properties. It also exhibited advantage over conventional synthetic hydroxyapatite due to presence of trace elements such as magnesium, sodium, zinc, and strontium, which contribute to enhancing osteoconductivity, bioactivity, and cellular response. Furthermore, the incorporation of fish-derived hydroxyapatite into polymeric and composite systems has shown considerable potential in improving the mechanical and biological performance of biomaterials intended for bone tissue regeneration.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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