SCAFFOLD FABRICATION FROM DOPING-REINFORCED RABBITFISH (*Siganus* sp.) BONE FOR BONE TISSUE ENGINEERING APPLICATIONS

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Study Program Physics

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To

STUDY PROGRAM PHYSICS
FACULTY OF MATHEMATICS AND NATURAL SCIENCES
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MAKASSAR, INDONESIA
2024

DISSERTATION

SCAFFOLD FABRICATION FROM DOPING-REINFORCED RABBITFISH (*Siganus* sp.) BONE FOR BONE TISSUE ENGINEERING APPLICATIONS

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With this, I declare that the dissertation entitled “Scaffold Fabrication From Doping-Reinforced Rabbitfish (*Siganus* sp.) Bone For Bone Tissue Engineering Applications” is my true work under the direction of the mentoring team of Prof. Dr. Dahlang Tahir, M.Si as Promotor and Prof. Dr. Bualkar Abdullah, M.Eng.Sc as Co-promotor-1 and Prof. Dr. Sri Suryani, DEA as Co-promotor-2. These scientific papers have not been submitted and are not being presented in any form to any college. Sources of information derived or quoted from published or unpublished works of other authors have been mentioned in the text and listed in the Library List of this dissertation. Part of the contents of this dissertation has been published in Journal AIP Conference Proceedings 2719, 020040 (2023) and https://doi.org/10.1063/5.0133232 as an article entitled “Hydroxyapatite derived from fish waste as a biomaterial for tissue engineering scaffold and its reinforcement,” in Journal IOP Conf. Ser.: Earth Environ. Sci. 1272 012040 and doi:10.1088/1755-1315/1272/1/012040 as an article under the title “Fish waste-derived biomaterials as a support of zero waste and Sustainable Development Goals (SDGs),” in Journal Polymer Bulletin and https://doi.org/10.1007/s00289-023-04794-6 as an article entitled “Hydroxyapatite based for bone tissue engineering: innovation and new insights in 3D printing technology,” in Journal BONE 183 (2024) 117075 dan https://doi.org/10.1016/j.bone.2024.117075 as an article entitled “Development and application of hydroxyapatite-based scaffolds for bone tissue regeneration: A systematic literature review,” in Journal IOP Conf. Ser.: Earth Environ. Sci. 1230 012042 and doi:10.1088/1755-1315/1230/1/012042 as an article entitled “The use of waste bones of rabbitfish (*Siganus* Sp.) for the synthesis of hydroxyapatite,” in Journal The Journal of The Minerals, Metal & Materials Society (TMS) and https://doi.org/10.1007/s11837-024-06760-7 as an article entitled “Hydroxyapatite extracted from rabbitfish (*Siganus* Sp.) bones and its potential for bone tissue engineering applications.” If it is later proven or can be proven that part or all of this dissertation is the work of someone else, then I am prepared to accept the sanction for the act according to the rules in force.

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ABSTRACT

FENDI. **Scaffold Fabrication from Doping-Reinforced Rabbitfish (*Siganus* sp.) Bone for Bone Tissue Engineering Applications** (supervised by Dahlang Tahir, Bualkar Abdullah, and Sri Suryani).

Background. The composition of fishbone is similar to natural human bone, which can be used as a biomaterial, particularly a bone scaffold. The potential of fish as a biomaterial can support zero waste and achieve the SDGs. Hydroxyapatite [HAp, $Ca_{10}(PO_4)_6(OH)_2$] material can extracted from the waste bones of rabbitfish (*Siganus* sp.). Besides being readily available in nature, their use reduces unwanted environmental impacts. HAp is the primary mineral constituent of bones, contributing significantly to their hardness and mechanical strength. HAp, both synthetic and natural, has become a popular composite material in bone tissue engineering because it is very similar to the structure and properties of bone. With its robust biocompatibility and bioactivity, HAp has found extensive utility in bone grafting, replacement therapies, and supplemental medical materials. **Aim.** This research aims to use fish bone waste as a biomaterial and the relationship between zero waste efforts and the SDGs. HAp from rabbitfish (*Siganus* sp.) bones can be synthesized using sintering. This research also discusses the use of HAp in bone implants, bone fillers, and HAp-based scaffolds. It also discusses the application of scaffold-based HAp in bone defects, bone regeneration, bone tissue engineering, and other applications. **Results.** Fishbone waste can be recycled into HAp as a biomaterial for bone tissue engineering, drug delivery, health, and pharmaceutical industries. HAp derived from fish bones has good biocompatibility and is non-toxic. The characteristics of HAp made from rabbitfish (*Siganus* sp.) bone waste show its potential for application in bone regeneration. 3D-printing technology is an innovation in making HA-based scaffolds and one of the advantages that can provide personalized bone regeneration. 3D scaffolds can be applied for bone defect repair, regeneration, and tissue engineering. In addition to bone-related applications, scaffolds show versatility in enhancing cartilage healing and serving as bioimplants. The diverse applications of scaffolds underscore their continued potential for further development in the field of medical science. **Conclusion.** Fishbone waste can be synthesized into HAp as a biomaterial that supports zero waste efforts and achieving SDGs, as well as a biomaterial source that has the potential to be applied in bone tissue engineering. The use of 3D-printing technology is an innovation in making HAp-based scaffolds, where 3D scaffolds can be applied for bone defect repair, regeneration, and bone tissue engineering.

Keywords: fishbone; hydroxyapatite; scaffolds; sintering; 3D-printing

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List of Abbreviations and Symbols

Symbol/Abbreviation	Meaning and Explanation
AFM	Atomic force microscope
Ag	Silver
AgNP	Silver nanoparticle
Al	Aluminium
ALP	Alkaline phosphatase
bFGF	Basic fibroblast growth factor
BMPs	Bone morphogenetic proteins
BMSCs	Bone marrow mesenchymal cells
BMP-2	Bone morphogenetic protein 2
BM-MSCs	Bone marrow-derived mesenchymal stem cells.
BTE	Bone tissue engineering
Ca	Calcium
Ca-P	Calcium Phosphate
CDHA	Calcium-deficient Hydroxyapatite
CH	Chitosan
CHA	Collagen-Hydroxyapatite
CHCS	Chitin-Hydroxyapatite-collagen composite scaffolds
CMCS	Carboxymethyl Chitosan
Col	Collagen
CPPH	Collagen/PVA/Propranolol/ Hydroxyapatite
Cro	Crocin
CS	Chitosan
Gd	Gadolinium
ECM	Extracellular Matrix
EPD	Electrophoretic Deposition
EPL	ε-poly-l-lysine
FB	Fish Bones
FS	Fish Scale
FTIR	Fourier transform infrared spectroscopy
GIONFH	Glucocorticoid-induced osteonecrosis of the femoral head
GO	Graphene Oxide
HA	Hydroxyapatite
HAp	Hydroxyapatite
hADMSCs	Human adipose-derived Mesenchymal Stem Cells
HAMT	Hydroxyapatite Microtube
hBMSCs	Human Bone Marrow-derived Stem Cells
hBN	hexagonal Boron Nitride
HDPE	High-Density Polyethylene
K	Kalium
La^{3+}	Lanthanum
LENS™	Laser Engineered Net Shaping
Li	Lithium
Mg	Magnesium
MG-63 cells	Human Osteosarcoma Cells

M-HAP	Mineralized Hydroxyapatite
MoS_2	Molybdenum Disulfide
MSCs	Mesenchymal Stem Cells
MW	Microwave
NaOH	Sodium Hydroxide
NHAP	Natural Hydroxyapatite
nHA	nanohydroxyapatite
n-HAp	nano-Hydroxyapatite
Na	Sodium
Nb	Niobium
OBC	Oxidized Bacterial Cellulose
P	Phosphate
PCL	Poly(ε-caprolactone)
PECVD	Plasma-Enhanced Chemical Vapor Deposition
PEEK	Polyether-Ether-Ketone
PHB	Polyhydroxy Butyrate
PICO	Population, Intervention, Control, and Outcome
PLA	Polylactic Acid
PLD	Pulsed Laser Deposition
PMMA	Polymethyl Methacrylate
PPG	Polypropylene Glycol
PRP	platelet-rich plasma
PRISMA	Preferred Reporting Items for Systematic Review
RGO	Reduced Graphene Oxide
ROS	Reactive Oxygen Species
SDGs	Sustainable Development Goals
SEM	Scanning Electron Microscope
SiC	Silicon Carbide
Sim-HA	Simvastatin and Hydroxyapatite
SiO_4^{4-}	Silicate
Sr	Strontium
Sr-HA	Strontium-doped Hydroxyapatite
TCP	Tricalcium Phosphate
XRD	X-ray Diffraction
XRF	X-ray Fluorescence
WS	Wollastonite
Zn	Zinc
Zn-MHMs	Zinc-doped mesoporous Hydroxyapatite microspheres
3DPP	Projection-based 3D printing
3DS	Three-dimensional composite scaffold

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Nilawati Usman A, Sartini S, Yulianti R *et al.* Turmeric extract gel and honey in post-cesarean section wound healing: A preliminary study [version 2; peer review: 1 approved, 1 approved with reservations]. *F1000Research* 2024, **12**:1095 (https://doi.org/10.12688/f1000research.134011.2)

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CHAPTER I
GENERAL INTRODUCTION

Bone defects are one of the health problems for which less expensive and safer materials are still being sought after. The scaffold plays a vital role in treating bone damage because it is a permanent or semi-permanent matrix that can support cell development and repair damaged tissue. Tissue engineering is the term commonly used to describe this process. Hydroxyapatite (HAp) is the preferred component for scaffolds due to the fact that it is non-hazardous to humans (Coelho et al., 2020; Safinsha and Mubarak Ali, 2020).

Commercial HAp is still prohibitively expensive, which means that even after the most recent publication in 2021, researchers will be attempting to innovate using less expensive local materials. In addition, recent studies that are synergistic with zero-waste efforts appear to be more focused on some waste that can be utilized (Hartatiek et al., 2021).

Zero waste is an effort to optimize the activity and use of goods or materials in various fields so that all are utilized and nothing is wasted. This zero waste effort has been linked to the Sustainable Development Goals (SDGs) (Dessie et al., 2023; Velazquez et al., 2023). Zero waste efforts have been carried out in various fields, including nutrition, industry, and health.

The use of waste from the sea, such as fish waste, clam shells, and crab shells, has received a lot of attention in the world, one of which is in the field of biomaterials (Ismail et al., 2021; Jeon and Yeom, 2009; Qin et al., 2022). The treatment and use of bio-waste have become a considerable concern, where improper waste disposal can cause environmental problems and result in wasting natural resources. Therefore, efforts are needed to convert useless bio-waste into value-added products. Waste materials from nature can be converted into biocompatible materials (biomaterials) (Abdulrahman et al., 2014).

Processing fish skin, scales, and bones into collagen, gelatin, HAp, and calcium phosphate converts fish waste into useful biomaterials. Its use is even precious for humans because it can be used as a biomaterial for medical treatments such as bone tissue engineering and drug delivery to collagen and gelatin, which are raw materials for the food, pharmaceutical, and cosmetic industries. Waste interconnects with the environment. Its reuse as a useful product means making people aware of responsible production and consumption. Utilizing biomaterial derived from fish waste has emerged as a valuable industrial resource with potential applications in human health, including treating various health conditions. This connection between the economy and health has the potential to contribute to achieving Sustainable Development Goals (SDGs), particularly in promoting good health and well-being (Dilekli and Cazcarro, 2019; Khairul Akter et al., 2022; Velazquez et al., 2023).

Research on HAp in bone tissue engineering seems to be ongoing. The initial findings were synthetic HAp, followed by the discovery of HAp derived from natural materials and developed in biomedical applications, including bone repair.

Weaknesses in HAp were then discovered, and the strengthening began from the aspect of the method of making HAp and substituting or doping other elements. Bone tissue engineering materials comprising various components, including biomaterials, are a promising trend for repairing bone damage. HAp is similar to bone and teeth minerals as a tissue engineering material. It has good bioactivity, biocompatibility, and osteoconductive. Its function can be bone filler, implant, and bone scaffold. HAp requires synergy with other metals, minerals, and collagen supports to improve its function, mechanical properties, and biocompatibility (Feng et al., 2022; Kim et al., 2022; Ma et al., 2021).

HAp to be used in bone tissue engineering requires performance improvement on its biological and biochemical properties (Balakrishnan et al., 2021). Even though natural materials are used, such as fish, eggs, shellfish, and others, improvement in mechanical and biological properties is still needed (Jyotsna and Vijayakumar, 2020).

HAp in the chemical form $Ca_{10}(PO_4)_6OH_2$ is a crucial mineral for the structural integrity of the human body. HAp has gained importance in bone tissue engineering research due to its customized features and resemblance to bone tissue. Besides that, calcium phosphate-based materials are becoming increasingly attractive in biological applications because of their chemical resemblance to the inorganic components of human bones and teeth. Calcium phosphate (Ca-P) biomaterials are advanced synthetic materials that are biocompatible and can enhance bone development and integration because of their natural osteoconductivity and osteoinductivity. Naturally derived HAp materials are extensively utilized in several medical domains owing to their remarkable bioactivity, bio-affinity, and biocompatibility, such as bone filling and coating in orthopedics and dental implantation. HAp possesses significant mechanical qualities, bioactivity, and non-toxicity and does not elicit any allergic response when implanted in the body (Mathina et al., 2022; Megha et al., 2023; Zhao et al., 2023).

The bioactive, biocompatible, and osteoconductive properties of nanohydroxyapatite (nHAp) can influence cellular behavior. This material demonstrates biocompatibility with non-osteoblast and osteoblast cells and has no harmful effects. The utilization of nanostructured biomaterials that mimic the architecture of native bone can enhance the process of bone regeneration. They also play a constructive role in the biomedical field in repairing bone defects and preventing post-surgery infections. Besides that, nanocrystalline HAp is among the biocompatible materials used to fill bone defects and as a bone substitute. Several cationic replacement ions, such as Mg, Zn, Boron, and others, are also doped into purified HAp chemical structures to boost their biological and physicochemical performance and improve the treatment process (Jodati et al., 2023; Kazimierczak et al., 2022; Liu et al., 2021; Shao et al., 2022; Singh et al., 2022; Tong et al., 2023).

HAp is a bioceramic biomaterial based on calcium phosphate (CP), which is potentially medically applied for its potential bioactivity, biocompatibility, osteoconductivity, and its Ca/P molar ratio of 1:67 (Surya et al., 2021). Studies on HAp that have been reported, including bones prepared from catfish (*Pangasius hypophthalmus*), tilapia (*Oreochoromis* sp.), seabass (*Lates calcarifer*), and yellowfin tuna (*Thunnus albacares*), which were calcined at 700 °C for 2 hours having a Ca/P ratio of about 1.80 (Nam et al., 2019), Nile tilapia *(Oreochromis niloticus)* bone was calcined at 800 °C for 5 h (Khamkongkaeo et al., 2021), types of fish bones (Salmo salar, Anoplopoma fimbria, and Sardine) which are calcined for one hour at different temperatures ranging from 600 °C to 1100 °C in a muffle furnace (Zhu et al., 2017), HAp extracted from rainbow trout, cod, and salmon reported atomic ratio of Ca/P is .47, 1.88, and 1.51, respectively (Shi et al., 2018).

In essence, the elemental composition of the samples has significant similarities to the chemical composition of natural bones. The Ca/P ratios calculated for the HAp-900 sample were 2.04 and 1.58, respectively, in terms of weight and atomic percentages. In general, the Ca/P atomic ratio of HAp samples in contact was 1.58-1.94 at a temperature of 900-1100°C. Comparatively, the Ca/P atomic ratio (1.58) of HAp-900 was the nearest to the HAp stoichiometric Ca/P ratio (1.67). One of the most important factors that could be responsible for the deviation of the Ca/P ratios from stoichiometry is the melting temperature, which affects the type and composition of calcium-based compounds that appear in the resulting HAp bioceramic (Obada et al., 2020).

HAp is the primary component of human bone, is highly biocompatible, compatible with biological systems, and can encourage the growth of new bone tissue, so it is widely used in tissue engineering and the medical profession (Hubadillah et al., 2023; Piccirillo et al., 2013). In addition, the similarity in chemical structure and crystallography between human bone tissue and HAp makes its applications related to bones increasingly widespread (Sharifianjazi et al., 2021). HAp has potential for medical applications due to its potential bioactivity, biocompatibility, osteoconductivity, and Ca/P molar ratio of 1:67 (Shi et al., 2018; Surya et al., 2021), as well as other applications such as bone tissue replacement, tissue engineering, drug delivery (Piccirillo et al., 2013), orthopedics, dentistry (Vinoth Kumar et al., 2021), and biomedical applications such as bone regeneration, wound dressing, and dental implants (Jyotsna and Vijayakumar, 2020; Saiful Firdaus Hussin et al., 2023).

Extracting HAp from Rabbitfish (*Siganus* sp.) bones is possible considering all these aspects. The obtained HAp was fully characterized in powder form to explore its potential application in bone tissue engineering.

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CHAPTER II
HYDROXYAPATITE DERIVED FROM FISH WASTE AS A BIOMATERIAL FOR TISSUE ENGINEERING SCAFFOLD AND ITS REINFORCEMENT

2.1 Abstract

The purpose of this study is to review explicitly wastes that are frequently overlooked in Indonesia, namely waste from fish in scales and bones, scaffolds derived from natural hydroxyapatite, and efforts to improve the synthesis of hydroxyapatite produced from fish waste. This study uses a narrative review by emphasizing fishbone waste as biomaterials in the health aspect. The databases used to find references were primarily focused on Science Direct, which contained 14 research articles included in the review (2018, 2019, 2020, and 2021). It is believed that the composition of fish scales is similar to human hard tissue, and the composition of fishbone is similar to natural human bone. In fish, scales and bones both contain a significant amount of hydroxyapatite, which can be used as a biomaterial, particularly a bone scaffold. Hydroxyapatite derived from scale and fishbone has good biocompatibility and is nontoxic. However, some researchers discovered that scaffolds made from fish scales and bones are fragile and need to be strengthened by adding other materials, such as chitosan or other polymers, to make them more durable.

2.2 Introduction

Bone defects are one of the health problems for which less expensive and safer materials are still being sought after. The scaffold plays a vital role in treating bone damage because it is a permanent or semi-permanent matrix that can support cell development and repair damaged tissue. Tissue engineering is the term commonly used to describe this process. Hydroxyapatite is the preferred component for scaffolds due to the fact that it is non-hazardous to humans (Coelho et al., 2020; Safinsha and Mubarak Ali, 2020).

Commercial hydroxyapatite is still prohibitively expensive, which means that even after the most recent publication in 2021, researchers will be attempting to innovate using less expensive local materials. In addition, recent studies that are synergistic with zero-waste efforts appear to be more focused on some waste that can be utilized (Hartatiek et al., 2021).

Zero waste has emerged as a current issue, along with environmental pollution, which is becoming more severe in all countries worldwide. Various products have been recycled or repurposed to put this effort into action (Bianchi et al., 2021). Fishbone waste is food waste that occurs in large quantities and is frequently overlooked (Harmita and Simbolon, 2020). Fish is a popular dish in Indonesia, particularly in the eastern part of the country, and it is served in many famous restaurants and is consumed daily at home. Bioceramic products for biomedicine can be made from the abundance of carp, fish bones, and scales available in the wild. It is possible to control the characteristics of this bioceramic in order to make it more cost-effective (Maidaniuc et al., 2020). Waste from fish in scales and bones is discussed explicitly in this study, as is the use of scaffolds derived from natural hydroxyapatite and the use of fish to improve the production of hydroxyapatite from fish waste.

2.3 Material and Methods

This study, a narrative review, is used to highlight the importance of fishbone waste as biomaterials in the context of health. The databases that were used to locate references were primarily centered on Science Direct. At first, the keywords were broad, such as fish waste, biomaterials, and health, and they were searched for in the years 2018, 2019, 2020, and 2021. Afterward, after discovering some relevant literature, the search became more specific, using the keywords fish waste and biomaterial and the years 2018, 2019, 2020, and 2021 as search parameters.

The journal's year of publication between 2018 and 2021 was the only criterion for inclusion; no literature review, systematic review, or meta-analysis was considered. The methodology was used as an exclusion criterion, and the conclusions were not clear. Additionally, books and papers presented at academic conferences will be executed.

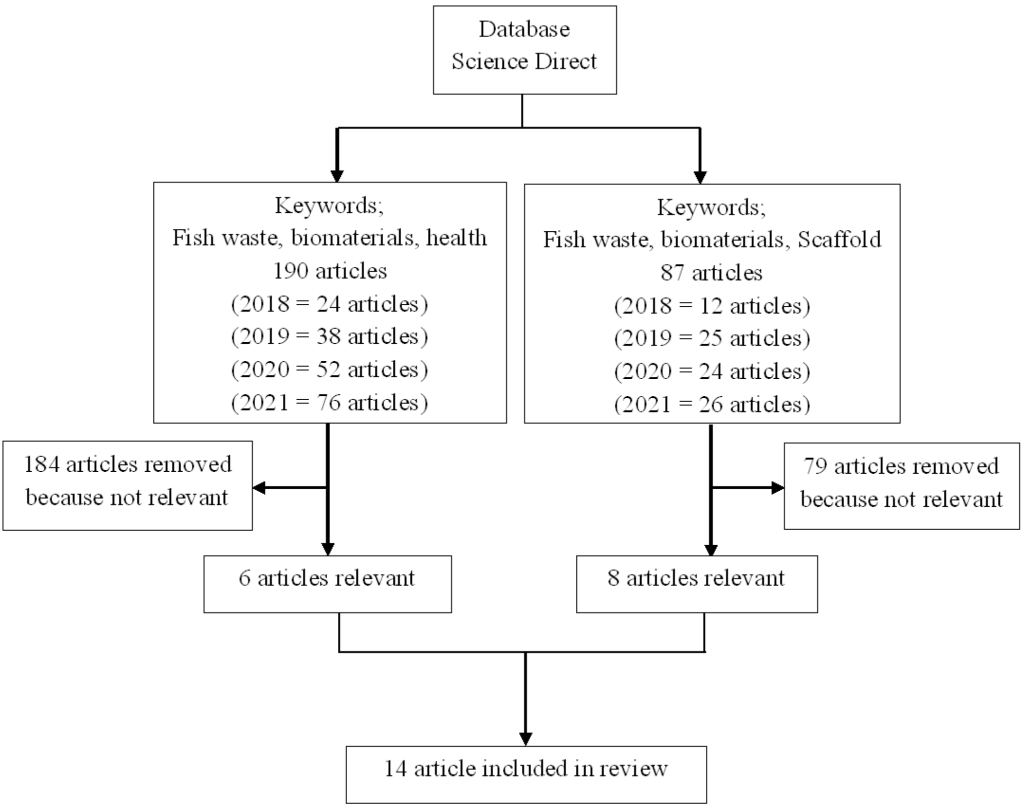

Figure 2.1. Search strategy flow chart

2.4 Result and Discussion

2.4.1. Fish Waste as Tissue Engineering Scaffold

Tissue engineering applications of scaffolds derived from fish scales had significant potential as a natural extracellular matrix. In vitro, the fish scales used as cell scaffolds demonstrated a high degree of cytocompatibility, allowing cells to adhere and proliferate more effectively. Thus, the use of fish scale scaffolds as a natural extracellular matrix for tissue engineering showed great promise, as demonstrated by the study results (Wu et al., 2021). Furthermore, the morphology, size, functional group, viability, and mineralization of hydroxyapatite synthesized from fish bones were also considered suitable for bone tissue engineering (Surya et al., 2021).

It is believed that the composition of fish scales is similar to the composition of human hard tissue and that the composition of fishbone is similar to the composition of natural human bone, respectively. Both hydroxyapatite and collagen, which are both minerals, are found in significant amounts in fish scales. When it comes to fish scales and bones, calcium and phosphate are the primary sources of hydroxyapatite, and both contain significant amounts of hydroxyapatite, which can be used as a biomaterial, specifically as a bone scaffold in bone regeneration procedures (Mondal et al., 2019; Sathiskumar et al. 2019).

2.4.2. Biocompatibility of Fish Scale and Bone-Derived Hydroxyapatite Scaffold

2.4.2.1. Fish Scale

Study of hydroxyapatite and nano-hydroxyapatite synthesis and the synthesis of hydroxyapatite scaffolds using various fish scales. Catla (Catla catla) fish scales, Tilapia (Oreochromis niloticus) fish scales, Labeo Rohita fish scales, Puntious conchonius fish scales, and Cirrhinus mrigala fish scales were used in this study.

The biocompatibility of hydroxyapatite (NHAp) prepared from fish scales showed encouraging results. A trial using MG-63 cells (human osteosarcoma cells) showed NHAp boosts up the growth of MG-63 cells, which showed superior cell viability and ALP activity when compared with commercial HAp (CHAp) (Sathiskumar et al., 2019). Furthermore, the fish scales used as cell scaffolds demonstrated a high degree of cytocompatibility, promoting cell adhesion and proliferation and the ability to guide cell migration along the ridge channels. This was obtained through culture testing of L929 cells and rat bone marrow mesenchymal stem cells (BMSCs) (Wu et al., 2021).

The hydroxyapatite derived from the fish scale used to develop the bone scaffold meets the criteria for bone tissue engineering application in terms of psycho-chemical, mechanical, structural, and bioactive properties (Deb et al., 2019b). Furthermore, HAp is thermally stable between 750 and 1000°C, and it has a highly porous morphology, indicating that it could be used to develop bone scaffolds in the near future (Buraiki et al., 2020; Deb et al., 2019a).

2.4.2.2. Fish Bone

Natural hydroxyapatite (nHAp) from fishbone has good biocompatibility. It was evaluated using nHAp from Salmon and trout fishbone with mouse preosteoblast MC3T3-E1. The experiment has proven to significantly promote osteoblast viability after three days and seven days of incubation. The trace elements CO_3^{2-} and Mg^{2+} were also present in this study (Shi et al., 2018). Another study revealed several trace elements present in hydroxyapatite: Na, K, Mg, Sr, Zn, and Al (Nam et al., 2019).

A study using hydroxyapatite derived from catfish bones revealed a microstructure with open pores suitable for cell adhesion. It also has a hardness value in the range of human cortisol bone (Akpan et al., 2020). In addition, the size, morphology, functional groups, viability, and mineralization of the synthesized n-HAp make it an excellent candidate for bone tissue engineering and other potential osteo and dental applications (Surya et al., 2021).

An essential concern in the practical use of the hydroxyapatite component of fishbone for the bone scaffold is that no inflammatory response or fibrosis was found. The scaffolds were biocompatible and bioactive, and they promoted bone formation via osteoinduction of osteogenic cells that migrated over and into the scaffolds (de Castro Prado et al., 2021).

2.4.3. Methods Used in The Manufacture of Hydroxyapatite

Several methods used in the manufacture of hydroxyapatite are a simple alkaline treatment process, calcification, a gradual mixing of $Ca(NO_3)_2$ $4H_2O$ (0.40 M) and $(NH_4)_2$ HPO_4 (0.24 M) solutions at room temperature, decellularized with the chemical method.

Characteristic analysis was used using scanning electron microscope (SEM), X-ray diffraction (XRD), atomic force microscope (AFM), and Fourier transform infrared spectroscopy (FTIR). In addition, biocompatibility was examined towards the cell viability of MG-63 cells (human osteosarcoma cells) with various dosages. (Buraiki et al., 2020; Deb et al., 2019a, 2019b; Sathiskumar et al., 2019; Surya et al., 2021; Wu et al., 2021).

2.4.4. Reinforcement of Scaffold-Derived Hydroxyapatite

Some researchers finally tried the objective that the scaffold from hydroxyapatite produced from fish scales makes it brittle. Solvent casting particulate leaching technique is one innovation that can improve this.The obtained results of the scaffold can meet the physiological demands to guide bone regeneration (Deb et al., 2019b). Kara and colleagues are also trying to make innovations by adding chitosan to hydroxyapatite made from fish scales. The addition of chitosan produced an excellent strengthening effect for the scaffold (Kara et al., 2019). Castro and his colleagues innovated by making nanocomposite scaffolds by mixing Nb_2O_5 and HAp powders (in vol%) at a ratio of 1:1. As a result, the HAp-Nb-1080 composite has physicochemical characteristics that meet the requirements for a mechanically reinforced biomaterial for bone regeneration (de Castro Prado et al., 2021).

Table 2.1. Relevant studies: fish scale waste.

Reference (years)	Source of biowaste	Method of fabrication	Fabrication product	Reference/SJR/ Quartile
(Sathiskumar et al., 2019)	Cirrhinus mrigala fish scale	Simple alkaline heat-treatment process.	Nanostructured hydroxyapatite (NHAp) crystalline powders)	Ceramics International/0.94/Q1
(Deb et al., 2019a)	Labeo rohita fish scales	Calcination	Hydroxyapatite (HAp)	Materials today; Proceeding/0.34 /Not yet assigned a quartile
(Buraiki et al., 2020)	Scales of Catla (Catla catla) and Tilapia (Oreochromis niloticus)	Gradual mixing of $Ca(NO_3)_2$ $4H_2O$ (0.40 M) and $(NH_4)_2$ HPO_4 (0.24 M) solutions at room temperature, with maintaining pH at 11 with NH4OH.	hydroxyapatite scaffolds and compared with synthetic	Materials today; Proceeding/0.34 /Not yet assigned a quartile
(Kara et al., 2019)	Fish Scale (FS) and Chitosan (CH)	FS were decellularized with the chemical method. CH/FS scaffolds were fabricated using the lyophilization technique.	Bio-composite scaffold	International Journal of Biological Macromolecules /1.14/Q2
(Wu et al., 2021)	Fish scale	Culturing L929 cells and rat bone marrow mesenchymal stem cells (BMSCs),	The scaffolds derived from fish scales	Materials Science and Engineering C/1.23/Q1

		decellularizing and decalcifying fish scales were then examined.		
(Deb et al., 2019b)	Puntius conchonius fish scales.	The solvent-casting particulate leaching technique	HAp bone scaffold derived	Ceramics International/0.94/Q1
(Athinarayanan et al., 2020)	Lethrinus lentjan fish scales	Thermal method	Carbon (CDs) and hydroxyapatite (HA NPs)	Materials Science and Engineering C/1.23/Q1

Table 2.2. Relevant studies: fishbone waste.

Reference (years)	Source of biowaste	Method	Fabrication product	Reference/SJR/ Quartile
(Akpan et al., 2020)	Non-separated animal bones (NB) and catfish (CF)	Low compaction pressure method	Hydroxyapatite	Results in Physics/0.74/Q2
(Surya et al., 2021)	Sardinella longiceps Bone	Alkaline hydrolysis method.	Nano-hydroxyapatite (n-HAp)	Journal of the Mechanical Behavior of Biomedical Materials/0.86/Q2
(Nam et al., 2019)	Bones of catfish (Pangasius hypophthalmus), tilapia (Oreochoromis sp.), seabass (Lates calcarifer), and yellowfin tuna (Thunnus albacares)	calcinating	Hydroxyapatite powder	Ceramics International/0.94/Q1
(Maidaniuc et al., 2020)	Carp fish bones	Thermal method	Calcium phosphate's	Ceramics International/0.94/Q1

(Mondal et al., 2019)	Tuna fish bones	Calcination	Hydroxyapatite (HAp	Materials Chemistry and Physics/0.76/Q2
(Shi et al., 2018)	rainbow trout (Onchorynchus mkiss), cod (Gadus), and salmon (Oncorhynchus keta) bones	Thermal calcination method	(Hydroxyapatite (HAp)	Materials Science and Engineering: C
(de Castro Prado et al., 2021)	Nile tilapia (Oreochromis niloticus) bones	Calcination	Hydroxyapatite, the nanocomposite, was produced by mixing Nb_2O_5 and HAp powders.	Materials Chemistry and Physics/0.76/Q2

All literature included in this study related to fish biowaste is from reputable international journals and, based on SCIMAGO's assessment, is in the first and second quartiles. Unfortunately, only one uses the proceeding. Studies reviewed showed that the resulting product from fish scale and fishbone is hydroxyapatite or nano-Hydroxyapatite. The synthesis of hydroxyapatite from fish biowaste indicates a potential use in the health sector, especially tissue engineering. Most products produced from fish biowaste are used for osteo problems or, more specifically, developing scaffolds. One of the exciting things is that research is not limited to hydroxyapatite or bone scaffold synthesis but has started developing bone scaffolds made of hydroxyapatite to cover its shortcomings.

The bone naturally cannot consist of only one composite material, so the designed scaffold should also contain several composite materials if damage occurs. The research was an attempt to create a scaffold by using polymer (natural and synthetic) and nHAp (12.5%) (Ma et al., 2021). In addition, hydroxyapatite nanocomposites reinforced by niobium (Nb) and silver (Ag) also increase their strength in the face of stress and fracture potential (Wei et al., 2019). A combination of composite materials is also a potential method to reduce the weakness of the scaffold made from fish waste.

The manufacture of scaffolds for bone using natural hydroxyapatite still requires innovation to increase its strength and biocompatibility. The majority of publications are still at the clinical trial stage and have not yet entered clinical trials. Studies regarding natural HAp must be continued, considering that synthetic hydroxyapatite does have advantages in manufacturing bone scaffolds; however, HAp from bone and fish scale materials is superior. Natural HAp has no toxic effects and is also cheaper (Coelho et al., 2020; Foroutan et al., 2021; Štoković et al., 2020).

The difference between hydroxyapatite produced from fish waste is trace elements such as CO_3^{2-} and Mg^{2+}, Na, K, Mg, Sr, Zn, and Al. Trace elements or various minerals found in HAp from fish are also found in human bones. A lack of trace elements or minerals will make bones brittle. HAp synthesis has a weakness in this aspect (Harkness and Darrah, 2019; Sathiskumar et al., 2019; Streli et al., 2019).

2.5 Conclusion

Biowaste from fish, namely scales and bones, can be used as a scaffold for tissue engineering by producing hydroxyapatite from both. Reinforcement of hydroxyapatite scaffold-derived fish scale and bone needs to be done by adding other materials. This innovation needs to be investigated further by using biomaterials as reinforcement.

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2.7. References

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