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Synthesis of Hydroxyapatite (HAp) from Green Mussel Shell As Bone Graft Material: A Review

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Abstract. Natural bone grafts that are widely used today such as autograft, allograft, and xenograft, have limitations in availability, compatibility, and risk of disease transfer. The use of synthetic bone grafts was developed as an alternative, one of which is hydroxyapatite. Hydroxyapatite (HAp) is the most commonly used calcium phosphate bioceramic in biomedical applications as it has a chemical composition similar to human bone and teeth. Hydroxyapatite can be synthesized using chemical precursors containing calcium such as $Ca(NO_3)_2$ and phosphate such as $(NH_4)2HPO_4$ and Na_2HPO_4. In addition to using chemicals, HAp can also be synthesized from natural sources, such as mussel shells, eggshells, fish bones, and coral that are rich in calcium content. Methods that can be used in HAp synthesis include dry, wet, thermal, or a combination of these methods. Each different synthesis method can produce different morphology, size, and crystallinity phase. This study summarizes several HAp synthesis methods, which include wet, high temperature, and sonochemical methods, aiming to determine the best method in the synthesis of hydroxyapatite (HAp) as bone graft material from green mussel shell waste. The analysis showed that HAp synthesized by microwave irradiation method is the most suitable for use as synthetic bone graft material. This method produces HAp with high crystallinity and has a Ca/P ratio of 1.68 which is closest to the stoichiometric ratio of HAp which is 1.67.

Keywords : Bone Graft, Green Mussel Shell, Hidroxyapatite, Synthesis Methode

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Introduction

The use of bone graft (allograft and xenograft) in Indonesia continues to increase from year to year. This is due to the widening field of bone graft use, which is not only in the orthopedic field but has also begun to be widely used in ophthalmology and periodontal (dental) [1][2][3]. In addition, the increased use of grafts is also due to the increasing prevalence of diseases that require bone grafts, such as in cases of bone cancer [4]. Currently, bone grafts that are widely used in the orthopedic field are natural bone, including autograft (bone from the same patient), allograft (bone from other human donors, either living or corpses) and xenograft (animal bone, generally cattle) [5]. Although proven to repair damaged bone tissue, the application of these materials still has obstacles that hinder the treatment process such as limited bone tissue that can be used, compatibility issues, and the potential for disease transfer. In the development of bone graft, the main obstacle faced is that it is difficult to obtain bone donors so that the availability of the three grafts is very limited in addition to the problems mentioned above. The use of synthetic bone graft that has properties resembling native bone is carried out as an effort to overcome problems in the bone tissue repair process. The requirements that must be met by synthetic bone graft are osteoconductivity, osteointegration, biocompatibility, non-toxicity, resorbability, have mechanical properties similar to bone, and meet all requirements for clinical use [6].

Hydroxyapatite (HAp) is a bioceramic known as one of the calcium phosphate family that has a Ca/P ratio = 1.67 and chemical formula $Ca_{10}(PO_4)6OH_2$. According to several studies, synthetic HAp nanostructures have been widely used in various biomedical applications such as bone tissue engineering scaffolds [7], bioactive material coatings [8], and drug or protein delivery systems [9] due to their similarity to the mineral components of the tissues found in human bones and teeth, as well as due to their biocompatibility, bioactivity, and osteoconductive properties. Although many synthesis methods have been developed, the preparation of HAp with specific characteristics remains a challenge due to the possible formation of toxic intermediate products during HAp synthesis [10]. Therefore, studies on new parameters in HAp synthesis are still ongoing. HAp can be chemically synthesized or extracted from natural sources. Previous studies have reported various methods for the synthesis of synthetic and natural HAp. It concluded that synthetic HAp can be synthesized through various methods including dry methods [11][12], wet methods [13][14][15], and high temperature processes [16][17].

The main problem in HAp synthesis is the expensive production cost because the production process uses relatively expensive chemicals. Therefore, more affordable raw materials are needed in the HAp production process. Indonesia is a country that has a vast marine area with a coastline of 81,000 km so that it has the potential for very abundant aquatic resources, one of which is shellfish [18]. Shells have a high calcium carbonate ($CaCO_3$) content so that it can be utilized as a source of calcium in HAp synthesis. HAp synthesized from natural materials has higher bioactivity compared to HAp synthesized with chemicals [19]. This is because HAp synthesized with natural materials has a chemical composition such as Mg^{2+}, K^{+}, Na^{+}, Sr^{2+} which plays an important role in the process of bone formation. One type of shellfish that can be used in HAp synthesis is green mussels. Green mussels have a $CaCO_3$ content of 95-98% [20]. In 2018, Indonesian green mussel production reached 309,886 tons. Assuming that the shells make up 70% of the total weight, the estimated waste from green mussel shells amounts to around 216,902 tons [21]. This suggests that green mussel waste is abundant and readily available in the region. If not managed properly, solid waste such as green mussels can cause environmental pollution.

Previous studies synthesized HAp based on green mussel shells and KH_2PO_4 by wet precipitation method and produced hydroxyapatite similar to commercial hydroxyapati powder, but the resulting Ca/P ratio has not reached the stoichiometric ratio of hydroxyapatite [22]. In the synthesis of HAp from mussel shells as a calcium source, the addition of phosphorus sources such as diammonium hydrogen phosphate $(NH_4)2HPO_4$, sodium hydrogen phosphate (Na_2HPO_4), phosphoric acid (H_3PO_4) is required to obtain a Ca/P ratio of 1.67 [23]. Therefore, a more in-depth review of a series of methods for the synthesis of HAp from green mussel shell waste will be conducted. In addition, this study also investigated the effect of source and processing method on the resulting properties such as Ca/P ratio, particle size, morphology, and crystallinity as well as the phase assemblage of natural HAp because the performance of synthesized HAp is affected by these

properties [24]. Thus, the results of this study can provide a benchmark for further studies on the synthesis of HAp from shell waste as bone graft material.

Experimental

This research uses the literature study method to collect information from credible sources, such as journals, books and proceedings. Literature study is a research approach that critically examines various knowledge, ideas, and findings that have been published in academic literature. The literature review used includes research results related to the synthesis method of HAp based on green mussel shells, with the main focus on studying the effect of source and processing method on properties such as Ca/P ratio, size, crystallinity, and morphology. This research is a descriptive analysis, in which the data obtained is systematically arranged, then explained and interpreted so that it is easily understood by the reader. In its preparation, this research uses a systematic approach with the help of a methodology chart, as shown in the research flow chart in **Figure 1**.

Result and Discussion

Green Mussel Shells as a Material for HAp Synthesis

Hydroxyapatite can be synthesized using chemicals such as $Ca(NO_3)_2$ as a calcium source and $(NH_4)2HPO_4$, Na_2HPO_4, KH_2PO_4, H_3PO_4 as phosphate sources. In addition, HAp can also be synthesized from natural materials, such as mussel shells, coral, eggshells, fish bones, gypsum, etc [25]. Among all calcium-rich natural resources, mussel shells have a composition consisting of 95% $CaCO_3$ and 1-5% other organic materials. The use of natural materials such as mussel shells as materials in HAp synthesis is increasingly in demand because it has several advantages compared to HAp synthesized from synthetic materials, such as its abundant availability, cheap, economical and efficient production [26]. The use of HAp synthesized from natural materials is considered to be an environmentally friendly, sustainable and economical process as these materials are available in large quantities. It can make a positive contribution to the economy, environment, and public health. According to [27], the utilization of natural materials such as mussel shells as a source of calcium in HAp synthesis is increasingly accepted by experts in orthopedic applications. This is because $CaCO_3$ derived from natural materials has a high surface area and unique morphology that can help improve implant solubility and facilitate cellular activity in bone. In addition, other studies who studied the synthesis of nanohydroxyapatite (nHA) from green mussel shells, HAp synthesized from natural materials can be more easily accepted by the body [28].

Green mussel shells represent about 55% of the total weight and are composed of 95-99% aragonite which is one of the crystalline forms of $CaCO_3$, so green mussels can be used as a source of calcium in HAp synthesis [29]. HAp synthesized from natural materials has higher bioactivity compared to HAp synthesized from chemicals [19]. This is because HAp synthesized from natural materials has a chemical composition consisting of important ions such as

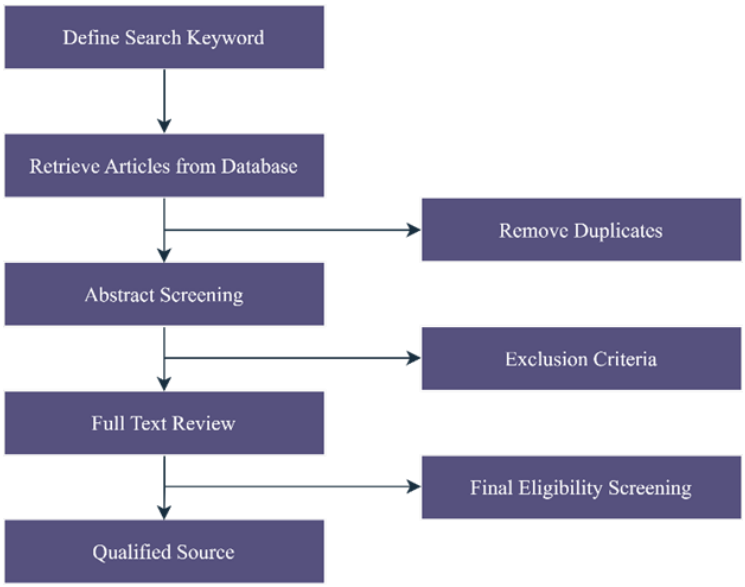

Figure 1. Flowchart of systematic literature review research

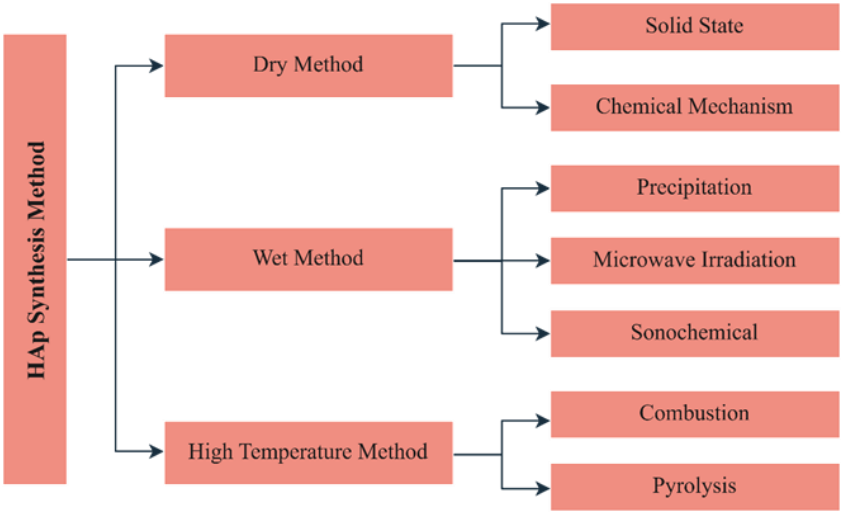

Figure 2. HAp Synthesis Method

Mg^{2+}, K^{+}, Na^{+}, Sr^{2+} which play an important role in the process of bone repair. HAp synthesized from natural materials also contains elements such as Na^{+}, Zn^{2+}, Mg^{2+}, K^{+}, Si^{2+}, Ba^{2+}, F^{-}, and CO_3^{2-} which makes it have a chemical composition similar to human bone.

Synthesis of HAp from Green Mussel Shells

HAp can be synthesized by various methods. Each HAp synthesis method can produce HAp with different size, morphology, Ca/P ratio, and also crystallinity phase besides pure crystalline HAp [24]. Therefore, the characteristics of HAp have a significant effect on its bioactivity, mechanical, and biological properties. According to [30], these characteristics determine the biomedical applications of the resulting HAp. The methods that can be used to synthesize HAp are shown in Figure 2.

HAp synthesis methods are classified into dry, wet, and high-temperature methods. HAp synthesis by dry method can be classified into two different methods, solid-state and chemical mechanism. In the dry method, chemicals (calcium and phosphate) in dry form are mixed to synthesize HAp. The dry method does not require controlled conditions so it is suitable for large quantity production [10]. HAp can also be synthesized using the high temperature method to decompose the material. The high temperature method consists of two different methods, combustion and pyrolysis. Among all types of HAp synthesis methods, combustion and pyrolysis methods are used less frequently than other methods. This is because these processes have poor control over the synthesis process [19]. The wet method of HAp synthesis leads to the use of solutions during the synthesis process. Some wet methods used for HAp synthesis include precipitation, microwave irradiation, and sonochemical [31]. HAp synthesis by wet method is the most commonly used method because it can control the morphology and average size of HAp produced. A summary of previous research on the potential utilization of green mussel shells in HAp synthesis by several wet methods is shown in Table 1.

Previous studies have used the microwave irradiation method to synthesize hydroxyapatite (HAp) based on green mussel shells as a calcium source and Na_2HPO_4 as a phosphorus source. In one study, green mussel shells were calcined at 900□ for 30 minutes, then the resulting CaO was mixed with 50 mL of 0.1 M EDTA solution to form a Ca-EDTA complex. A 0.06 M Na_2HPO_4 solution was added slowly at a rate of 4 mL/min, stirred for 15 minutes, and the pH of the mixture was maintained at 13. The precipitate formed was washed to remove residual Na and EDTA, and then dried in a vacuum oven at 80□ for 6 hours. To evaluate the effect of heating on the crystal properties of HAp, the synthesized powder was heated at 650° for 1 hour [28]. Another study used a similar approach, where 1 gram of shell powder that had been dried at 110 □ for 5 hours was mixed with 100 mL of 0.1 M EDTA and 0.06 M Na_2HPO_4 solution slowly. The pH of the mixture was raised to 13 by adding NaOH solution, then stirred for 1 hour before being irradiated in a microwave oven for 15 minutes. The white precipitate obtained was washed and dried at 110° for 5 hours [29].

Table 1. Synthesis HAp from green mussel shell using wet method

Method	Phospate Source	Ca/P Ratio	Pore Shape	Ref.
Microwave irradiation	Na_2HPO_4	1.68	Solid ball-shaped and lumpy	[28]
Microwave irradiation	Na_2HPO_4	1.79	Flower-shaped with w = 100-200 nm; l = 2-5 µm	[29]
Sonochemical	$(NH_4)_2HPO_4$	-	Rod-shaped with d = 12-18 nm; l = 30-80 nm	[32]
Wet precipitation	$(NH_4)_2HPO_4$	1.67	Fine granules with uniform size	[20]
Wet precipitation	H_3PO_4	-	Rod-shaped with d = 87 nm; l = 20 nm	[33]

The sonochemical method has also been applied in the synthesis of HAp using green mussel shells calcined at 1000 °C for 3 hours. A total of 1 gram of CaO obtained was dissolved in 20 mL of 2.5 M HNO_3 solution, then the pH of the mixture was adjusted to 10 by adding NH_4OH solution. Next, 25 mL of $(NH_4)_2HPO_4$ 0.427 M solution was added slowly during the irradiation process which lasted for 1 hour. Afterward, the mixture was centrifuged, washed, and dried in an oven at 80 °C for 12 hours [32].

n addition, the wet chemical precipitation method has also been used in the synthesis of HAp by varying the calcination temperature of the shell at 650 °C, 750 °C, 850 °C, and 950 °C for 2 hours. The synthesis process was carried out by stirring the mixture at a speed of 300 rpm for 60 minutes at 70 °C, and maintaining the pH above 9 by adding 3 M NH_4OH solution. The mixture was then stirred using a magnetic stirrer at 70 °C for 50 minutes before undergoing aging treatment for 24 hours. After filtration, the precipitate was dried at 100 °C for 2 hours, then calcined again at 950 °C for 3 hours [20]. Another study used a similar approach by utilizing H_3PO_4 solution as a phosphate source and green mussel shells as a calcium source. In one study, the shells were calcined at 900 °C for 3 hours, then the CaO powder obtained was dissolved in 1 M $Ca(OH)_2$ solution. H_3PO_4 0.6 M solution was then added slowly at a rate of 2.5 mL/min, while stirring at 1500 rpm at 40 °C [33].

XRD characterization of HAp from Green Mussel Shell

Previous studies have demonstrated that the XRD pattern peaks for samples dried in a vacuum oven are higher and sharper than the pattern peaks of samples heated at 650 °C for 1 hour, proving that heating treatment after HAp formation can increase the crystallinity of the resulting HAp. The XRD patterns of both samples also show that both have pure hexagonal HAp phase [28]. In addition, the XRD pattern of the HAp synthesis using microwave irradiation method shows that the product obtained is pure crystalline HAp with hexagonal crystal structure, with lattice constants a = b = 9.4360 Å, c = 6.8796 Å, and unit cell volume V = 530.49 $Å^3$ [29]. In the case of HAp synthesis via the sonochemical method, XRD results confirm that the X-ray diffraction peaks of the hydroxyapatite align with the JCPDS file (No. 09-0432), indicating the formation of a pure hexagonal phase [32]. Meanwhile, other results showed that the lack of several characteristic peaks of $Ca_3(PO_4)_2$ at 27° and 37.5°, CaO at 37.5°, and $CaCO_3$ at 30° indicated the absence of residual reactants and the formation of by-products in the sample. In addition, the XRD pattern of the resulting HAp crystal is in accordance with the standard diffraction data of JCPDS Card No: 09-0432 [33]. The XRD pattern of the HAp synthesized by the precipitation method shows a peak at 31.70° with an hkl index close to 211, which is in accordance with the data from the Joint Crystal Powder Diffraction Standard (JCPDS) No. 09-0432. The resulting crystal size is 82.5 ± 5.3 nm [20]. The results showed that HAp synthesis using microwave irradiation method can produce HAp with high crystallinity, especially with reheating treatment after the formation of HAp [28][29]. In addition, HAp synthesis using sonochemical method is proven to produce products with high purity [32]. However, the wet precipitation method has the disadvantage of potentially incorporating various ions from the solution or reactants used during the synthesis process into the crystal structure. This can lead to HAp contamination as well as the formation of other products that affect the resulting crystallinity [20][33].

FTIR characterization of HAp from Green Mussel Shell

FTIR spectrum analysis from previous stud-

ies indicates that the synthesized HAp contains an O-H functional group, with a stretching mode at 3750 cm^{-1} and vibration at 630 cm^{-1}. Weak bands at 1406 and 1478 cm^{-1} suggest the presence of CO_2 in the HAp, while the absence of a C-H peak and the presence of a C-N peak confirm that the synthesized HAp does not contain EDTA [28]. Examination of HAp synthesized from green mussel shells using the microwave irradiation method reveals that the resulting sample is B-type carbonate-substituted HAp, indicating that a portion of the phosphate group in HAp has been replaced by carbonate groups [29]. Meanwhile, weak bands at 1458, 1417, and 878 cm^{-1} corresponding to CO_3^{2-} peaks suggest that CO_2 may have been adsorbed from the atmosphere, leading to the replacement of phosphate groups during synthesis [32]. Further FTIR spectra analysis of HAp synthesized from green mussel shells confirms the presence of characteristic HAp functional groups [20]. Additionally, bands observed at 567 and 600 cm^{-1} align with the v_4 PO_4^{3-} vibration mode, while bands at 954 and 1027 cm^{-1} correspond to the vibrational modes of v_1 PO_4^{3-} and v_3 PO_4^{3-}. The detection of CO_3^{2-} bands between 1365-1565 cm^{-1} (v_3, asymmetric stretching) and at 875 cm^{-1} (v_2) indicates the formation of carbonated HAp with a nanocrystalline structure [33].

Elemental Composition of HAp from Green Mussel Shell

HAp synthesized using the microwave irradiation method exhibits a composition of Ca (20.63%), O (40.58%), P (12.36%), Na (0.88%), and C (25.55%). The Ca/P ratio, determined through ICP and EDS analysis, was found to be 1.68, which is close to the stoichiometric ratio of HAp at 1.67 [28]. Further analysis of HAp synthesized from green mussel shells using the same method confirms the presence of Ca (16.09%), P (8.97%), C (2.83%), and O (72.11%) in the resulting sample. Quantitative evaluation of the HAp revealed a Ca/P ratio of 1.790, indicating that the product obtained was non-stoichiometric HAp [29]. Similarly, EDX analysis of HAp synthesized through precipitation shows a Ca/P ratio of 1.67, aligning with the expected stoichiometric ratio [20]. Findings from HAp synthesis using the microwave irradiation method with green mussel shells as a calcium source suggest that this technique is effective in producing HAp with a pure hexagonal phase. However, the use of EDTA acid in the process presents potential environmental concerns if not properly managed [28].

Morphology of HAp from Green Mussel Shell

SEM analysis of HAp synthesized using the microwave irradiation method reveals a thick and dense particle structure, while TEM images indicate a spherical particle morphology with dense agglomeration [28]. Another study using the same synthesis method exhibits a flower-like morphology with a width of 100–200 nm and a length of 2–5 µm [29]. Observations from the sonochemical method display a uniform rod-like shape with a diameter of 12–18 nm and a length of 30–80 nm. This method offers advantages in terms of faster reaction speed and greater energy efficiency compared to other synthesis techniques such as hydrothermal, chemical precipitation, and sol-gel [32].

In contrast, TEM images of HAp synthesized through wet precipitation present a rod-like structure with diameters of approximately 87 nm and 20 nm. SEM images confirm that the precipitation method results in fine-grained HAp with uniform size [20][33]. The wet method is considered more suitable for producing nanoscale HAp with regular morphology [34]. However, chemical precipitation tends to yield non-stoichiometric HAp with weak crystallinity and irregularity. Despite these drawbacks, this method remains widely used due to its cost-effectiveness compared to other techniques [10]. Nonetheless, among various HAp synthesis methods, wet precipitation requires a longer time and a more complex process [35].

Conclusion

In this HAp can be synthesized by various methods. Each HAp synthesis method can produce HAp with different size, morphology, Ca/P ratio, and also crystallinity phase. Each method requires several processing parameters such as pH, temperature, molar ratio of chemicals, etc. to produce pure HAp phase. After comparing research data from several previous research journals, it is known that the sonochemical method, provides more advantages, in terms of increased reaction speed and energy efficiency compared to other HAp synthesis methods such as hydrothermal, chemical precipitation, and others. HAp synthesis using chemical precipitation method showed that the synthesis process must be considered properly so that no impurities are substituted into the synthesized HAp. Meanwhile, the synthesis of HAp through microwave irra-

diation method shows that the synthesis with this method can produce HAp with high crystallinity with heating treatment. This method produces HAp with a composition of Ca (20.63%), O (40.58%), P (12.36%), Na (0.88%), and C (25.55%) and a Ca/P HAp ratio of 1.68 making it the most suitable method to be used as a synthetic bone graft material. This is because the Ca/P ratio is close to the HAp stoichiometric ratio of 1.67.

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