Hydroxyapatite from natural sources: methods and its characteristics

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Abstract. Hydroxyapatite is widely known as bioceramics with excellent biocompatibility. Also, hydroxyapatite has biodegradable properties that can support the bone therapy process and can be applied as a bone scaffolds. In recent years, the preparation of hydroxyapatite from natural sources is exciting to be developed. This is because, natural resources, especially in the form of waste, are very abundant and can produce hydroxyapatite with good osteoconductive properties. In this review article, we conducted a study related to the comparative preparation methods and characteristics of hydroxyapatite derived from natural waste such as eggshells, animal bones, and cockle shells. Some of the main characteristics reviewed include crystal characteristics, particle size, and morphology of hydroxyapatite particles.

1. Introduction
Bone scaffolding is an alternative method for bone therapy purposes [1,2]. Compared to other methods such as autograft and allograft, the use of bone scaffolding has a smaller risk of rejection and viral infection and it is not prone to donor shortages [3]. However, the selection of material as a bone scaffold must meet various requirements such as (i) biocompatible, (ii) biodegradable, (iii) has good mechanical properties, and (iv) can be fabricated into porous 3-dimensional scaffolding [4]. These factors are very important because the scaffold will be a medium of cell interaction and the formation of extracellular matrix to form new tissue until bone tissue has successfully regenerated [5].

Hydroxyapatite is a bioceramic material that has been widely developed for the purpose of scaffolding development [6,7]. Besides having an identical composition to human bones, hydroxyapatite has excellent biocompatibility and it is stable [8]. At the application level, hydroxyapatite has been developed into 3-dimensional scaffolding through various methods such as freeze-drying [9], polymer template methods [10], even 3D-printing [11].

In addition to the development of 3-dimensional scaffolding, research related to hydroxyapatite also focuses on the search for raw materials derived from natural sources [12-14]. Some researchers believe that the use of calcium from natural organisms is less risk to health, has better mechanical properties, and has better cell proliferation [15]. In this paper, we compile a review related to several natural resources that can be used as raw materials for hydroxyapatite along with the characteristics of hydroxyapatite produced. More specifically, our study focused on Indonesian local raw materials.
2. Basic characteristics of hydroxyapatite

Hydroxyapatite is a family member of the calcium phosphate compound with the chemical formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. Specifically, to distinguish hydroxyapatite from other members of the calcium phosphate family, hydroxyapatite has a molar ratio of $\text{Ca/P} = 1.67$ [16]. Comparison of Ca/P molar ratios of various types of calcium phosphate is presented in Table 1.

| Ca/P molar ratio | Compounds                                           | Formula                        |
|------------------|-----------------------------------------------------|--------------------------------|
| 0,5              | Monocalcium phosphate monohydrate (MCPM)            | $\text{Ca(H}_2\text{PO}_4\text{)}_2\cdot\text{H}_2\text{O}$ |
| 0,5              | Monocalcium phosphate anhydrous (MCPA)              | $\text{Ca(H}_2\text{PO}_4\text{)}_2$ |
| 1,0              | Dicalcium phosphate dihydrate (DCPD), mineral brushite | $\text{CaHPO}_4\cdot\text{H}_2\text{O}$ |
| 1,0              | Dicalcium phosphate anhydrous (DCPA), mineral monetite | $\text{CaHPO}_4$ |
| 1,33             | Octacalcium phosphate (OCP)                         | $\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4\cdot\text{H}_2\text{O}$ |
| 1,5              | $\alpha$-Tricalcium phosphate ($\alpha$-TCP)        | $\alpha\cdot\text{Ca}_3(\text{PO}_4)_2$ |
| 1,5              | $\beta$-Tricalcium phosphate ($\beta$-TCP)          | $\beta\cdot\text{Ca}_3(\text{PO}_4)_2$ |
| 1,0-2,2          | Amorphous calcium phosphate (ACP)                   | $\text{Ca}_x\text{H}_y(\text{PO}_4)_z\cdot\text{nH}_2\text{O}$, $\text{n} = 3-4,5$; $15-20\% \text{H}_2\text{O}$ |
| 1,5-1,67         | Calcium-deficient hydroxyapatite (CDHA)             | $\text{Ca}_{10-x}(\text{HPO}_4)_x(\text{PO}_4)_{6-x}(\text{OH})_{2-x}$, $(0 < x < 1)$ |
| 1,67             | Hydroxyapatite (HA, HA atau OHA)                    | $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ |
| 1,67             | Fluorapatite                                        | $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$ |
| 1,67             | Oxyapatite                                          | $\text{Ca}_{10}(\text{PO}_4)_6\text{O}$ |
| 2,0              | Tetracalcium phosphate (TTCP atau TetCP), mineral hilgenstockite | $\text{Ca}_4(\text{PO}_4)_3\text{O}$ |

In the body of humans and other mammals, hydroxyapatite is easily found as a constituent of bones and teeth. Hydroxyapatite has a monoclinic or hexagonal crystal structure. However, hexagonal crystal type is more commonly found with the P63/M symmetry space group. Hydroxyapatite crystals have lattice parameters: $a=b=9.432$ Å, $c=6.881$ Å dan $\gamma=120^\circ$ [18]. In some researchs it is found that the x-ray diffraction pattern of hydroxyapatite depend on the crystal size. X-ray diffraction pattern of nanocrystal hydroxyapatite has FWHM bigger than microcrystal hydroxyapatite. The x-ray diffraction pattern of hydroxyapatite as a function of the crystal size can be seen in Fig. 1.
Figure 1. X-ray diffraction patterns of hydroxyapatite as function of the crystal size [19].

3. Hydroxyapatite from natural sources

In this section, we describe various types of calcium sources from nature that can be used for hydroxyapatite synthesis. Some of the sources that will be discussed include (i) bovine bones, (ii) fish bones, (iii) shells of marine animals, and (iv) eggshells.

3.1 Hydroxyapatite from cow bones

The cow is one animal that is often consumed by humans. Therefore, the abundance of cow bone waste is interesting for further use. Cow bones contain calcium phosphate up to 58.3%. Thus, the cow bone can be synthesized into hydroxyapatite because it contains the main compounds of hydroxyapatite: calcium and phosphate [20]. The surface morphology of cow bone derived Hap under scanning electron microscopy can be seen in Fig. 2(a). In addition, in Fig. 2(b) we present a comparison of x-ray diffraction patterns of HAp synthesized from animal bones such as cow bones, goat bones, and fish bones: Osphronemus goramy and Euthynnus affinis.

Figure 2. (a) Surface morphology of cow bone derived Hap [21]; (b) x-ray diffraction patterns of hydroxypatite isolated from different types of bones [20]
Some methods that can be used to produce hydroxyapatite from cow bones are precipitation methods [22], dry methods [23], and wet methods [24]. Hydroxyapatite synthesis from cow bone by precipitation method conducted by Wahdah et al. (2014) uses the cow bone as a source of calcium (CaO) and it reacted with H$_3$PO$_4$ to produce hydroxyapatite. One important thing that is noteworthy in the method is that the bone of the cow used must be fat-free. The process of removing fat can be done by soaking cow bones in 1% NaOH solution and 96% acetone [22].

In the hydroxyapatite synthesis by the dry method carried out by Wathi et al. (2014) used a mixture of cow bone powder and (NH$_4$)$_2$PO$_4$ which was then heated at a temperature of 1000°C for 2 hours. Cow’s bones act as calcium precursors while (NH$_4$)$_2$PO$_4$ acts as a source of phosphorus. The weakness of this method is the presence of impurities associated with the carbonate group [23].

Yuliana et al. (2017) has synthesized hydroxyapatite from bovine bones using the wet method. Before further use, bovine bones are calcined at 1000°C to produce CaO powder which is then converted to a Ca(OH)$_2$ suspension. The wet method is carried out by reacting a suspension of Ca(OH)$_2$ with (NH$_4$)$_2$HPO$_4$. The resulting mixture is then sintered at 850°C to produce hydroxyapatite [24].

### 3.2 Hydroxyapatite from fish bones

Apart from bovine bones, some researchers have also developed a method of synthesis of hydroxyapatite from fish bones for reasons of abundance. In Indonesia, several types of fish that have been used to synthesize hydroxyapatite are mackerel and tuna. The choice of fishbone type is often associated with abundance due to consumption and bone structure that is easily separated from the meat. The comparison between hydroxyapatite synthesized using fishbone as raw material is shown in Table 2.

| No | Type of Fish | Methods      | Microstructure | Particle Size (μm) | Impurity         | Ref. |
|----|--------------|--------------|----------------|--------------------|------------------|------|
| 1  | Mackerel     | Precipitaion |                | 1.109              | -                | [25] |
| 2  | Tuna         | Precipitaion |                | 0.21               | Carbonates ion   | [26] |
| 3  | Mackerel     | Sol-gel      | 0.79 – 1.06     | -                  |                  | [27] |
| 4  | Tuna         | Hydrothermal |                | 0.1                | Carbonates ion   | [28] |

Through the precipitation method, Anggresani et al. (2020) have succeeded in obtaining hydroxyapatite from the mackerel bone. Before precipitation, mackerel fish bones are calcined at 800°C to produce CaO. Precipitation is done by mixing Ca(OH)$_2$ suspension as a calcium precursor and (NH$_4$)$_2$HPO$_4$ as a phosphate precursor and then sintered at 900°C for 5 hours [25].

With a slightly different precipitation method, Mutmainnah et al. (2017) synthesized hydroxyapatite from the bones of tuna. Just like previous studies, before using tuna fish bones are calcined to produce CaO. To perform hydroxyapatite synthesis, Mutmainnah uses phosphoric acid solution as a phosphorus precursor then the precipitates obtained are sintered [26].

In addition to the precipitation method, Zein et al. (2020) have succeeded in synthesizing mackerel fishbone-based hydroxyapatite with the sol-gel method. After calcining, CaO from mackerel fish bones
is mixed with HCl to produce CaCl$_2$ deposits. Hydroxyapatite synthesis was made by mixing H$_3$PO$_4$ with CaCl$_2$ to produce a gel. The formed gel is then heated so that it transforms into hydroxyapatite [27].

Through the hydrothermal method, Hardiyanti (2016) has also succeeded in producing hydroxyapatite from tuna fish bones [28]. After calcining, CaO powder from tuna bones obtained was mixed into a solution of ammonium dihydrogen phosphate. The solution is then heated in an autoclave and continued with heating at 900°C to produce hydroxyapatite powder. However, this method has a weakness that is, in addition to hydroxyapatite it may also produce fluorapatite [29].

### 3.3 Hydroxyapatite from Shell-Based Materials

The shell of animals is one of the many wastes produced by the community besides bone in Indonesia. Some shells that are abundant and can be used as raw materials for hydroxyapatite include crab shells, clamshells, and eggshells. The use of the shell as a raw material for hydroxyapatite because the shells are rich in calcium. Table 3 presents the comparative method and hydroxyapatite produced from shell waste.

Crab shell can be used in hydroxyapatite synthesis because the shell has a high calcium content, which is around 54 - 78% of the weight of the dried shell. Hydroxyapatite synthesis from crab shells can be done through the wet deposition method as done by Supangat et al. (2017). Before being used in wet deposition, the crab shell is calcined to produce CaO or Ca(OH)$_2$ when it has been reacted with water. Hydroxyapatite synthesis was carried out by mixing H$_3$PO$_4$ solution into solution (CaOH)$_2$ in a single drop and then sintering at 900°C for 2 hours [30].

| No | Type of shells | Methods         | Microstructure | Particle Size ($\mu$m) | Impurity         | Ref. |
|----|----------------|-----------------|----------------|------------------------|------------------|------|
| 1  | Crab shell     | Precipitation   | –              | –                      | Carbonate ion    | [30] |
| 2  | Eggshell       | Precipitation   | –              | 1.25                   | Carbonate ion    | [31] |
| 3  | Eggshell       | Hydrothermal    | –              | 0.05                   | –                | [32] |
| 4  | Clamshell      | Hydrothermal    | –              | –                      | Carbonate ion    | [33] |

Andika et al., (2015) using precipitation methods to synthesize hydroxyapatite from eggshells. Basically, shells contain high amounts of calcium carbonate, which is around 94 - 97%. As with other methods, the shell is chosen because it can be a candidate source of calcium through calcination. As a source of phosphate used (NH$_4$)$_2$HPO$_4$ then continued by heating at 900°C. According to microscopy analysis, it is known that the characteristics of hydroxyapatite by the precipitation method are also influenced by the time of deposition. The longer the deposition time, the greater the size of the hydroxyapatite particles formed [31].

Through the hydrothermal method, Sitohang et al. (2016) have successfully synthesized eggshell-based hydroxyapatite. The synthesis process is carried out by mixing the eggshell powder with (NH$_4$)$_2$HPO$_4$ in a hydrothermal vessel under alkaline conditions (pH = 11). The analysis showed that
hydroxyapatite produced through this method has a morphology in the form of agglomerates with a size of about 50 nm [32].

Through the hydrothermal method, Arrafiqie et al. (2016) have also succeeded in synthesizing hydroxyapatite from the waste shells of the clamshells. The shell is calcined in the furnace at 900°C for 3 hours to obtain CaO. Calcined CaO is converted into PCC (precipitated calcium carbonate) using the carbonation method. Furthermore, the PCC obtained was mixed with \((\text{NH}_4)_2\text{HPO}_4\) under alkaline conditions in a hydrothermal vessel. Morphological analysis using SEM showed that the hydroxyapatite formed was in the form of agglomerates with a size range of 15 - 50 nm [33].

4. Discussion and Conclusion
Hydroxyapatite can be synthesized from natural resources that are rich in calcium. Some natural resources that are abundant in Indonesia and can be used include cow bones, fish bones, and shell-based materials such as clamshells, crab shells and eggshells. The majority of methods that have been widely used to synthesize hydroxyapatite from these natural sources are the wet deposition method, precipitation, hydrothermal, sol-gel, and dry method. To produce hydroxyapatite, calcium from these natural sources needs to be added phosphate-based compounds such as \((\text{NH}_4)_2\text{HPO}_4\) or phosphoric acid.

Some of the impurities that may arise in the process of synthesis of hydroxyapatite based on natural sources are related to carbonate groups that may arise because calcium in some natural resources is in the form of calcium carbonate.

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