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Synthesis of hydroxyapatite from biogenic wastes

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Abstract

Hydroxyapatite (HAp) is a major component of human bone, teeth and hard tissue. It is one of only a few bioactive materials. Since HAp is the most widely used ceramic biomaterial, various techniques have been developed to synthesize HAp. In recent years, the use of natural biogenic structures and materials for medical proposes has been motivated by limitations in producing synthetic materials. This article mainly focuses on the use of biogenic wastes to prepare HAp. These include bio-wastes, marine corals, eggshells, seashells and bio-membranes. In the present review, useful information about HAp preparation methodologies has been summarized for further research and development.

Keywords : Hydroxyapatite, Calcium phosphate, Biogenic wastes, Biomaterials, Bioceramics

1. Introduction

For thousands of years, ceramics in the form of pottery have been used by humans. During the past five decades, another application has developed in the use of ceramics to improve the quality of life. Specially designed ceramics have been developed for repair, restoration of function and replacement of diseased or damaged parts of the body. Ceramics used for these purposes are termed ‘‘bioceramics’’.

Calcium phosphate-based bioceramics are currently used in various types of orthopedic and maxillofacial applications because of their osteoconductive properties and tissue responses. Applications include coatings of orthopedic and oral implants, alveolar ridge augmentation, maxillofacial surgery, otolaryngology, and scaffolds for bone growth and as powders in total hip and knee surgery [1-7]. Nowadays, calcium phosphate-based bioceramics are also used for drug delivery applications. Advanced processing and new chemical techniques allow the integration of drugs within these materials or on their surfaces. In this regard, bioceramics act as local drug delivery systems to treat bone defects, osteoporotic fractures, bone infections and bone tumours [8]. The tissue responses of bioceramics can be categorized into three types; (i) nearly bioinert (e.g., alumina and zirconia), (ii) bioactive (e.g., hydroxyapatite (HAp) and bioglass), and (iii) bioresorbable (tricalcium phosphate (TCP)).

Hydroxyapatite (HAp), has a chemical composition of $Ca_{10}(PO_4)_6(OH)_2$ with a calcium/phosphorus (Ca/P) molar ratio of 1.67. It is a class of calcium phosphate-based bioceramics. HAp in powder form is frequently used in biomedical applications, such as prosthetic implants and coating implants [9-11]. This is due to its excellent biocompatibility (ability of material to perform with an appropriate host response under specific conditions), bioactivity (ability of material to provide an appropriate scaffold for bone formation) and osteoconductivity (formation of bone-like apatite on their surface with a strong bone-calcium phosphate biomaterial interface) and its chemical and structural similarity with natural bone minerals. HAp is also useful for drug delivery and controlled antibiotic release for treating bone defects and inflections [12-13]. Physiochemical, mechanical and biological properties, which make HAp an appropriate choice for medical applications, are shown in Table 1 [14].

Various synthesis techniques, such as sol-gel synthesis, hydrothermal reaction, solid-state reaction and chemical precipitation have been developed to prepare HAp powders. Using these techniques, powders with different morphologies, stoichiometries, grain sizes and levels of crystallinity can be obtained. Some properties of HAp powders prepared using a variety of techniques are summarized in Table 2.

In last two decades, the use of biogenic structures and materials to produce HAp powders using various synthesis techniques has been widely studied using such materials as egg shells, seashells, animal bone and corals. The preparation of biogenic materials is an interesting procedure to produce HAp powders not only because it uses biogenic resources, but economic and environmental benefits are obtained through waste recovery. Additionally, HAp generated from biogenic sources, such as animal bones, may be less likely to be rejected by living organs due to its physicochemical similarity to human bone [22-23].

In this study, the author purposes to demonstrate the usefulness of preparing HAp powders from various kinds of biogenic sources.

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Table 1 Physiochemical, mechanical and biological properties of HAp [14]

Properties	Experimental data
Chemical composition	$Ca_{10}(PO_4)_6(OH)_2$
Ca/P molar ratio	1.67
Crystal system	Hexagonal
Young's modulus (GPa)	80 - 110
Elastic modulus (GPa)	114
Compressive strength (MPa)	400 – 900
Bending strength (MPa)	115 – 200
Density (g/cm^3)	3.16
Relative density (%)	95 – 99.5
Fracture toughness (MPa. Mm$^{1/2}$)	0.7 – 1.2
Hardness (HV)	600
Decomposition Temp. (ºC)	>1000
Melting point (ºC)	1614
Thermal conductivity (W/cm. K)	0.013
Biocompatibility	High
Bioactivity	High
Biodegradation	Low
Cellular-compatibility	High
Osteoconductivity	High

2. Hydroxyapatite extraction from bio-wastes

Hydroxyapatite extraction from bio-wastes is the most well known method for preparation of HAp from biogenic sources. Bio-wastes from many animals have been used to prepare HAp. These include bovine and pig bone [22-23, 26-27], fish bone [24] and fish scale [25]. Simple thermal decomposition is the method that has been used to extract HAp from bio-waste. Sobczak et al. [22-23] synthesized HAp from pig bone using a simple thermal decomposition method. Deproteinized and defatted bone pulp (bone sludge) was used as a raw material. To prepare HAp, a two stage calcination process was applied to bone sludge. After calcination of bone sludge at the maximum temperature of 950ºC for 3 hours, XRD analysis showed that HAp was the only crystalline phase in the calcined product as shown in Figure 1. Calcium to phosphorus (Ca/P) molar ratios greater than 1.67 can be obtained after calcination. This shows a nonstochiometric Ca/P molar ratio in HAp. The result showed the tendency of the Ca/P molar ratio to increase with increasing calcining temperature. Additionally, Ozawa and Suzuki [24] used a thermal decomposition method to prepare HAp from fish bone waste. In this method, fish waste was washed using a strong water jet to remove the fish meat after boiling in water for 1 hour. The washed fish bone waste was then dried and calcined at a temperature of 600-1300ºC in air. They reported that highly crystalline HAp was obtained by calcination at 800-1200ºC. This highly crystalline HAp coexisted with a tricalcium phosphate (TCP) obtained at a calcining temperature of 1300°C. The Ca/P molar ratio indicate nonstochiometric HAp at all calcining temperatures. Moreover, an enzymatic hydrolysis method was used to extract HAp from fish scale [25]. According to the results, HAp was synthesized with low crystallinity and nano-particle sizes with a high Ca/P molar ratio (1.76). Other extraction methods have been used including subcritical water processing, alkaline hydrothermal hydrolysis and transfer arc plasma processing [26-27].

3. Hydroxyapatite preparation from marine corals

Additionally, calcium carbonates present in the skeletons of marine corals are another source of raw biogenic materials for synthesis of HAp [28-31]. For example, Sivakumar et al. [28] conducted a hydrothermal reaction between pre-heated coral and diammonium phosphate ($(NH_4)_2HPO_4$). The corals were cleaned of macroscopic impurities and were then heated to 900ºC for 2 hours to remove organic matter and impurities. High purity diammonium phosphate, preheated coral powder and water were well mixed and heated in a simple pressure vessel under high water pressure for a few hours to obtain HAp. They reported that both aragonite and calcite phases of calcium carbonate were observed on the XRD pattern of raw coral. Pure calcite phase was observed on 900ºC preheated coral. After hydrothermal processing, peaks of both HAp and β-tricalcium phosphate (β-TCP) were observed. Xu et al. [29] prepared HAp using a hydrothermal conversion of Porites skeletal material and diammonium phosphate with and without the use of a mineralizer, potassium dihydrogen phosphate (KH_2PO_4). Complete conversion of coral to HAp was achieved after 17 days without the use of mineralizer. Conversions of coral (aragonite) into calcite, calcite into β-TCP and β-TCP into HAp were observed. By contrast, the only reported conversion of aragonite into HAp was when hydrothermal conversion was done with a mineralizer. The conversion was complete within 6-10 days.

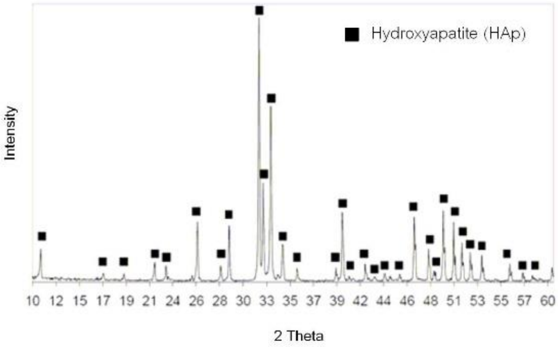

Figure 1 XRD pattern of sample obtained at 950ºC. [23]

4. Hydroxyapatite preparation from eggshells

Some researchers also tried to use eggshells as a calcium source in the synthesis of HAp [32-41]. Eggshells consist of about 95% calcium carbonate, with a $CaCO_3$ calcite phase. The rest is comprised of an organic component, i.e., several layers of interlaced protein fibers, as well as various mineral salts [35, 39-41]. Commonly, eggshells are first calcined at 900 - 1100ºC in order to remove organic substances, i.e., several layers of interlaced protein fibers. At this temperature, the eggshells convert into calcium oxide (CaO), releasing carbon dioxide (CO_2) according to the following equation:

$$CaCO_3 + \text{Heat} \rightarrow CaO + CO_2 \qquad (1)$$

Chaudhuri et al. [32] presented a simple method to synthesize nanoparticle HAp from calcined eggshells and dipotassium phosphate (K_2HPO_4). CaO obtained from calcined eggshells was slowly dissolved into appropriately mixed K_2HPO_4 with deionised water and then well mixed for about 10 min. Its pH value was held at about 12 using ammonium hydroxide (NH_4OH). The solution was placed in a heating chamber at 37ºC. Nanoparticle HAp was obtained after a day of heating. Only the peaks corresponding to HAp

Table 2 Different methods for preparation of HAp powder

Methods	General remarks	Advantage	Disadvantage
Wet methods			
Precipitation [15]	- High crystallinity after sintering at 900ºC - Grain size increased with increasing sintering temperature. - Grain size of 7.7-59.0 nm	- Low cost - Few number of chemicals - Nano size of HAp	- Diverse of HAp morphology - Low crystallinity of HAp - High crystallinity of HAp after sintering at high temperature
Hydrolysis [16]	- The grain size decreased when synthesized with cetyltrimethylammonium. - Grain size of 20-50 nm - Ca/P ratio: 1.33 – 1.67	- Low temperature process - Few number of chemicals - High phase purity of HAp - Nano size of HAp	- High cost - Diverse of HAp morphology
Emulsion [17]	- The crystallinity increased with increasing heat treatment temperature. - The stability of the apatite phase achieved when being heat treated at 800°C – 1300°C. - Particle size of 200-1300 μm - Ca/P ratio: 1.5 – 1.2	- High phase purity of HAp	- High cost - Many number of chemicals - Non-stoichometric HAp - Low crystallinity of HAp
Hydrothermal [18]	- Rod-like morphology with hexagonal shape - Ca/P ratio: 1.67	- High crystallinity of HAp - Stoichometric HAp	- High cost
Sol-gel [19]	- Variation of Ca/P molar ratio depends on the calcinations temperature. - Crystalline size of 20-60 nm - Ca/P ratio: 1.66 – 1.77	- Nano size of HAp - High phase purity of HAp	- Diverse of HAp morphology
Dry methods			
Solid state [20]	- The calcining temperatures were critical in controlling the size and shape of HAp particle. - Particle size of 747-1510 nm - Ca/P ratio: 1.67	- Low cost - Few number of chemicals - Micron size of HAp	- Low phase purity of HAp - High crystallinity of HAp after sintering at high temperature
Mechanochemical [21]	- Particle size decreased with increasing mechanical activation duration. - Average particle size of ~25 nm	- Easy production and room temperature process - Low cost - Nano size of HAp	- Low phase purity of HAp - Non-stoichometric HAp - High crystallinity of HAp after sintering at high temperature

phase were observed in the XRD pattern. HAp was also synthesized via a hydrothermal method [33-35]. Recently, Hui et al. [33] synthesized HAp from calcined eggshells and tricalcium phosphate (TCP, $Ca_3(PO_4)_2$) solution in the presence of steam at 900ºC and subsequent aging for 24 hours. Spherically shaped nanosized particles were observed in the HAp product. Additionally, Booyang et al. [34] conducted hydrothermal processing at 250ºC using crocodile egg shells and three different phosphate precursors. This included diammonium phosphate ($(NH_4)_2HPO_4$), tricalcium phosphate ($Ca_3(PO_4)_2$) and phosphoric acid (H_3PO_4). They reported that only $(NH_4)_2HPO_4$ and $Ca_3(PO_4)_2$ gave solely a HAp phase within 25 and 8 hours of heat treatment, respectively. It was also reported that the morphology of the resulting HAp showed clusters of agglomerated plate-like crystals. Rivera et al. [35] reported the use of calcined eggshells and tricalcium phosphate (TCP, $Ca_3(PO_4)_2$) with a hydrothermal process at 1050ºC for 3 hours to prepare HAp. However, they reported that the final product was primarily composed of HAp with minute fractions of calcium oxide (CaO) and calcium hydroxide ($Ca(OH_2)$).

The mechanochemical process is another simple method for fabrication of HAp from eggshells. Balazsi et al. [36], Gergely et al. [37], and Lee and Oh [38] synthesized HAp using calcined eggshells and phosphoric acid (H_3PO_4) via the mechanochemical method. In the regular process, the materials are ground in a planetary mill. The processing variables include the type and mixing ratio of reagents, the type of milling medium, the milling duration and the rotational speed. They found that the characteristics of the final HAp product were influenced by CaO/acid mixing ratio, milling time and heat treatment temperature. A uniform HAp particle size in the micrometer range was reported.

In another study, Goloshchapov et al. [39] synthesized HAp using a precipitation technique with calcined hen's eggshells and phosphoric acid (H_3PO_4) as raw materials. It was reported that the resulting HAp powders consisted of nanocrystals with an average size of about 30 nm. Moreover, Kumar et al. [40-41] proposed a rapid microwave conversion process to obtain HAp from eggshells, ethylene diammine tetra acetic acid (EDTA), disodium hydrogen phosphate (Na_2HPO_4) and sodium hydroxide (NaOH). They reported that the resulting product was HAp containing magnesium (Mg). The HAp prepared in this manner had a flower-like nanostructure as shown in Figure 2. It exhibited typical flower-like nanostructured HAp with 100–200 nm width and 0.5–1 μm length which are stretching radially from center.

5. Hydroxyapatite preparation from seashells

Some attempts have been made to produce HAp from seashells. Various seashells contain 95-99% $CaCO_3$ by weight. This can be used as a calcium precursor in the synthesis of HAp [42-48]. Ni and Ratner [42] found that nacre chip surfaces can be transformed to HAp in a phosphate buffer solution at atmospheric temperature via a surface reaction process. The mineral phase of the nacre surface was found to convert from $CaCO_3$, aragonite phase to the HAp phase. They found that a small amount of nacre surface immediately converted to HAp after 1 day of soaking in a buffer solution. The nacre was completely covered by a HAp layer after soaking for 14 days. They suggested that nacre surface reactions occur by a dissolution precipitation mechanism.

Guo et al. [43] studied the transformation of $CaCO_3$ particles from nacre by treatment with a phosphate buffer solution. They reported that the phase of $CaCO_3$ plays an important role in the conversion rate because of solubility products. For example, if the main phase is vaterite of $CaCO_3$, it is completely converted to HAp after a day of soaking. If the main phase is calcite, the conversion percentages of HAp increase with increasing immersion period in buffer solution. Recently, Guo et al. [44] fabricated nanoparticles of HAp from nacre and disodium hydrogen phosphate (Na_2HPO_4) under hydrothermal condition at 140°C for 12 hours. Microspheres composed of many nanoparticles were observed using TEM and SEM as shown in Figure 3. The whole microspheres are composed of lots of nanoparticles. The aggregates of the nanoparticles induce the formation of mesopores among them.

Additionally, oyster shell has been successfully used to produce HAp [45-46]. Lamos et al. [45] synthesized HAp nano-powder via a hydrothermal transformation of milled oyster shell powders with dibasic ammonium phosphate $(NH_4)_2HPO_4$ and additional potassium dihydrogen phosphate (KH_2PO_4) at 200°C for various periods. They reported that the transformation reaction from aragonite to HAp exhibited higher efficiency compared to the reaction from the calcite phase of calcium carbonate to HAp. Recently, Wu et al. [46] synthesized HAp from oyster shell powder using a mechanochemical reaction and heat treatment. In this study, the $CaCO_3$ calcite phase of oyster shell powders and calcium pyrophosphate ($Ca_2P_2O_7$) or dicalcium phosphate dehydrate ($CaHPO_4.2H_2O$, DCPD) were done by ball milling and subsequent heat treatment. From the results, oyster shell powders mixed with $Ca_2P_2O_7$ were completely transformed into a pure HAp phase after milling for 5 hours and heat treatment at 1100°C for 5 hours. Pure HAp was successfully synthesized using oyster shell powder mixed with DCPD, milled for 5 hours and subsequent heat treatment at 1000°C for 1 hour.

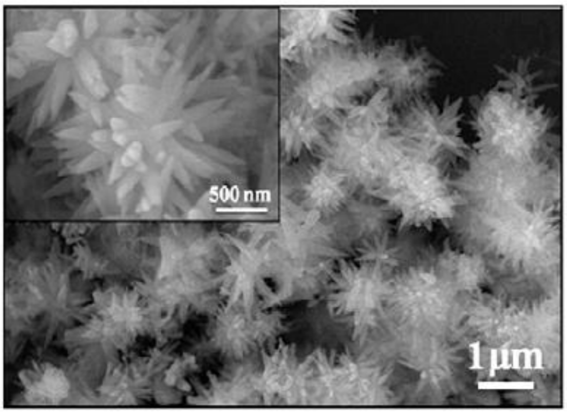

Figure 2 SEM micrograph of nanostructure of flower-like HAp prepared from rapid microwave conversion process [40]

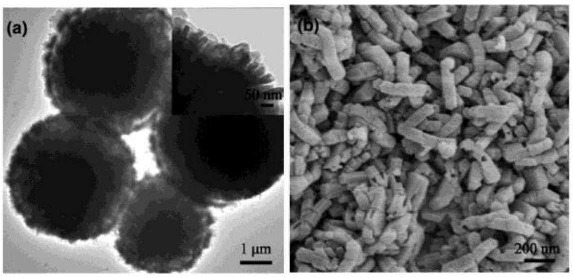

Figure 3 (a) TEM micrograph and (b) TEM micrograph of microsphere HAp [44]

Calcium carbonate from other shells, such as garden snail, conch and giant clam shells has been used to fabricate HAp [47-48]. The garden snail shell has been used to synthesize HAp using a chemical precipitation method with nitric acid (HNO_3) and diammonium phosphate ($(NH_4)_2HPO_4$) [47]. From these results, XRD analysis indicated HAp powder with phase purity and crystallinity. The average particle size of this material was reported to be 2.63 μm. Conch and giant clam shells with $(NH_4)_2HPO_4$ have been used to fabricated HAp via a hydrothermal reaction at 180-240°C [48]. The authors reported that the conch and giant clam shells were completely converted to HAp at 200°C after 10 days of hydrothermal reaction. The thickness of the HAp layer increased with reaction time.

6. Hydroxyapatite preparation from bio-membranes

Recently, studies have reported the utilization of natural membranes, such as those of eggshell or bamboo, to produce HAp [49-50]. HAp nanostructures are formed on the membranes through a diffusion-controlled nucleation mechanism. Zhang et al. [49-50] recently fabricated HAp with a flower-like structure using eggshell and bamboo membranes with calcium acetate ($Ca(CH_3COO)_2$) and dipotassium phosphate (K_2HPO_4). Figure 4 shows the schematic diagram of the HAp crystallizer used in their studies. They reported that flower-like HAp agglomerates with high crystallinity could be produced on the upper and lower sides of both membranes. The morphology and crystal structure of agglomerated HAp were influenced by temperature, pH value and holding time.

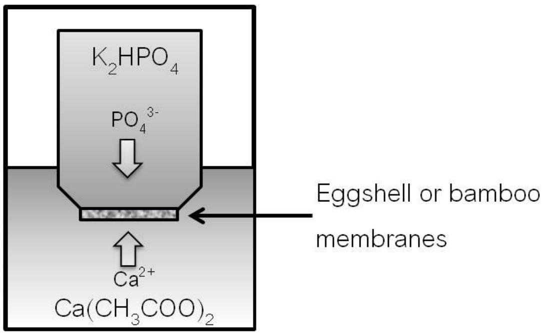

Figure 4 Schematic diagram of the HAp crystallizer (adapt from 49, 50)

7. Summarization

A careful consideration from review articles reveals three different groups, including extraction from bio-wastes, synthesis from calcium carbonate based biogenic materials and synthesis using bio-membranes. This can schematically be summarized in Figure 5. Preparation methodologies and significant factors to prepare HAp from different biogenic wastes are summarized in Table 3.

8. Conclusion

This article primarily focuses on the use of various biogenic wastes to produce HAp. These include bio-wastes, marine corals, eggshells, seashells and bio-membranes. In this review article, the preparation methodologies of HAp form biogenic wastes were classified into three groups as follows:

1. HAp extraction from bio-wastes: To obtain HAp, biowastes, especially animal bones, usually involves a few hours of heat treatment. During this processes, the organic materials in animal bones are removed. Pure HAp can be obtained from the residue. Other extraction methods have been used including enzymatic hydrolysis, subcritical water processing, alkaline hydrothermal hydrolysis and transfer arc plasma processing.
2. HAp synthesis from calcium carbonate based biogenic materials: The biogenic materials, i.e., corals, eggshells and seashells, are composed mainly of calcium carbonate and can be used as calcium precursors in the synthesis of HAp. The calcium precursors are $CaCO_3$ or CaO which subsequently react with a suitable phosphate precursor to produce HAp. There are a wide variety of methods to do this. They include hydrothermal reaction, chemical precipitation, mechanochemical reaction and rapid microwave conversion.
3. HAp synthesis using bio-membranes: Natural membranes can be used to produce HAp from calcium and phosphorus solutions. Eggshell or bamboo membranes were used as a diffusion controller and formation sites of calcium and phosphate ions for HAp nucleation.

9. Acknowledgements

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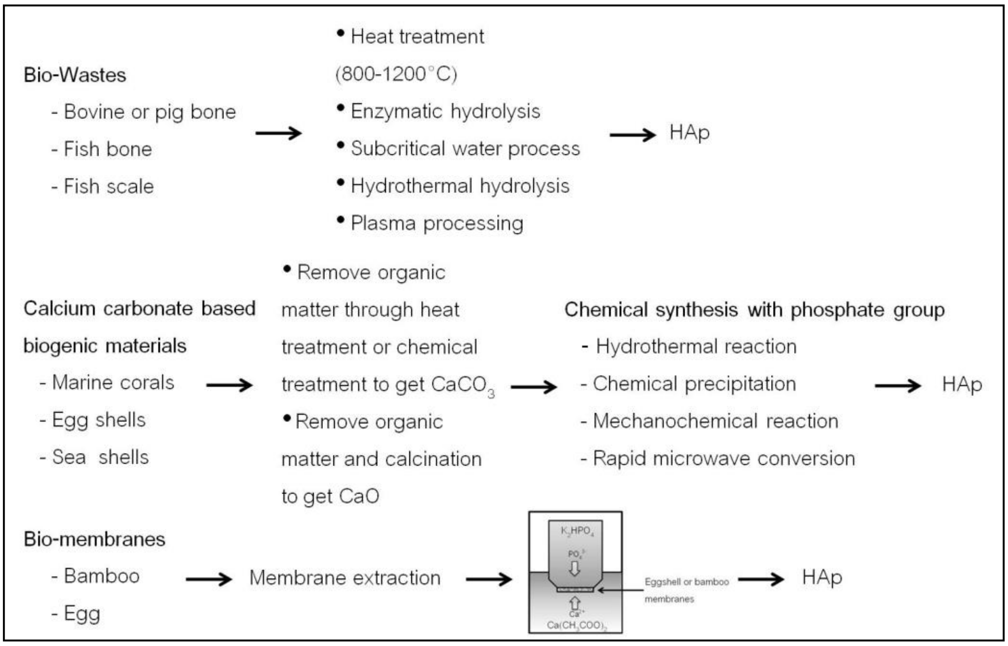

Figure 5 Preparation methodologies of HAp from biogenic materials

Table 3 Preparation methodologies and significant factors to prepare HAp from different biogenic wastes

Methods	General remarks	Significant factors
HAp extraction from bio-wastes		
• Bovine and Pig bone [22, 23]		
- Heat treatment	- 650-950ºC calcination temperatures - 2 and 3 hours holding times - Non-stoichometric Ca/P molar ratio - Pure HAp phase - Ca/P molar ratio increased with increasing calcination temperatures.	- Calcination temperatures - Type of animal bones
• Fish bone [24]		
- Heat treatment	- 600-1300ºC calcination temperatures - Non-stoichometric HAp - High crystalline HAp from 800-1200ºC	- Calcination temperatures
HAp synthesis from calcium carbonate based biogenic materials		
• Marine corals		
- Hydrothermal reaction [28, 29]	- Hydrothermal reaction between $CaCO_3$ from marine corals and phosphate precursor - HAp coexisted with β-TCP was observed during hydrothermal process.	- Processing temperature and time duration - KH_2PO_4 accelerated the direct conversion into HAp
• Egg shell		
- Simple reaction [32]	- Simple reaction between CaO calcined from egg shell and KH_2PO_4 in deionised water - Pure HAp phase	- pH value of solution - Temperature during reaction periods
- Hydrothermal reaction [33-35]	- Hydrothermal reaction between $CaCO_3$ or CaO from egg shell or calcined egg shell and different phosphate precursors	- Type of phosphate precursors - Processing temperature and
- Mechanochemical reaction [36-38]	- Mechanochemical reaction between CaO calcined from egg shell and phosphate precursor - Usually low phase purity of HAp	- Mixed ratio - Milling duration, medium and speed - Heat treated temperature
- Precipitation method [39]	- Preparation from CaO calcined from egg shell and phosphate acid solution - Nano size of HAp	- pH value during reaction
- Rapid microwave conversion [40, 41]	- Many number of chemicals - Low purity of HAp	- Mixing procedure - pH value during reaction
• Sea shells		
- Precipitation method [42, 43, 47]	- Preparation from $CaCO_3$ from various sea shells and various phosphate solutions - Usually high purity of HAp	- pH value during reaction - Phase of $CaCO_3$ - Immersion period
- Hydrothermal reaction [44, 45, 48]	- Hydrothermal reaction between $CaCO_3$ from various sea shells and different phosphate precursors	- Phase of $CaCO_3$ - Type of phosphate precursors - Processing temperature and time duration
- Mechanochemical reaction [46]	- Mechanochemical reaction between $CaCO_3$ from oyster shell and phosphate precursors and then heat treatment	- Mixed ratio - Milling duration, medium and speed - Heat treated temperature - Type of phosphate precursors
HAp synthesis using bio-membranes		
• Bamboo and egg shell membranes [49,50]	- HAp growth from diffusion mechanism using calcium and phosphate solutions - Nano structure of HAp	- pH value during diffusion - Temperature during diffusion - Holding duration

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