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Review

Review on the natural sources-based hydroxyapatite for biomedical applications

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ABSTRACT

Hydroxyapatite (HAp) is a bio-ceramic material in the family of calcium phosphate. Due to its biocompatibility nature, it is used widely for bone regeneration, orthopedic implants, development of dental materials, and also used in environmental applications. HAp can be prepared using both chemical precursors and natural sources. Chemical precursors of calcium and phosphate are commonly used in synthesis of HAp via various methods such as wet, dry, hydrothermal and precipitation, emulsion, freeze-drying, and electrochemical deposition, among others. However, it affects human cells slightly when using the chemical precursor-mediated synthesis of HAp. Therefore, many researchers have been focusing on natural source materials or biological synthesis methods rather than the chemical methods. So, it is necessary to synthesize toxic free materials from natural bio-waste materials that have been converted into valuable bio-compounds. Natural derived HAp has been applicable for various biomedical applications due to its excellent biocompatibility, bio-degradability and bioactivity nature. This review discusses the recent development of derived HAp from various natural sources, including marine, mammalian and shell sources, for biomedical applications particularly in bone tissue engineering.

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1. Introduction

Hydroxyapatite (HAp) is an essential mineral in the form of calcium apatite that constitutes about 70% of human bone inorganic compounds and 30% of the organic materials of the bone marrow cells and collagen. HAp is highly biocompatible, and the most common hexagonal structural form is $Ca_{10}(PO_4)_6(OH)_2$ (Roopalakshmi et al., 2017) which is similar composition material of bone. Due to the metabolic activity of this HAp, it can be used as alternative for the bone material. Moreover, it used broadly in many biomedical applications such as dental materials, bioactive coating on metallic implants, middle ear implants and bone tissue engineering, because of its high affinity to biopolymers, biocompatibility, bio-degradability, non-toxic, non-inflammatory and potential osteoconductive properties (Sadat-Shojai et al., 2013; Mohd Puad et al., 2019; Sathiyavimal et al., 2020). Biomedical research plays a significant role in repairing or replacing specific sites of the body's tissue in order to extend its lifespan of living. Numerous chemical compositions of some ceramic materials, such as hyaluronic acid, β-tricalcium phosphate, saturated aliphatic polyesters of poly (glycolic acid) (PGA), poly (lactic acid) PLA), and poly (lactic- co- glycolide), which resemble human bone minerals (Singhvi et al., 2019; Bohner et al., 2020). These ceramic compounds are appropriate for a wide range of orthopaedic applications, including joints, bones, human bodies, and dental materials.

There are three types of bone transplantation is available around the world such as autografts, allografts, and xenografts. Most commercial bone transplantation is auto grafts which is suitable for orthopedic surgery, but the major issue of this is a lack of materials and desired structure, which causes donor site pain and morbidity. In the case of allografts and xenografts, they have the major disadvantages of easy pathogen transfer and host immunity rejection. As a result, more research is needed to develop various biomedical beneficial materials of HAp (Wang et al., 2018; Wang et al., 2019). Synthetic HAp has been synthesized using different physicochemical methods such as dry processing, wet processing, hydrothermal, emulsion, ultrasonic spray freezedrying and electrochemical deposition etc (Phatai et al., 2019; Chen et al., 2020; Nyoo et al., 2014; Banerjee et al., 2018). However, the techniques described the above using of some HAp precursor forms, chemical precursors have induced an inflammatory response and limited bone regeneration (Pujari-Palmer et al., 2016). For those reasons, many researchers have been focusing on the synthesis of HAp from natural bio-waste resources. In recent years, research has been reported based on HAp derived from various bio-waste materials such as fish bone, chicken bone, bovine bone, pig bone, eggshell, fish scale, mussel shell, snail shell, abalone shell, oyster shell and, as shown (Fig. 1), etc., (Alshemary et al., 2018; Hidroksiapatit et al., 2017; Vanitha et al., 2017). Bio-resource materials have been transformed into valuable products in the desired form of HAp. It's not only waste management to reduce environmental pollution and also to reduce the cost production of HAp. Therefore, this review letter focuses on the recent developments and investigations of HAp derived from various natural bio-waste materials and their various biomedical applications.

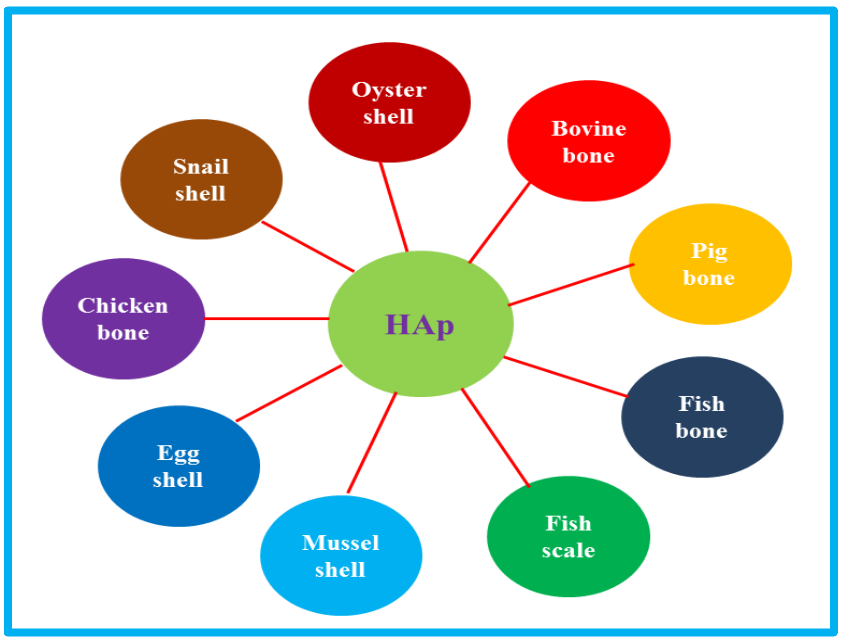

Fig 1. Various natural sources of HAp

2. Natural sources of hydroxyapatite (HAp)

2.1. Bovine bone

Meat production causes environmental pollution around the world due to the impact of generating waste products that are harmful to aquatic animals, the environment, and human's health. Bovine bone is a bio-waste material which is low in cost, and easily available on the market in large quantities, and has a low environmental impact. Furthermore, Bovine bone the presence of 65-70% of inorganic (hydroxyapatite) and 30-35 % of organic (collagen and bone marrow cells) compounds. In the natural bone of organic compounds, 95% of the collage is present. Besides that, there are other important organic compounds, such as keratin sulfate, chondroitin sulfate and lipids (triglycerides, phospholipids and cholesterol, etc.). The bovine bones were collected and washed with running tap water and dried at ambient temperature, after being soaked in 0.1 mol/dm^{-3} of the NaOH solution at 80°C for 4hrs to remove unwanted organics. The bones were dried at room temperature and calcined at 800°C for 1 hour, which process completely removed all of the organic components. The prepared powder was hot-pressed for 30 minutes at 1000 °C under a 30 MPa atmosphere pressure. The physicochemical properties were characterized and could be a promising substance for biomedical applications (Kim et al., 2008). Another researcher reported that the pre-treatment method of washing the bovine bone pieces is used by using alkali and surfactant solutions to remove the organic composition (Sun et al., 2017). Similar research reported by many researchers has reported that the bovine bone was cut into smaller pieces before pretreatment or boiling with the solvent solutions, which helped to remove the unwanted organic matter inside of the bone marrow cells (Londono-Restrepo et al., 2016; Sun et al., 2017; Hosseinzadeh et al., 2014).

2.2. Pig bone

Several researchers have reported that HAp is derived from the mammalian bone of a pig, and Ofudje et al., reported cortical bones of pigs. Bones were collected and washed with hot water to remove dirty particles. The bones were subjected to a washing de-proteinization process using 1M NaOH and 1M HCl solution shocked into boiling at 100 °C for 5 to 10 min, which resulted in the removal of the organic substance of collagen and bone marrow cells. After that boiling process, the bones were dried at 100 °C for overnight. The dried bone samples were cut into smaller pieces and calcinated in a muffle furnace at different temperatures (600 °C, 800 °C and 1000 °C). The samples were cooled to room temperature, and the resulting materials were identified as HAp (Ofudje et al., 2018). The bone bio-waste can be utilized alternative biomaterial form for bone tissue engineering application (Ofudje et al., 2018). Haberko et al

S. No	Bio-resource	Method of extraction	Reference
1.	Bovine bone	Alkaline and hydrothermal treatment	Ramesh et al., 2020
2.	Bovine bone	Alkaline and heat treatment	Pires et al., 2020
3.	Bovine Femur Bones	Calcination treatment	Khoo et al., 2015
4.	Bovine bone	Calcination treatment	Krishnamurithy et al., 2014
5.	Bovine bone	Hydrothermal and calcination treatment	Londono-Restrepo et al., 2016
6.	Bovine bone	Subcritical water process calcination	Sun et al., 2017
7.	Bovine bone	Subcritical water process calcination	Hosseinzadeh et al., 2014
8.	Pig bone	Calcination treatment	Zelaya-Laineza et al., 2020
9.	Pig bone	Milling and hydrothermal treatment	Ramirez-Gutierrez et al., 2017
10.	Pig bone	Calcination treatment	Ofudje et al., 2017
11.	Pig bone	Alkaline and Calcination treatment	Janus et al., 2008

Table 1. Recent literature of HAp derived from mammalian source.

and Janus et al. have reported the HAp derived from pig bone followed a combination treatment of an alkaline de-proteinization process and calcination of the removal of organic components. The collected pig bone was washed and treated with a 4 M NaOH solution at 100 °C for 24 hrs. This process was again repeated until the filtrated solution reached the pH of 7 and then dried. The samples were calcinated at 800 °C and 1200 °C. The pig bone derived HAp was performed in a cytotoxic test on the CAL-72 (human osteosarcoma) cell line that revealed microstructure features of prepared HAp. It is alternatively used for bone reconstruction or implant (Janus et al., 2008; Haberko et al., 2006) Table 1, shows recently literature of HAp derived from mammalian source.

2.3. Fish bone

Due to rapid growing fish industry significantly generates a large amount of bio-waste materials during processing. Worldwide, every year, 75 million tons of fish bio-waste are generated (Athinarayanan et al., 2020; Kannan et al., 2017). However, by-products of fish waste are hazardous to aquatic animals as well as environmental systems that induce high biological oxygen demands (BOD), chemical oxygen demands (COD) when dumped into the oceans without any pre-treatment and also increase the pathogenic microbial population. Worldwide fish waste management is a major issue due to its unusual practices, which can cause health and environmental problems (Chowdhury et al., 2010; Arvanitoyannis et al., 2008). The fish by-products are contained organic forms of collagen, lipids, proteins and inorganic materials of calcium phosphate ceramics. Therefore, researchers have been focusing the biomaterial materials for fabricating the bone tissue engineering application due to their potential biocompatibility and biodegradability.

Pal et al., reported that *Lates calcarifer* fish bones were collected and cleaned in boiling water to remove unwanted debris and fleshy materials, then the bones were dried at 80 °C for 24hrs in an oven. After that process, the bones were subjected to calcination at different temperatures, from 200, 400, 800, 1000 and 1200 °C for an hour. It was synthesized from the biogenic form of HAp and cytotoxic was performed by *in vitro* cell culture techniques. It revealed the non-cytotoxic nature of fish bones derived HAp can be suggested to develop bone implantation and tissue engineering applications (Pal et al., 2017). To remove unwanted debris and flesh meat materials, fish bones/scales have been used in the pretreatment methods of boiling and washing. Venkatesan et al., have reported HAp derived from thunnus obesus fish bones through the comparative study of alkaline hydrolysis and calcination methods. The fish bones were calcined at 900 °C for 5 hours to form the HAp powder. The other alkaline treatment technique, pretreated fish bone was immersed 2 M of NaOH solution and heated at 250 °C for 5hrs. Eventually, the precipitate product was adjusted to the pH with distilled water and dried. Both techniques generate pure HAp, but their crystallinity and particle sizes differ. The HAp material was produced from calcination results that revealed large particle size and higher crystallinity compared to the alkaline heat treatment method (Venkatesan et al., 2011).

2.4. Fish scale

HAp can also be derived from another source of fish scales. According to Athinarayanan et al., the Lethrinus lentjan fish scales were collected hygienically and gently washed with distilled water to remove unwanted debris particles and air. 5g of dried fish scales were mixed with 250 mL of MilliQ water before being placed in a hydrothermal autoclave at 280 °C for 3 hours. Following that, the obtained mixture of solid and liquid portions was separated via filtration and purified dialysis and freeze-dried, yielding a fine powder containing HAp.The synthesized HAp NPs were studied through morphological investigation and cytotoxic study in human mesenchymal stem cells, demonstrating that they are biocompatible and non-toxic,

S. No	Bio-resource	Method of extraction	Reference
1.	Fish scale	Hydrothermal treatment	Athinarayanan et al., 2020
2.	Fish scale	HCl acid and calcination treatment	Deb et al., 2019
3.	Fish scale	Calcination treatment	Paul et al., 2017
4.	Fish scale	Calcination treatment	Mondal et al., 2016
5.	Fish scale	Alkaline heat treatment and calcination treatment	Panda et al., 2014
6.	Fish scale	Alkaline heat treatment	Pon-On et al., 2016
7.	Fish scale	Ionic liquid pretreatment	Muhammad et al., 2016
8.	Fish bone	Calcination treatment	Pal et al., 2017
9.	Fish bone	Calcination treatment	Sunil et al., 2016
10.	Fish bone	Alkaline heat treatment	Venkatesan et al., 2011
11.	Fish bone	Calcination treatment	Sathiyavimal et al., 2020

Table 2. Recent literature of HAp derived from marine source.

making them suitable for bone tissue engineering and bio-imaging applications (Athinarayanan et al., 2020). Panda et al., HAp can be extracted from fish scales using a combination treatment of calcination and alkaline heat treatment. In this method, fish scales were treated with 1N NaOH solution and combined with calcium chloride dehydrate heated at 75°C and then followed by calcination at 800 °C for 1 hour. Athour obtained the agglomerated crystalline HAp with an average particle size of 76.62 nm and a Ca/P ratio of 1.62 using this method. The prepared HAp can be used as an alternative biomaterial for bone tissue engineering (Panda et al., 2014) Table 2, shows comprehensive literature of HAp derived from marine source.

2.5. Egg shell

Eggs are a highly nutritious and easily available food that is widely consumed around the world. It is high in protein and contains a variety of important nutrients, such as folic acid, selenium, choline, iron, and vitamins A, B, D, E, and K. It also contains antioxidant carotenoids, zeaxanthin, and lutein. Furthermore, egg shells contain abundant minerals that can be used in a variety of biomedical applications, such as bone implant development, dental applications, food industry applications, and as a fertilizer component in agriculture (Oliveira et al., 2013; Murakami et al., 2007; Boron, 2004). The egg shell membranes are made up of glycoproteins and contain type I, V, and X collagen, which is useful in the production of cosmetics. Others are made up of dermatan sulfate, keratin, and glycosaminoglycans, which can be used to make collagen and other products, as well as to synthesize chitin.

Agbabiaka et al., have reported that Gallus domesticus egg shells were collected and washed with distilled water to remove the debris particles, and then boiled in distilled water for 10 min for the removal of the membranes. The shells were dried at 80 °C for 24 hours before being heated at various temperatures and converted into powder samples, which were labeled as HA800, HA900, and HA1000. The obtained HAp powder (HA1000) has a Ca/P stoichiometry ratio that is similar to that of natural bone (Ca/P=1.65). Goloshchapov et al. applied a combination treatment of precipitation and calcination for the synthesis of HAp from eggshells. The cleaned shells were calcined at 900 °C for 2 hours to obtain CaO powder that was mixed with distilled water and heated at 100 °C to transform it into the calcium hydroxide form. The Ca (OH)2 was treated with orthophosphoric acid (0.6 M). It was formed a precipitate and calcined at 400 °C, 700 °C and 900 °C for 1 hour. The pH of the obtained HAp powder sample was adjusted to be closer to the stoichiometric ratio (Agbabiaka et al., 2019).

2.6. Mussel shells

Mussel shells are in coastal areas that can synthesis the HAp. Kumar et al., used a fresh mussel collected from the Kaveri River, Tamil Nadu, India and washed with distilled water to remove unwanted dust particles. The cleaned shells were heated at 110 °C for 5 hours in a hot air oven and then cooled at room temperature. The shells were then crushed into fine powder, and 1g of mussel shell powder was mixed with 100 mL of 0.1 M EDTA before being added to a 0.06M disodium hydrogen phosphate solution. During the reaction, the pH was adjusted with NaOH solution to maintain a stoichiometric ratio of 1.67. After 1 hour of reaction, the obtained mixture was transferred into a microwave oven for 15 min of irradiation. White precipitation was obtained and washed with distilled water and followed under 110 °C for 5hrs dried in hot air oven to get final product of HAp (Kumar et al., 2016). Shavandi et al., used a microwave irradiation method to synthesis of HAp derived from mussel shells. In this method, mussel shells were cleaned with distilled water and air dried, and they were calcined at 900 °C for 30 min to form CaO, which was treated with a 0.1 M EDTA solution to produce a Ca-EDTA complex. The drop was then treated in the microwave after adding 0.06 M of Na_2HPO_4 solution at a rate of 4 mL/min for 15 minutes while stirring, and the pH was adjusted to a similar stoichiometric

Table3. Recent literature of HAp derived from shell source.

S. No	Bio-resource	Method of extraction	Reference
	Egg shell	acetic acid and calcination treatment	Shafiei et al., 2019
	Egg shell	Calcination treatment	Agbabiaka et al., 2019
	Egg shell	Calcination treatment	Ayawanna et al., 2019
	Egg shell	Alkaline and Calcination treatment	Lala et al., 2019
	Egg shell	Enzymatic hydrolysis and heat treatment	Nam et al., 2019
	Egg shell	Precipitation and calcination treatment	Sirait et al., 2020
	Mussel shell	Calcination and microwave irradiation treatment	Shavandi et al., 2015
	Mussel shell	Acid treatment	Karunakarana et al., 2020
	Mussel shell	Heat and EDTA treatment	Irfan et al., 2021
	Mussel shell	Calcination and ultrasound irradiation	Edralin et al., 2017

ratio. The powder was dried in vacuum and further calcined at 650 °C for 1 hour to finally get the pure form of HAp (Shavandi et al., 2015). Table 3, Shows recent report of HAp derived from shell.

3. Conclusion

In summary, this review article summarized the derived HAp can be synthesized from various natural sources of mammalian, marine, and shell. The synthesized HAp was evaluated as calcium phosphate, a renewable by-product that is converted into valuable material production with a stoichiometry ratio as well as waste management. Renewable food by-products are considered to be useless. However, there are low-cost and rich resources of calcium phosphate present in the materials. The biogenic synthesized HAp has excellent properties of being non-toxic, biodegradable, biocompatible, and reveals good biological activities. Therefore, natural derived HAp can be used for several biomedical applications, including bone tissue engineering, drug delivery, dental and fertilizer for agriculture purposes. In this review, the naturally derived HAp ensures biological properties and sustainable use of the CaP family of materials. To be commercially viable, future research should be focused on naturally available materials as well as on developing new materials for various biomedical applications.

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