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Hydroxyapatite: Preparation, Properties and Its Biomedical Applications

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Abstract

Hydroxyapatite, a naturally occurring form of calcium phosphate, is the main mineral component of bones and teeth. Natural hydroxyapatite and bone have similar physical and chemical characteristics make it biocompatible. Its porous structure resembles native bone. The biocompatibility, biodegradability and bioactivity make it extensively useful in interdisciplinary fields of sciences like chemistry, biology, and medicine. Calcium phosphate-based ceramics are of great interest as substitutes of synthetic bone graft due to their similarities in composition to bone mineral and bioactivity as well as osteoconductivity. This article gives an overview of hydroxyapatite from its preparation and properties to biomedical applications of its composites.

Keywords

Applications, Bioactivity, Bioceramics, Composites, Hydroxyapatite, Tissue Engineering

1. Introduction

Hydroxyapatites (HAP) is a naturally occurring mineral form of calcium apatite comprising of about 50% of the weight of the bone, which accounts for its excellent osteoconductive and osteointegrative properties [1] [2] [3]. It is a main component of bone mineral but in some cases carbonate-apatite is a main hard tissue component, as in dental enamel [4]. One of the most common apatites used as bioceramic in medicine and dentistry is hydroxyapatite (HAP) due to its bioactivity and osteoconductive properties *in vivo* [5] [6] [7] [8]. The advantage of using HAP as a bioceramic or biomaterial compared to other bioceramics, such as Bioglass or A-W glass-ceramic, is its chemical similarity to the inorganic component of bone and tooth. Chemically hydroxyapatite is $Ca_5(PO_4)_3OH$ but often written as $Ca_{10}(PO_4)_6(OH)_2$. Naturally, hydroxyapatite is an inorganic

component found in human hard tissues such as tooth and bone. These materials are generally used as human body implant materials. Natural hydroxyapatite can be prepared from eggshells, coral, fish bone, chicken bone, etc. [9]. Recently, hydroxyapatite has attracted interests because of its hemostatic properties, and bone healing function [10] [11] [12].

This article gives an overview on different ways of hydroxyapatite preparation, its properties and biomedical applications of its composites.

2. Preparation of Hydroxyapatite

Hydroxyapatite can be prepared by different methods such as sol-gel process [13], chemical precipitation [14], etc. Chaudhari *et al.* prepared the HAP by applying the following reaction [15].

$$10\mathrm{CaO} + \mathrm{K_2HPO_4} + 4\mathrm{H_2O} \rightarrow \mathrm{Ca_{10}\left(PO_4\right)_6\left(OH\right)_2} + 12\mathrm{KOH}$$

Khoo *et al.* prepared natural HAP from the bovine femur *via* calcinations at different temperature. It was observed that particle size and calcination temperature affect the composition, crystallinity and crystallite size of the extracted natural HAP [16].

HAP can be produced from coral [17], seashell [18], eggshell [19] [20] [21] and also from body fluids [22]. There are numerous methods have been reported for the preparation of hydroxyapatite from eggshell. One of them is the hydrothermal method. It is extensively reported method of HAP production from eggshell [23]. This method of preparing HAP from eggshells in a phosphate solution at a high temperature is a novel approach for synthesizing valuable biomedical materials [19]. In this method, fine hydroxyapatite single crystals are prepared by a hydrothermal method with $Ca(OH)_2$ and $CaHPO_4{\cdot}2H_2O$ as starting materials. HAP prepared from hydrothermal methods has more crystallinity and good homogeneity, the major advantage of hydrothermal method. This method is direct and straight forward which gives all the characteristics band of HAP but it is laborious and time consuming [19].

Next is the microwave irradiation method, it requires a chelating agent *i.e.* ethylenediamine tetra acetic acid (EDTA) (**Figure 1**) [24]. This is an indirect way where synthesis of HAP is generally led by formation of calcium precursor from eggshells as the first step. Thus, prepared HAP shows higher sinterability and stability at high temperatures with better stoichiometry, morphology, and osteoblast cell adhesion [23]. Türk *et al.* reported that microwave assisted biomimetic synthesis can be a promising technique of preparing HAP powders in shorter time [25].

High energy mechanochemical activation method is also applied to produce HAP. It involves two processes: attrition milling and ball milling [26]. The mechanochemical reaction supplies enough amount of hydroxyl group to the starting powders to form a single phase of hydroxyapatite. This is relatively simple and recommended for the mass production of high crystalline hydroxyapatite [27].

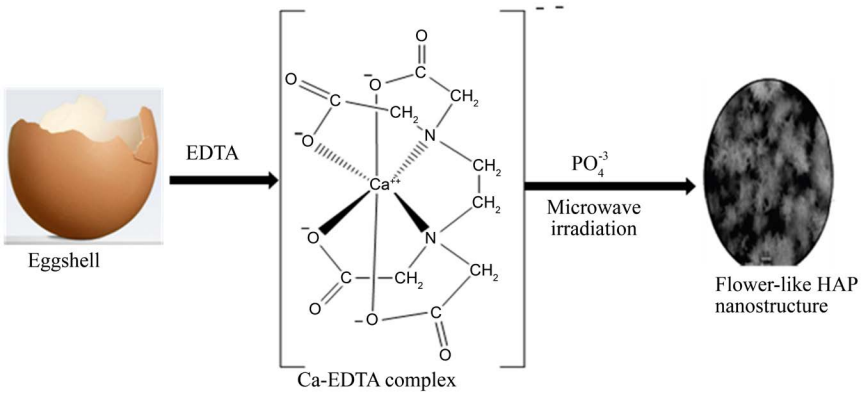

Figure 1. Microwave irradiation method to prepare hydroxyapatite nanostructure from egg shell (adapted from Ref. 24).

A simple sol-gel precipitation technique can be used to prepare nanohydroxyapatite from egg shell. The powder particles are polycrystalline in nature with an average size of 5 - 90 nm. The produced nano-HAP was found in pure form [28] with higher bioactivity than HAP coarser crystals [29]. Bernard *et al.* reported the preparation of HAP by neutralizing suspension of lime $Ca(OH)_2$ with solution of orthophosphoric acid at low temperature. It is a simple and non-polluting method [30].

$$6H_3PO_4 + 10Ca(OH)_2 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18H_2O$$

Guo *et al.* synthesized nanosized HAP particles *via* reverse microemulsion method with different values of hydrophile-lipophile balance (HLB). HAP particles prepared by the microemulsion route led to a smaller particle size and the improve degree of particle agglomeration as compared to conventional precipitation method [31].

Basically, biomimetic processing is based on biologic systems store and process information at molecular level [32] [33] [34] [35]. The extension of this concept has upgraded in processing of synthetic bone in last few decades [36]. Hydroxyapatite (HAP)-gelatin (GEL) nanocomposites were synthesized using a biomimetic process [37].

3. Properties of Hydroxyapatite

Sobczak-Kupiec *et al.* reported that the physicochemical properties and morphology of HAP depended on the origin/preparation method [38]. Synthetic hydroxyapatite exhibited low crystallinity, with high porosity and more surface area. On the otherhand, HAP obtained from animal bone *via* calcination at 800˚C possesses highest crystallinity [38].

Hydroxyapatite has the capability to form chemical bonds with surrounding hard tissues [39] [40] with the formation of a HAP interfacial layer [41]. The similar physical and chemical characteristics of natural hydroxyapatite with bone make it biocompatible [8].

Bowen co-workers studied the relationship between the composition and di-

electric and piezoelectric composites for polarized bone substitutes. It was observed that the addition of $BaTiO_3$ increases permittivity and ac conductivity of the material [42]. It is summarized that HAP-$BaTiO_3$ composites can be used as polarized bone substitutes [42].

Gao *et al.* prepared three porous scaffolds by sintering of bovine bone and three-dimensional gel-lamination method. The results demonstrated that three types of HAP scaffolds showed good attachment, proliferation and differentiation of osteoblasts [43].

Hydroxyapatite ceramic, derived from bovine bone by sintering, has a porosity and pore structure which resembles that of native bone. The porosity and the good wettability with water and organic solvents permit ceramic loading with drugs such as antibiotics, or substances that improve healing of bone [44].

According to Zhang and Darvell, the morphology and structural characteristics of hydroxyapatite whiskers depend on the initial Ca/P ratio (iCa/P) and pH (ipH), as well as the initial calcium concentration (i[Ca]) [45]. Deviation in these values did not affect on constitution, which was crystallographically indistinguishable from HAP. Ca/P ratio gradually improved with increase in both ipH and iCa/P, but was independent of i[Ca]. Uniform whiskers were obtained at high iCa/P and low ipH, or at high ipH and low iCa/P. Uniform whiskers were obtained at high iCa/P and low ipH, or at high ipH and low iCa/P. At low iCa/P and a low ipH branch-like whiskers and irregular plate-like particles were produced, while a high ipH supported the formation of lath-like HAP at high iCa/P. Preferred growth along the c-axis was greater at higher iCa/P and ipH as well as at low i[Ca] [45].

Werner and coworkers manufactured osteo implants having graded porosity by multilayer casting of HAP tapes with controlled pore structure [46]. The results proved that sintering temperature is a critical factor influencing density, microstructure and stability of HAP phase. The optimum sintering temperature to obtain maximum flexural strength for three layered structures was found to be 1250˚C. Pore-graded three-layer structures revealed approximately 40% higher flexural strength than a homogeneous three-layer structure with single pore size. The macroporous HAP network gives access for osteoblast-like cells which can attach, spread and propagate throughout the macropores and their interconnections [46].

Several studies have been reported the scanning electron micrographs of hydroxyapatite. Here, representative SEM of sample *i.e.* calcined at 900˚C is presented in **Figure 2**. In this image, the morphology of hydroxyapatite was found porous with pore size less than 1 μm in average and nonhomogeneous [8]. **Figure 3(a)** and **Figure 3(b)** presented representative SEM pictures of received bovine bone (raw material) and bones annealed at 900˚C, respectively. The microstructure of received bovine seemed dense due to the presence of organic substances in the bovine bone matrix. A typical bone-like matrix was obtained for samples annealed at 900˚C as shown in **Figure 3(a)**. Surface morphology showed the interconnected porous structure [47]. Rahavi *et al.* studied the

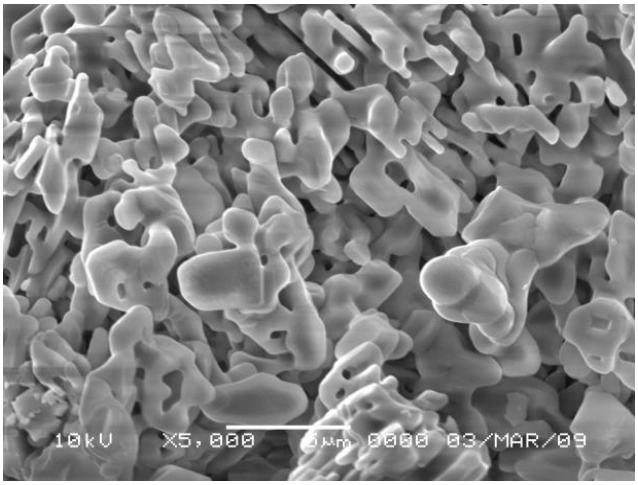

Figure 2. Scanning electron microscope image of sample calcined at 900˚C [8].

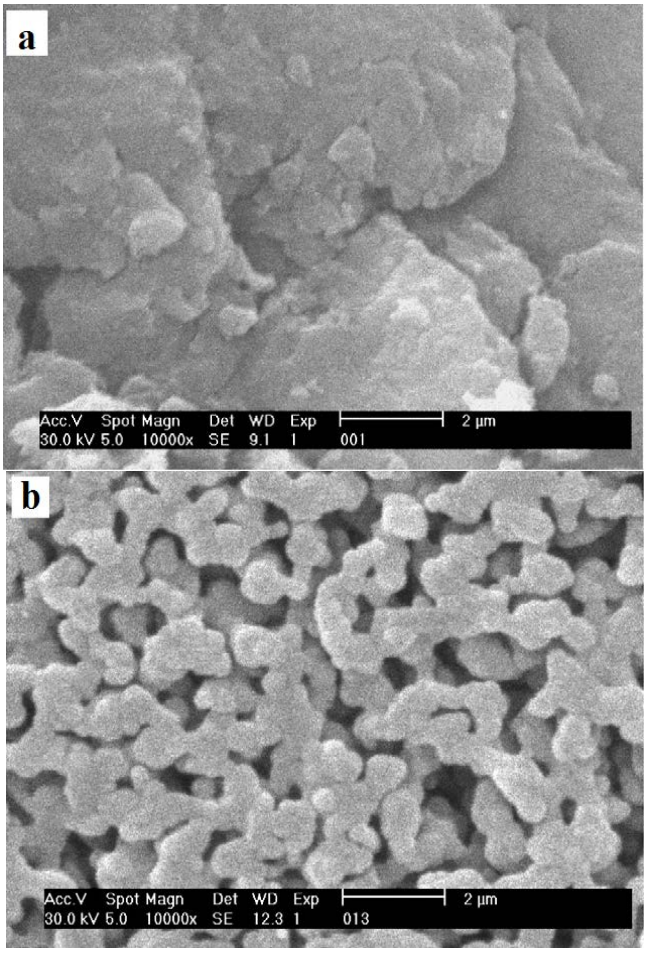

Figure 3. SEM images of (a) bovine bone and (b) bone annealed at 900˚C [47].

surface morphology of the prepared hydroxypatite (HAP) ceramic particles via calcinations of natural bones and synthetic sol-gel method and observed the aggregation of particles with rough and granular to dense surfaces. The size of HAP particles was predicted to the range between 50 - 500 nm [48].

4. Applications of Hydroxyapatite (HAP)

Historically, the first broadly tested artificial bioceramics was plaster of Paris (calcium sulfate) but they have *ex vivo* applications. By the end of 19th century, surgeons already used plaster of Paris as a bone-filling substitute [49] [50]. References [51] [52] [53] [54] [55] give details on recent history of $CaPO_4$, bioceramics and biomaterials. Fred Houdlette Albee (1876-1945), who invented bone grafting [56] made the first attempt to implant a laboratory produced $CaPO_4$ as an artificial material to repair surgically created defects in rabbit bones in 1920 [57]. He also invented some other advances in orthopedic surgery [50]. Presently hydroxyapatite has received much more interest as an implant material with applications in dentistry and orthopedics [58] [59] [60].

Synthetic HAP has been used widely as an implant material for bone substitute because of its excellent osteo inductive properties [61]. Oonishi explained the use of HAP composites in clinical orthopaedics for spacing or filling bone defects because of its important biological properties such as lack of immuno-reaction and absence of postoperative morphological change or volume decrease. HAP implants fixed with cement avoids problems of high density polyethylene wear particles [62]. Other applications of HAP include femoral plugs in total hip replacement and HAP coating on metal components for cementless fixation. For rapid and strong cementless fixation porous metal surfaces are used; HAP coating of porous metal gives improved results. Bioactive interfacial bone cementation technique was also developed by introducing fine HAP granules between the bone and polymethyl methacrylate (PMMA) cement [62].

Blends of polycarpolactone (PCL)/HAP, PCL/collagen (Col)/HAP, PCL/gelatin (Gel)/HAP, poly-L-lactic acid (PLLA)/Col/HAP and poly3-hydroxy-butyrate-co-3-hydroxyvalerate (PHBV)/HAP were studied by various research groups as a substitute for bone tissue engineering [63]-[68]. Scaffolds with HAP polymeric composites improved the new bone tissue development with increased osteointegration, osteoblast adhesion and calcium mineral deposition on its surface [68]. HAP-enhanced surface properties can be used to increase cell response and proliferation to induce mineralization in bone tissue engineering. Hydroxyapatite has been used in diversity biomedical fields such as matrices for bone cements, controlled drug release, tooth paste additive, dental implants, etc. [65].

Prabhakaran *et al.* fabricated poly-L-lactic acid (PLLA)/HAP and PLLA/Collagen (Col)/HAP nanofibres by electrospinning and found that PLLA/Col/HAP nanofibres biocomposite are better than PLLA/HAP nanofibres for effective bone regeneration and mineralization [68]. Polycaprolactone (PCL)/HAP/Col nanofibres has interconnected porous structure which provided mechanical support and facilitated extracellular matrix (ECM) production for bone tissue formation [65]. Marra *et al.* examined the blends of biodegradable polymers, poly (caprolactone) and poly (D,L-lactic-co-glycolic acid), as scaffolds for applications in bone tissue engineering. HAP granules were introduced into the blends and porous discs were prepared. Mechanical properties and degradation rates *in vi-*

tro of the composites were determined. The discs were seeded with rabbit bone marrow or cultured bone marrow stromal cells and incubated under physiological conditions. This study suggested the feasible use of novel polymer/ceramic composites as scaffold in bone tissue engineering applications [69].

Calcium phosphate-based ceramics, such as HAP, are of great interest as synthetic bone graft substitutes due to their similarity in composition to bone mineral and bioactivity as well as osteoconductivity [70].

Wang *et al.* blended hydroxyapatite (HAP) into poly (3-hydroxybutyrate) (PHB) and poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx) to build films and scaffolds [71]. HAP blending, showed improvement in mechanical properties of PHB including compressive elastic modulus and maximum stress as well as enhancement in osteoblast responses including cell growth and alkaline phosphatase activity. On the other hand, the blending of HAP particles into PHBHHx scaffolds fabricated by salt leaching was unable to either strengthen its mechanical properties or enhance osteoblast responses. Although HAP is bioactive and osteoconductive, its blending with PHBHHx cannot generate a better performance on bone reconstruction [71].

Petricca *et al.* reported the composites of HAP and PLGA; poly (D,L-lactic-co-glycolic acid) and found the improved mechanical properties as well as increased osteogenic response of the HAP/PLGA composites are appropriate as bone substitution scaffolds [72].

Palazzo *et al.* investigated the adsorption and desorption of anticancer drugs cis-diamminedichloroplatinum (II) (CDDP, cisplatin) and new platinum (II) complex di(ethylenediamineplatinum) medronate (DPM), as well as the clinically relevant bisphosphonate alendronate, towards two biomimetic synthetic HAP nanocrystalline materials with either needle-shaped (HAP) or plate-shaped (HAP) morphologies and different physico-chemical properties. This work demonstrated that the properties of HAP nanocrystals can be modulated to produce HAP/biomolecule conjugates that are tailored for specific therapeutic applications [73].

A transparent and slight yellow chitosan (CS)/HAP nanocomposite rods reported high performed, potential application as internal fixation of bone fracture. The method resolves the problem of the nano-sized particle aggregation in polymer matrix [74]. Hoffmann *et al.* fabricated HAP/starch/chitosan composites hemostatic material and proposed as a substitute for bone wax or even as a bone filling material for orthopedic surgery applications [75].

Madhumathi *et al.* deposited HAP on the surface of chitosan hydrogel membranes and evaluated the biocompatibility of these membranes using MG-63 osteosarcoma cells and suggested that chitosan hydrogel-HAP composite membranes is applicable for tissue-engineering [76].

Electrospinning is cost effective and appropriate technique for the production of nanofibers for fabricating scaffolds with biomolecules and has been used across a wide range of biocomposite polymer systems and bone tissue engineer-

ing actions [62]. Calcium phosphate ceramics has great importance in the field of tissue engineering for the biological applications [77]. Ngiam *et al.* fabricated the nanofibrous composites for mimicking the bone components and observed that deposition of HAP on PLLA/collagen nanofibers results in better early osteoblast attachment to mineralized nanofibers [78]. Rodríguez-Lorenzo *et al.* reported that HAP ceramic bodies with controlled porosity could be appropriate for hard tissue substitution or as carriers for controlled delivery of drugs or as scaffolds for tissue engineering [79]. Ramier *et al.* investigated PHB/nHAP biocomposite scaffolds with structural, mechanical, and biological properties appropriate for tissue engineering applications [80].

Yang *et al.* reported the comparative study of blood clotting activity of HAP with other potential bone repairing materials such as calcium silicate, calcium combined attapulgite, calcium triphosphate, and chitosan to show HAP as recommended hemostatic constituent to replace bone wax. HAP is recommended as a promising constituent in fabricating hemostatic material in orthopedic application as alternatives to bone wax [12]. Rahavi *et al.* mentioned that cells proliferations were stimulated in the presence of HAP nanopowders obtained from horse and human bones via MTT assay. This HAP can be a viable and economical graft material for clinical applications [48]. Baradaran *et al.* prepared reduced grapheneoxide (rGO) reinforced hydroxyapatite nano-tube (nHAP) composites *in situ* via simple hydrothermal method in a mixed solvent system of ethylene glycol (EG), *N*,*N*-dimethylformamide (DMF) and water, without using any reducing agents. Study of cell culture and viability test showed that the addition of the reduced graphene oxide improves osteoblast adhesion and proliferation, and hence increase the biocompatibility of the nHAP/rGO composite [81]. Zeng *et al.* fabricated graphene oxide/hydroxyapatite (GO/HAP) composite by electrochemical deposition method. The bioactivity of the synthesized GO/HAP composite implant coatings showed better results, *i.e.* the improved MG63 cells adhesion, proliferation and differentiation compared with the pure Titanium and pure HAP coating [82].

Many investigations have been developed for 3D printing of polymer-ceramic composites, among them polymer-hydroxyapatite composites are of great interest [83] [84]. It was found both *in vitro* and/or *in vivo* tests that 3D-printed bone tissue engineering scaffolds, based on polylactide (PLA)/HAP [83] [85], polycaprolactone (PCL)/HAP [84] [86], or poly(propylene fumarate) (PPF)/HAP [87] allow bone healing.

Nano HAP has been applied for both bioimaging as well as therapeutic applications [88]. Morgan *et al.* reported that 20 - 30 nm diameter organically doped calcium phosphate nanoparticles can be prepared using various fluorescent dyes such as cascade blue, 10-(3-sulfopropyl) acridinium betaine (SAB), rhodamine WT, fluoresce in sodium salt, and Cy3 amidite and found that fluorescence quantum efficiency can be increased by 4-fold from 0.045 to 0.202 for the free and encapsulated dye respectively [89]. Nano HAP can be used as an antigen

carrier. Goyal *et al.* used cellobiose-coated, spherical nHAP ranging from 50 to 150 nm to deliver a hepatitis B surface antigen (HBsAg) [90].

The antibacterial properties of nano-hydroxyapatite can be increased by adding silver ions in the HAP structure [91] [92]. Dubnika *et al.* developed a method to prepare a novel carrier system based on the silver-doped hydroxyapatite and loaded with lidocaine hydrochloride in the presence of chitosan or sodium alginate (HAP/Ag/polymer/drug composite) [92].

5. Conclusions

Hydroxyapatite is shown to be a significant material for biomedical applications due to its biodegradability, biocompatibility and bioactivity. HAP is a beneficial biomaterial for dental and medical applications. The HAP nanoparticles are more useful than conventional sized HAP bulk ceramics based on large surface-to-volume ratio, reactivity, and biomimetic morphology of the HAP nanoparticles for applications such as fillers for composites, reparative materials for damaged enamel and carriers for drugs. This review gives an overview about the synthesis, properties and applications of HAP in biomedical domain.

It can be concluded from the above presented investigations that despite numerous methods elaborated the synthesis of HAP which are used as bone scaffolds and in dentistry, there is still a huge demand for developing a simple efficient and green method for the production of HAP.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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