MINI-REVIEW

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Green Synthesis of Hydroxyapatite Nanoparticles for Biomedical Applications: A Brief Review

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Abstract: Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] (HAp) represents an outstanding source for the development of materials with biomedical interest. This material is a part of the mineral phase of bones and teeth of animals and humans. Due to its osteointegrity, biocompatibility, and bioactivity, this material is considered one of the most promising materials for biomedical applications. Hydroxyapatite nanoparticles are synthesized mainly by traditional wet chemical precipitation, that uses non-environmentally friendly templates. In the last years, many attempts were directed to turn the hydroxyapatite nanoparticles synthesis greener, by application of the green synthesis approach. Recently, special attention has been given to the application of plant extracts as substitutes for synthetic templates. Therefore, this work aims to provide a general state of the art on the green synthesis of hydroxyapatite nanoparticles for biomedical application. Additionally, the main gaps regarding the green synthesis are carefully stated.

Keywords: Biomaterials.Bone Tissue Engineering. Nanotechnology.

Introduction

Among the emerging biomaterials, nanoparticles of the bioceramic hydroxyapatite (HAp) have gathered attention due to their promising applications in bone and teeth reconstruction. HAp [$Ca_{10}(PO_4)_6(OH)_2$] is a calcium mineral with a hexagonal arrangement of calcium and phosphate ions around columns of hydroxyl ions. It constitutes approximately 70 wt% of human bones.[1,2] Favorably, synthetic HAp is similar to naturally occurring HAp as shown in crystallographic and chemical studies.[3] This type of calcium phosphate is naturally found in bones and teeth, hence its importance. Synthetic HAp presents high biocompatibility, solubility, bioactivity, but poor mechanical properties. Moreover, some properties can be altered through partial ion substitution in the ionic sites of HAp particles.[4] As demonstrated in different studies, HAp nanoparticles' functionality is affected by their structural properties. Nowadays, with environmental pressure, nanotechnology has been aiming to adopt the green productions approaches[5] green synthesis is a potential alternative to obtain nanoparticles, sustainably, using ecological sources. This study developed a method of synthesizing the green of zirconia (ZrO2. Then, in the synthesis of hydroxyapatite nanoparticles, green routes have been reported as alternatives to conventional methods.[6-9] Therefore, this brief review aims to provide a state of the art regarding the green synthesis of hydroxyapatite nanoparticles and their biomedical applications. Besides that, an outlook of their benefits in biomedical applications will be critically analyzed, with an emphasis on the possible enhancement of properties of hydroxyapatite bonded with plant secondary metabolites.

Hydroxyapatite

Hydroxyapatite is the main inorganic constituent of bones, dentin, and enamel. Studies show that natural HAp has a non-stoichiometric ratio of Ca/P =1.67[10] and has carbonate groups with traces of ions such as HPO_4^{2-}, Na^{1+}, Mg^{2+}, Sr^{+2}, K^{+1}, Cl^{-1}, and F^{-1} in its structure. Marković et al. [11] compared biological apatite (BHAp) from mandible bone with synthetic HAp (CHAp) in their research. Results from Fourier Transform Infrared (FTIR) spectra exhibited that structural (phosphate, hydroxyl, and carbonate) bands were very similar, concluding that both were a mixed AB-type of carbonated apatite. A thermogravimetry/differential thermal analysis (TG/DTA) showed that both apatites have 1 wt% of carbonate in their composition demonstrating that CHAp mimics the composition and crystal structure of biological apatite, proving its potential as a biomimetic agent.

Conventional Synthesis

Several synthetic procedures have been developed and improved to control the HAp's morphology. The nanoparticles' shape, size, and distribution affect their properties, extending or limiting its applications. Rod-like, spherical, irregular, and flake-like shapes prevail as the shapes found in procedures. Desired shapes are often induced by adding organic modifiers to the anisotropic crystals with simpler shapes.[12] The great affinity between the crystals and the additives allows these alterations to happen at the nucleation center.

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To obtain hydroxyapatite nanoparticles, approaches such as dry and wet methods are used. Dry methods do not require the use of solvents, unlike wet methods.[12] However, both methods require reactants containing calcium and phosphate to form calcium phosphates minerals. The hydroxyapatite nanoparticles obtained from dry methods generally present a mixture of products with various shapes. Thus, can be applied for mass production of HAp. Well-known dry methods include solid-state synthesis and mechanochemical processes. During solid-state synthesis, grinned reactants containing calcium and phosphate are calcined at elevated temperatures. The elevated temperature allows the formation of crystals. Resulting crystals exhibit irregular shapes in their phase composition, hence the addition of agents. Common agents added are sulfates, carbonates, alkali chlorides, hydroxides, polyethylene glycol (PEG), and others.[12]

Mechanochemical synthesis offers the simplicity and practicality of solid-state synthesis yet results in crystals with a well-defined structure. Briefly, this synthetic approach consists of grounding in a ball mill (planetary or another type). Additionally, during the milling process, the molar ratio of Ca and P sources is maintained at the stoichiometric ratio (Ca/P is at 1.67).[13] The common milling media used is polymeric.

Wet chemical methods represent the main processes for the synthesis of Hap nanoparticles. The particles produced by these techniques tend to have a better outcome regarding the crystal's size. By altering the processing parameters, the desired features of HAp nanoparticles are achieved. However, low temperatures of the procedure result in lower crystallinity in the powder. Water or organic solvents are usually employed; other chemicals and additives can be additionally incorporated. Common techniques include chemical precipitation, sol-gel method, hydrothermal method, and emulsion synthesis.[14]

Chemical precipitation is the simplest procedure to produce HAp particles. The technique involves the dropping of one reactant over the other under stirring. It usually occurs at a pH higher than 4.2 and at atmospheric temperature. With these conditions, HAp particles are the least soluble and most stable form of CaP.[12] Simple precipitation does not guarantee a well-crystallized powder. Therefore, several additives are used to modify the procedure. Under this approach, various types of macromolecules interact on the crystal surface and readjust their morphology. Surfactants are constantly macromolecules used in which at equilibrium they act as micelles and allow the formation of nucleation centers where crystal growth can be controlled. Commonly used macromolecules include cetyl trimethyl ammonium bromide (CTAB), polyethylene glycol (PEG), ethylenediaminetetraacetic acid (EDTA), ethanolamine, citric acid, and amino acids.[10,12,13]

A traditional procedure for sol-gel synthesis requires a mixture of alkoxides to form a tridimensional inorganic network either using water or an organic compound as a solvent. After the mixtures are prepared, the slow-rate reaction is left for aging, galeated, dried, and calcined. A secondary phase of CaO can form if the aging time is not enough or if the impurities are not removed during the calcination.[13]

During a hydrothermal synthesis, the reaction occurs using water as a solvent and under conditions of elevated pressure and temperature. This procedure produces crystalline particles, although it has a higher cost due to the necessary conditions. Likewise, this technique uses organic additives to improve the crystal's morphology. Calcium chelating agents such as EDTA and other organic surfactants are added.[15]

When two immiscible liquids come in contact, a thermodynamically stable dispersion is achieved. For this reason, an emulsion synthesis is required. When a surfactant is added to the emulsion formed, the surface tension reduces and forms nanosized particles in the dispersion. The commonly used routes are water-in-oil emulsion, oil-in-water emulsion, and water-in-oil-in-water double emulsion. For the stabilization of the dispersion, ionic, nonionic, and block-copolymer surfactants are employed.[16]

A Greener Route

Conventional routes for synthesis use organic solvents and toxic additives to modify the crystals´ morphology and crystallinity. Therefore, due to environmental concerns, is urgent to turn the synthetic methods greener. The search of more ecologically friendly synthetic routes aiming to substitute synthetic organic modifiers rose in the last years. Biological substitutes, known as bioapatites, confer the advantages of producing non-toxic renewable particles with higher biocompatibility at a reasonable cost. Existent green routes follow a similar procedure as their conventional counterparts. The same wet and dry methods are applicable, though there are no organic agents involved (Fig. 1).

Green materials that have been studied include eggshells, red algae, various plant and vegetable extracts, and bamboo membranes[17,18]. The synthesis of hydroxyapatite nanoparticles from biowastes provides two environmental advantages. Firstly, using biowaste as a source of templates turns conventional synthesis into a green synthesis. On the other hand, biowaste-assisted synthesis contributes to increasing the value-added of biowastes. Products such as cuttlefish shells, porcine teeth and bones, bovine bones, and fish scales are considered bio-waste and are a source rich in calcium [18]. Additionally, the HAp nanoparticles obtained from natural resources present antibacterial properties. The hydroxyapatite nanoparticles inhibited two Gram-negative bacteria like *Escherichia coli* and *Klebsiella* spp. on an agar surface[7]. The presence of minerals such as Na^{+}, Mg^{2+}, and Zn^{2+} in some of the extracts from bioapatite is credited with this antibacterial activity.

Similarly to bio-waste-related resources, fruit extracts have been used due to their availability and cost-effectiveness. Hydroxyapatite has been synthesized using malic acid found in apples, tartaric acid found in bananas, tamarind, and grapes, and from banana peels.[2,7,19] Hydroxyapatite crystals obtained from these synthetic routes have different morphologies, sizes, and crystallinity levels which will depend on the method, procedure conditions, and mainly, on its biogenic source. For the HAp particles synthesized using banana extract, agglomerated particles that resembled ice cubes were formed. The length, width, and aspect ratio of the few discrete nanorods formed ranged from 85.92 nm – 192.18 nm, 24.21 nm – 40.12 nm, and 2.43 nm – 6.77 nm, respectively.6 For the particles obtained from grape extract, the rod-like particles exhibited a length that ranged from 110.36 nm – 276.34 nm, width from 22.26 nm – 86.37 nm, and an aspect ratio from 2.86 nm – 8.97 nm. The particles obtained were not uniform. Interestingly, uniform and small HAp particles were obtained using tamarind extract as a template. The length ranged from 162.16 nm – 298.54 nm, width from 26.86 nm – 34.81 nm, and the aspect ratio ranged from 5.38 nm – 9.65 nm.[6]

Differently from bio-waste resources, plant leaf or roots extracts are rarely reported in the scientific literature.[8,20,21] HAp nanoparticles were synthesized using *Azadirachta indica* (NHA) and *Coccinia grandi* (GHA) leaf extracts. The obtained nanoparticles are pure HAp, though they presented poor crystallinity when compared to control HAp (prepared without leaf extract). The HAp (NHA) particles presented a rod-like morphology with a length varying from 40 nm – 80 nm and a width from 30 nm – 50 nm. Similarly, HAp (GHA) particles presented a nanorod morphology with lengths that ranged from 80 nm – 150 nm and a width from 30 nm – 50 nm. For comparison, the control sample exhibited a spherical morphology with a width ranging from 30 nm – 40 nm and a length from 70 nm – 80 nm. In addition, the green synthesized hydroxyapatite nanoparticles inhibited *Escherichia coli* and *Staphylococcus aureus* probably due to the presence of biomolecules such as flavonoids, terpenoids, and proteins in their composition.[22]

In summary, the green synthesis of hydroxyapatite nanoparticles represents an affordable alternative to deal with environmental concerns. Hydroxyapatite nanoparticles synthesis assisted by plant extracts is interesting because superficial biomolecules add new perspective applications, such as antimicrobial.

Biomedical applications

The rapid increase of studies regarding biomaterials is directly correlated to their possible benefits towards human health. Due to its biocompatibility with biological apatite in hard tissue, and high bioactivity, HAp is extensively studied for biomedical applications (Fig. 2). Recently, studies show that HAp exhibits high biocompatibility with soft tissue as well, such as skin, muscles, and gums, playing an important role in regenerative medicine and pharmacology.[23–26]

Figure 1 – Schematic illustration of synthesis methods.

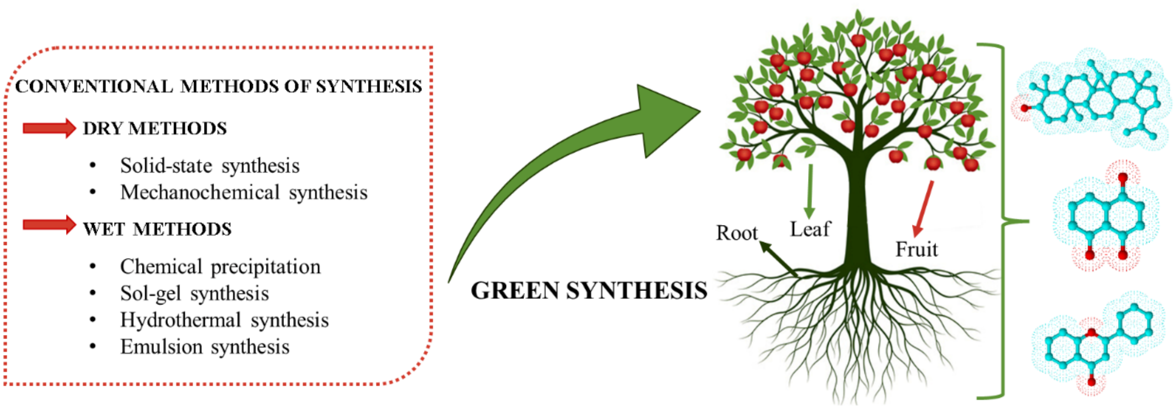
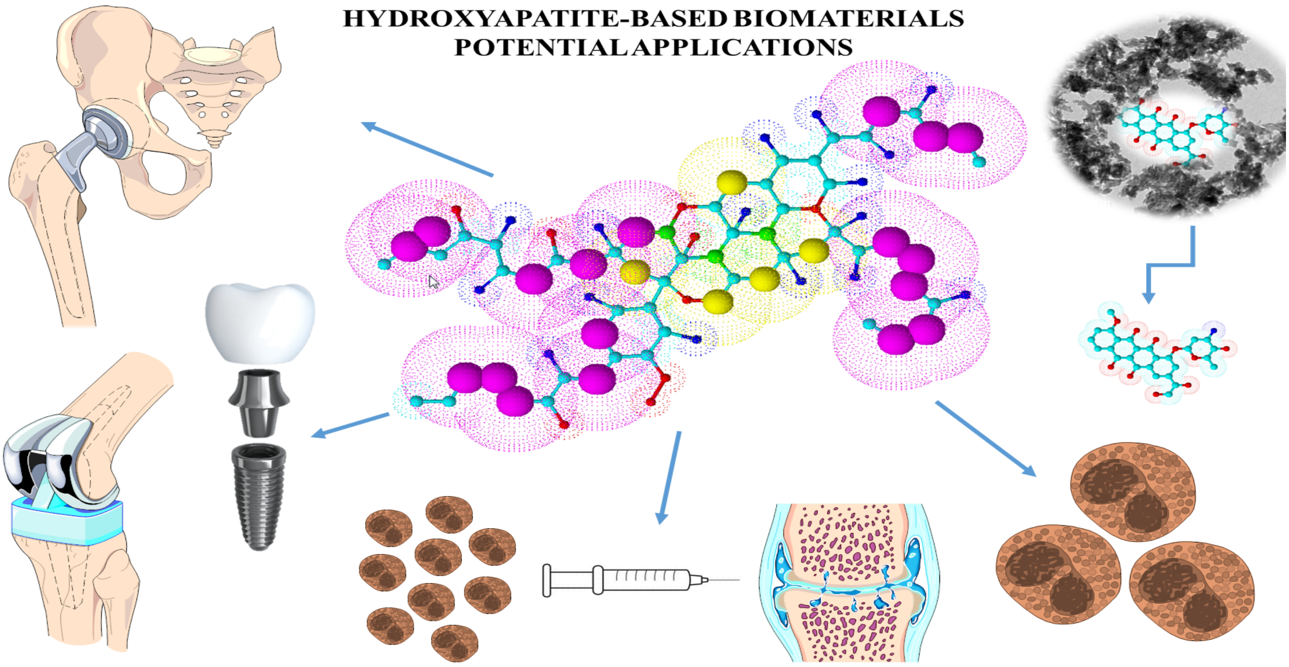


Figure 2 – Schematic illustration of applications in the biomedical field.



The bone is a complex composite material consisting of collagen fibrils reinforced with hydroxyapatite crystals.[13,27] However, it can suffer trauma that can need a repair or a total or partial replacement.[28] Standard hard tissue repair involves the use of autographs and allografts, although these techniques have limited availability. Furthermore the latter increases the risk of infection and rejection.[29] Hence, there is a need for biomaterials that mimic natural apatite.

To recover or improve tissue functioning, a temporary scaffold or matrix is utilized to direct this tissue recovery. Given that synthetic HAp has the potential to mimic the chemical composition and structure of the natural matrix, several studies show its advantage as a substitute.[30] HAp has been used in tissue engineering for bone repair, bone augmentation, coating of implants, and fillers for bones and teeth.[13,27] Rabbits with bone defects that were treated with nano-HAp artificial bone exhibited a callus bonier than those who were treated with HAp artificial bone and those without their defect treated.[31,32]

HAp nanoparticles are a drug delivery system due to their porous nature and larger surface area. They deliver precisely some drugs such as anti-inflammatory drugs, vitamins, hormones, proteins, and anti-osteoporotics drugs.[23,27] HAp has great compatibility with versatile substances, and it was evaluated as a protein drug carrier.[33] Spherical particles of HAp nanoparticles conferred high adsorption of protein and a slow release of it, granting a lasting effect of the drug on the body. Besides, it is known that protein adsorption is caused by electrical interaction and that there is a positive plane containing calcium ions and a negative plane containing hydroxide ions on the crystal surface. The proteins used in the study were bovine serum albumin (BSA–negative charged protein) and lysozyme hydrochloride (LSZ–positive charged protein). The adsorption ratio depended heavily on the particle diameter and the plane area of the crystal. Large particles have smaller specific surface areas whereas small particles have larger specific surface areas. It was noticed that an increase in the positively charged crystal plane increased BSA. Moreover, when the positively charged plane was decreased and the negatively charged one was left unchanged, the adsorption of LSZ was favorable. The particles showed an initial burst during the first 2 days and a sustained release for 2 weeks.[33]

HAp nanoparticles have poor mechanical properties, such as intrinsic brittleness, low fracture toughness, and wear resistance. Modern bioengineering involves the improvement of synthetic HAp nanoparticles through composite materials. The combination of HAp nanoparticles with others such as polymer, silicon, and carbon nanomaterials can aid in the stress transfer from the matrix to the nano-fillers.[34] Conveniently, HAp has the capacity for partial ion substitution, which makes the crystals much smaller and gives an extensively hydrated surface layer.[35]

This approach substitutes PO43– for ionic impurities like Na+, K+, Mg2+, Zn2+, HPO42–, Cl–, F–, and SiO44– that are present in bioapatite.35 For instance, natural bone contains Mg2+ and Zn2+. The former is significant for the calcification process and on bone fragility while the latter promotes osteoblastic cell proliferation and differentiation.[6]

Thus, the use of biomaterials is a valuable tool for biomedicine. These research areas are expanding, as there are still challenges to be overcome, as it is a multidisciplinary field. However, the opportunity to improve quality of life through human health drives scientific research in this area.

An insight into green HAp in future applications for the biomedical field

Green synthesis does not only guarantee a less harmful ecological impact but it can enhance some of HAp's properties. Particular attention can be given to the study and development of composites. Sumathra et al. (2017) developed a composite using HAp particles and pectin, a polysaccharide found in the cell walls of plants. The results obtained showed that the pectin reduced particle size, reduced agglomeration, and generated a spherical form. After observing the effect of the composite on human osteosarcoma cells MG63 using MTT for 7 days, the authors concluded that the MG63 cells can proliferate in the matrix, proving it is non-toxic and is favorable for adhesion. Applying the alkaline phosphate (ALP) enzymatic activity to examine cell differentiation on MG63 osteoblast-like cells on the HAp/pectin matrix showed that the composite favored the cell's ability to differentiate compared to the HAp scaffold.[36]

Furthermore, the green synthesized HAp nanoparticles surrounded or bonded with secondary metabolites from plants might enhance some properties of HAp based biomaterials. While developing a polymer/HAp nanocomposite, some complications arise when the polymer used is apolar, such as poor distribution of filler in the matrix. Nowadays, to overcome this drawback, compatibilizers and silanization of HAp have been employed and they have shown outstanding results.[37,38] We hypothesize that HAp nanoparticles obtained from greener procedures will enhance the interaction with the polymer matrix, being unnecessary the addition of compatibilizers. Moreover, most plant metabolites have antioxidant properties, which come as an advantage because the polymer matrix can be protected from oxidation. Besides, green synthesized HAp nanoparticles have been reported as having antimicrobial properties.[7] For these reasons, we hypothesize that this biomaterial can boost existing applications in the biomedical field.

Conclusions

Hydroxyapatite is one of the most relevant biomaterials in the biomedical field due to its wide range of applications. Conventional methods of synthesis involve the use of toxic and expensive templates such as surfactants. Over the years, substantial progress has been made towards the synthesis of HAp using plant-related templates, which enhance the nanoparticles' properties. Prospects include an easier adhesion of HAp to an apolar polymeric matrix, the increased antioxidant effect obtained from plant metabolites, and improved interactions between HAp and substances in drug delivery systems. Additionally, the optimization of green synthesis aiming to control the morphology of obtained nanoparticles is an interesting area to be addressed in the future. Still, further research is needed, though HAp has presented itself as an attractive option for biomedical applications.

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