Synthesis Methods of Doped Hydroxyapatite: A Brief Review

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Abstract. Hydroxyapatite (HA) has drawn great attention to biomedical applications due to their bone mineral similarity, strong bioactivity, biocompatibility and osteoconductive. Despite the fact that HA has many advantages, several properties are still lacking, emphasising the crucial need for ion doping/substitution. Many attempts have been made to incorporate ions into HA structure to increase their physical, chemical, and biological properties. With such a diverse range of methods available for the synthesis of doped HA, this article discussed the importance of doping for HA and summarizes four common techniques used to prepare doped hydroxyapatites which include precipitation, hydrothermal, sol-gel and mechanochemical method.

1. Introduction

Recovery of bone tissue is one of the most important areas of regenerative medicine, where applications of various types of calcium-based biomaterials are being extensively researched to build new tissue or replace damaged bone. Among the calcium-based biomaterials that have been investigated, hydroxyapatite is the material of choice since it is similar with the main inorganic element of bones and teeth [1]. The pure stoichiometric hydroxyapatite, (Ca₁₀(PO₄)₆(OH)₂, HA) is well known as a bone substitute or replacement materials in dentistry and orthopaedics [2]. HA is chemically and crystallographic similar to bone. Therefore, it may have a significant biological advantage in terms of improved protein and cell adhesion [3]. It is also demonstrated exceptional biocompatibility and bioactivity properties [4, 5], where HA able to integrate with host hard tissues through the formation of direct chemical bonds with the orthopaedic surroundings. Besides that, HA enhance osteoblast activity which lead to the formation of bone and responsible for cell adhesion and stability.

The main inorganic element of bones and teeth, also knowns as biological apatite. In contrast to pure stoichiometric HA, which contains no other ionic impurities, biological apatite is nonstoichiometric,
poorly crystalline, and often contain several ionic impurities, such as CO$_3^{2-}$, Mg$^{2+}$, Na$^+$, Fe$^{3+}$, HPO$_4^{2-}$, F$^-$ and Cl$^-$. Carbonated (CO$_3^{2-}$) ions, in particular, play an important role in the biochemical reactions of bone cells. In regards to that, carbonated hydroxyapatite (CHA) is a nonstoichiometric version of HA which is represented by Ca$_{10}$(PO$_4$)$_6$(CO$_3$)(OH)$_2$. The CHA is produced by incorporating the ions of hydroxyl and phosphate with the carbonate group into structure of HA, thus resulting in A-type and B-type carbonation respectively. The presence of B-carbonates in the HA structure decreases the latter's crystallinity, leading to rise in the solubility of both in vitro and in vivo biomaterials. Other than that, CHA, which is chemically comparable to biological apatite than HA, has demonstrated outstanding biocompatibility, bioactivity, and resorbability [6]. Furthermore, CHA is a great option for any bone-related use, treatment, and therapy due to its nonstoichiometric, low crystallinity, and nanosized properties [7].

Therefore, doping HA with various types of ions has been another alternative for improving the material properties of HA. The choice of cations that are used to dope is determined by the similarity of their high-coordination crystal radius to that of Ca$^{2+}$. Some of the common types of ions that have been substituted to HA are Ag$^{+}$, Sr$^{2+}$, Mn$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Zn$^{2+}$, Mg$^{2+}$, Zr$^{2+}$, Eu$^{2+}$, and Te$^{2+}$ [7]. Thus, the objective of this paper is to review the methods used to synthesise doped hydroxyapatite.

2. The importance of doping for hydroxyapatite

Hydroxyapatite (HA) commonly has been known as bioactive, biocompatible, osteoconductive and chemically similar with bone mineral. Despite of these advantages, there are still some characteristics that are lacking, highlighting the critical necessity for doping. One of the important factors that contributed to doping is HA lacks of the prerequisite criteria for a material design for bone tissue engineering applications which is angiogenesis properties [8].

Besides that, doping also able to reduce the high degree of crystallinity structure of HA. This is because high degree of crystallinity prevents resorption during bone formation which also led to high brittleness and low fracture durability [1]. The inclusion of carbonates in the HA structure reduces the crystallinity of the material, resulting in an increase in the biomaterial’s in vitro and in vivo solubilities, with a higher presence of neoformed bone [9]. CHA exhibits higher bioactivity than HA, and stronger tissue-implant interactions will inevitably result from the smaller particle size of CHA.

Moreover, based on past research, substitution of cerium ions had proved that its presence increases the solubility of HA which indirectly improved the biodegradability and antibacterial properties [10]. In addition to that, doping silicate into HA also increases bone density and mineralization [11]. This is because during the completion of calcination, the silica content decreases drastically. Another benefit of doping is that it helps to prevent metallic implant failure due to infections, corrosion, fatigue, and poor osteointegrability, all of which jeopardise the healing process despite their widespread usage as fixation media in orthopaedics [12].

3. Synthesis methods of doped hydroxyapatite

As interest in doped hydroxyapatite grow, numerous synthesis methods have been used to produce HA. However, the preparation of doped HA with specific properties remains difficult due to the possibility of toxic intermediary products being formed during the synthesis of HA [13]. The processes involved in the synthesis of substituted HA are critical in order for this material to be generated with a controlled particle size, shape, chemical composition and degree of crystallinity [14]. Precipitation, hydrothermal, sol-gel, and solid-state synthesis are among of the most prevalent methods for producing doped HA.

3.1 Precipitation

The precipitation technique is the most widely used and researched method for synthesis of doped HA. Wet precipitation, chemical precipitation, and aqueous precipitation are named upon this technique. This method is commonly used due to its ease of use and ability to generate a wide range of particle sizes and morphologies. In this process, two or more compounds are precipitated simultaneously in a solvent
due to supersaturation. Also, precipitation method consists of mixing, stirring, aging, filtering, drying and calcination. Phosphate solution is added dropwise into a stirring calcium solution [15]. Besides, addition of ammonium hydroxide is necessary to maintain the alkalinity of the resultant mixture. Moreover, in order to prevent the formation of a secondary phase in HA and the elevated calcining temperature to form the crystalline HA, precipitation technique requires high pH value. Some common types of ions doped into HA using precipitation method are \( \text{Mg}^{2+}, \ \text{Co}^{2+}, \ \text{Sr}^{2+}, \ \text{Li}^{+} \) and \( \text{Zn}^{2+} \) [16-20] as shown in Table 1.

Table 1. Doped HA using precipitation method.

| No | Author & Year | Ions Doped | Stirring/Aging Time | Washing Solvent | Drying/Calcination | Remarks |
|----|---------------|------------|---------------------|----------------|-------------------|---------|
| 1  | Kanasan N et al. 2018 [16] | \( \text{Mg}^{2+} \) | 4h/24h at room temp | DI Water | 80°C for 24h | The peak intensity and adsorption bands have decreased due to the doping of magnesium. |
| 2  | Nagyné-Kovács T et al. 2018 [17] | \( \text{Sr}^{2+} \) | 24h at room temp | DI Water | 80°C for 12h | Lattice parameters and the unit cell volume of Sr-doped HAs increased slightly. |
| 3  | Wang Y et al. 2016 [18] | \( \text{Li}^{+} \) | 1h at 60°C/48h at room temp | Distilled Water | 80°C | Li-HA show higher degradation rate than pure HA. |
| 4  | Kulanthaivel S et al. 2016 [19] | \( \text{Co}^{2+} \) | 80°C/24h at 35°C | Distilled Water | 55°C for 24h | Co-HA is biocompatible and osteogenic. |
| 5  | Ofudje E A et al. 2019 [20] | \( \text{Zn}^{2+} \) | 24h | DI Water | 100°C for 24h | The doping causes a decrease in the crystallite size of the HA with an increase in zinc ions concentrations. |

3.2 Hydrothermal

Hydrothermal is a process that create crystalline substances at high vapours pressure from a hot aqueous solution where it utilizes single or heterogeneous phase reactions in aqueous at elevated temperature of \( T > 25^\circ\text{C} \) [21-24] and pressure, \( P > 100 \text{ kPa} \) to crystallize ceramic materials directly from solutions. Hydrothermal method commonly used in the synthesis of doped HA due to its good repeatability and crystallinity control [21]. This method also produces fine HA powders [22-24]. In addition, this method is often used to establish high temperature and high-pressure roughness on the surface of the substrate. Furthermore, as hydrothermal pressure or temperature is increased, phase purity and the Ca/P ratio of the precipitates improve [25]. Also, the change in the solvent and reactant properties at high temperature could indirectly control the experimental variables. Table 2 shows the examples of ions doped into HA using hydrothermal method.
Table 2. Doped HA using hydrothermal method.

| No | Author & Year | Ions Doped | Stirring/Aging Time | Washing Solvent | Drying/Calcination | Remarks |
|----|---------------|------------|---------------------|-----------------|-------------------|---------|
| 1  | Pal A et al. 2018 [22] | Sr$^{2+}$ | Autoclave for 130℃ at 8h | DI Water         | 80℃              | Sr doping affect the crystallinity, size and cell parameter of HA. |
| 2  | Rafique M et al. 2018 [23] | TiO$_2$ | Treated in vessel for 6h at 130℃ | -               | 80℃ for 24h       | The sample retained sufficient properties to be used in more advanced applications. |
| 3  | Moreno-Perez B et al. 2020 [24] | Si$^{4+}$ | Teflon vessel using convection furnace at 150℃ for 10h | DI Water         | Freeze Drier      | Si ion promotes a small but significant crystalline and size reduction for the powder of the prepared samples. |

3.3 Sol–gel

Numerous papers have proved that sol-gel method can be used to synthesise doped HA [26-29]. The sol-gel synthesis route is a method of converting reactants into gels at any stage of the reaction scheme. The sol-gel method produces homogeneous molecular mixing of calcium and phosphorus precursors, maintaining a low processing temperature, and does not necessitate long hydrolysis times for the formation of nano crystalline powders [25]. The production of HA nanoparticles via traditional sol–gel process involves the preparation of a 3D inorganic network by mixing alkoxides (or other suitable precursors) in either an aqueous or organic phase, proceeded by aging at room temperature, gelation (due to increase in temperature), drying on a hot plate, and finally organic residues removal from the dried gel by calcination [25]. Table 3 shows lists of research works that use sol-gel method to synthesise doped HA.

Table 3. Doped HA using sol gel method.

| No | Author & Year | Ions Doped | Stirring/Aging Time | Washing Solvent | Drying/Calcination | Remarks |
|----|---------------|------------|---------------------|-----------------|-------------------|---------|
| 1  | Singh J et al. 2015 [26] | Mg$^{2+}$ | 70℃ for 1h          | DI water         | 22℃ for 24h/70℃ for 24h | There is a growth of apatite on the surface of Mg-HA. |
| 2  | Predoi M et al. 2018 [27] | Zn$^{2+}$ | 100℃ for 48h        | DI water, Ethanol | -                 | The non-destructive ultrasonic method is suitable to characterize undiluted bioceramic gels. |
| 3  | Phatai P et al. 2018 [28] | Ce$^{3+}$ & Ce$^{4+}$ | 25℃ for 30min | DI water         | 100℃/600℃ for 2h | The substitution of Ca$^{2+}$ with Ce$^{3+}$ and Ce$^{4+}$ increased the lattice parameters. |
| 4  | Kamonwannasit S et al. 2020 [29] | Cu$^{2+}$ & Ag$^{+}$ | 0.5h | DI water         | 100℃/600℃ for 4h | Cu-Ag Doped Ha possess excellent antibacterial activity against Gram-positive and Gram-negative bacteria. |
3.4 Mechanochemical method

Mechanochemical methods are solid-state techniques that are used to synthesise phase-pure and ion-doped HA as shown in Table 4 [30-32]. This technique is quick and easy to use, and it does not require the addition of any solvents or organic surfactants during the process. Ball milling is a commonly used in this procedure, in which the powder mixture is placed in a ball mill and subjected to high energy collisions from the balls, resulting in chemical processing and transformation using mechanical force. Generally, the method involves the mixing calcium and phosphate precursor with the various amount of dopant. The preparation process is carried out in the typical setting at room temperature and at natural atmospheric pressure. It is also energy intensive and needs high temperatures compared to the precipitation method, but it can fix aggregation. Different type if ions has been doped into HA using the solid-state method as shown in Table 4.

| No | Author & Year | Ions Doped | Milling time | rpm | Ball to powder weight ratio (BPR) | Remarks |
|----|---------------|------------|--------------|-----|----------------------------------|---------|
| 1  | Fakharzadeh A et al. 2017 [30] | Ag⁺ | 3 hours | 600 | 15: 1 | The synthesized sample in the absence and presence of different levels of dopant showed a high tendency to agglomerate. |
| 2  | Fahami A et al. 2016 [31] | Cl⁻ & F⁻ | 5 hours | 1060 | 10:1 | Structural features influenced by the ions doped and bioactivity is confirmed with apatite formation. |
| 3  | Jia B et al. 2020 [32] | Li⁺ | 60 minutes | 1725 | 10:1 | Crystallinity of HA significantly decreases with the increase of dopant fraction. Undoped and low-doped samples consist of spheroidal particles, whereas the high-doped samples demonstrated a high tendency to agglomerate. |

4. Conclusion

The data presented in this paper provides evidence of the growing interest in ion doping to improve biological performances of HA. According to the review, the traditional precipitation method is the most commonly employed due to its convenience of use and the ability to form various size of particles. Much effort remains to be done to improve the biocompatibility of HA. Plant-derived organic compound can be considered as a new alternative substitution element to improve biocompatibility and antibacterial properties of HA for use in medical applications.

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