Development of Zinc Doped Hydroxyapatite for Bone Implant Applications

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ABSTRACT

Hydroxyapatite (HA) has widely been used as bone substitutes and as coatings on metallic prostheses because of its good biocompatibility and bioactivity. In order to improve bioactivity of HA, hydroxyapatite has been doped with zinc. It was also proven that doping zinc into calcium phosphate structure gave better mechanical strength of calcium phosphate implants. In this research, zinc-doped hydroxyapatite powder was synthesized through sol-gel method. The obtained powders were physically characterized using FESEM, XRD, FTIR, TG/DTA, and particle size analyzer. In this method, ammonium solution, EDTA, Ca(NO$_3$)$_2$.4H$_2$O, (NH$_4$)$_2$HPO$_4$ and urea have been used as the raw materials and Zn(NO$_3$)$_2$.6H$_2$O as dopant with the concentrations of 2, 5, 10, and 15 mol%. Calcination temperatures were varied between 500 – 900°C. It was shown that synthesized Zn-doped HA powders resulted in high purity after calcination up to 700°C, and TCP started to appear after 800 ºC calcination. The highly crystalline powders had been obtained for the calcination temperature above 700°C. Moreover, FTIR analysis shown that the bands of HPO$_4$ increased meanwhile O-H decreased with the increasing zinc doping. Individual particle s become larger with the increased calcination temperature and the decreasing concentration of zinc doping.

Keywords: Hydroxyapatite, Zinc doped, Sol-gel method, Synthesis, Characterization

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1. INTRODUCTION

Hydroxyapatite (HA; Ca_{10}(PO_4)_6(OH)_2), a synthetic material analogous to calcium phosphate found in bone is considered for orthopedic and dental applications. This biomaterial is highly biocompatible and presents bioactive properties. HA physicochemically bonds to bone and promotes bone formation necessary for implant osseointegration. This property of osseointegration is needed to minimize damages to surrounding tissues and to increase the implant efficiency [1]. Biological and physicochemical properties of HA can be improved by the substitution with ions usually present in natural apatites of bone. Most natural apatites are non-stoichiometric because of the presence of minor constituents such as cations (Mg^{2+}, Mn^{2+}, Zn^{2+}, Na^+, Sr^{2+}) or anions (HPO_4^{2-} or CO_3^{2-}). Trace ions substituted in apatites can have effect on the lattice parameters, the crystallinity, the dissolution kinetics and other physical properties of apatites [2]. Synthesis of Zn substituted hydroxyapatite is of major interest because biological tissues like bone and teeth are composed with hydroxyapatite containing zinc. Zinc is present in small amount in the enamel of human teeth and in bone. This element is probably the most important element in medicine because of its role in as much as 200 enzymes [3].

The present study in this research has focused on the effect of doped zinc on the microstructure and properties of HA. The doped hydroxyapatite can be produced using Sol-Gel method. Sol-Gel technique has been developed and employed to prepare various materials because it has main advantage of easy control of chemical composition and low temperature synthesis that are very important for thin film formation. The advantages of sol-gel technique are numerous; it results in a stoichiometric, homogeneous and pure coating due to mixing on the molecular scale; reduced firing temperatures due to small particles sizes with high surface areas and it has the ability to produce uniform fine-grained structures [4].

2. BASIC THEORY

Hydroxyapatite is chemically similar to the mineral component of bones and hard tissues in mammals. These materials can be classed as bioactive, meaning that it will support bone ingrowths and osseointegration when used in orthopedic, dental and maxillofacial applications. In 1981, an application for the first time shows that Hydroxyapatite (HA) has no toxicity, no inflammatory response, no pyrogenetic response, no fibrous tissue formation between implant and bone and also the ability to bond directly to the host bone. Moreover, it is a permanent implant material, thus eliminating the need of replacement after implantation. The chemical nature of hydroxyapatite lends itself to substitution, meaning that it is not uncommon for non-stoichiometric hydroxyapatites to exist. The most common substitutions involve carbonate, fluoride and chloride substitutions for hydroxyl groups, while defects can also exist resulting in deficient hydroxyapatites [5].

Hydroxyapatite has the unique characteristics that make it very suitable and compatible for implantation with human body. When implanted into the body the synthetic implant is accepted by the body and because of its porous nature allows normal tissue integration to take place. Most people tolerate solid hydroxyapatite implants very well. The body accepts the implant and bone and soft tissue ingrowths throughout the implant takes place immediately after implantation. Solid hydroxyapatite implants are permanent. It is thought that due to the porous nature of these implants they lack strength but this is possibly made up for by the ease of bone tissue growth into the pores of the implant once inserted. Over time they are partially resorbed and replaced by natural bone.
Bone substitute and resorbable implants can be produced when hydroxyapatite and other calcium phosphate variants are used as the ceramic phase. These foams have a good ability to sustain and encourage the growth of human cells; pore sizes of 100-200 μm allow osteoblasts to grow over and into the pores, leading to osteoid formation that can mineralize within the pores. Processing conditions that result in the presence of ~1 μm diameter microporosity in the pore walls are important for effective cell attachment and ingrowth and both the microporosity and the macroporosity have been found to affect the cell morphology and degree of cellular infiltration. In vitro cytotoxicity tests have revealed that the purity of biomedical-grade hydroxyapatite powder is not compromised by the reagents employed or the process route itself [6, 7].

3. METAL DOPED HYDROXYAPATITE THEORY

Hydroxyapatite (HA) \([\text{Ca}_{11} \left(\text{PO}_4\right)_6(\text{OH})_2]\) is a bone-like ceramic used as a coating for dental and orthopedic implants. It is well known for its good cytocompatibility properties, but is limited in use due to its high solubility within the body and mechanical properties that differ from surrounding tissue and bone [8]. The mineral part of bone is composed of carbonated and nonstoichiometric HA structure containing substitutions of \(\text{Na}^+, \text{K}^+, \text{Mg}^{2+}, \text{Sr}^{2+}, \text{Cl}^-, \text{F}^-, \text{HPO}_4^{2-}\) ions originated in the extracellular matrix-mediated process of mineralization found in in vivo body fluid medium. HA is capable of accepting substitute ions within its lattice [9]. The present in vitro study investigated a variety of dopants as a way to further enhance the good cytocompatibility properties of HA as well as address these problematic properties. The dopants investigated were divalent (magnesium and zinc) and trivalent (yttrium, lanthanum, indium, and bismuth) ions [8]. Thus, to enhance the mechanical properties of HA, those metals are doped to HA to substitute into its structure.

4. ZINC DOPED HA THEORY

With its good properties of biocompatibility and bioactivity hydroxyapatite (HA) is highly used as bone substitutes and as coatings on metallic prostheses. In order to improve the bioactive properties of HA, Zn\(^{2+}\) doped hydroxyapatite has been elaborated. Zn\(^{2+}\) ions substitute for Ca\(^{2+}\) cations in the HA structure.

HA doped with 5% of Zn dissolves more rapidly than pure HA or HA doped with low concentrations of Zn. Moreover, this concentration of Zn leads to the formation of a Ca-P layer which contains Mg. This layer is created in a biological environment and represents bioactive properties of the bioceramic. The Ca-P layer bridges chemically the bone and the implant. The reactions between hydroxyapatite and bone, and dependence of these reactions on the hydroxyapatite composition are important in bioactivity mechanisms. This literature demonstrates an increase of bioactive properties of HA doped with 5% of Zn [10]. Zinc plays an important role in proliferative effects on osteoblastic cells apart from inhibiting osteoclastic resorption. Zinc-substituted calcium phosphate has thus received considerable attention. In addition, hydroxyapatite solubilizes high fractions of Zn, approximately 15%mol10, which is a necessary condition for delivering efficacy. The possibilities of increasing Zn releasing rate by controlling the surface area and/or phase transformation are a promising means to achieve such purposes [9].
Morphology of the powders was determined using transmission electron microscopy. TEM results revealed that the calcination of Zn doped powders at 500 °C resulted into agglomerated nano HAp powders having particle size in the range of 20-50 nm in diameter. In particular, the doping with Zn$^{2+}$ ions appears to be very efficient as an advantage of using soft chemistry routes, like sol-gel process. On SEM micrograph, we observe a hydroxyapatite particle with micropores (Figure 1). All the prepared hydroxyapatites exhibit the same porous morphology [11].

Phase analyses were carried out using powder X-ray diffraction technique. The XRD pattern of non-calcined HA-granules showed good correlation with stoichiometric hydroxyapatite (JCPDS 09-0432 card), with a small shift to higher angles, probably due to zinc substitution. The non-calcined Zn-granules were characterized as low-crystalline hydroxyapatite since the diffraction peaks have low intensity and are relatively broad [9]. In this case, zinc affects the structure when it partially replaces calcium in the hydroxyapatite crystal sites as illustrated in Figure 2.

![Figure 1: SEM micrograph of a hydroxyapatite doped with 5% of Zn (HAZn5) [11]](image1)

![Figure 2: X-ray diffraction patterns of samples with and without calcination: a) HA granules; b) Zn-granules [9]](image2)
As for infrared spectroscopy, the spectra of HA-granules and Zn-granules (Figure 3) present similar bands, though, with different relative intensities. For the Zn-granules treated at 1200 °C, we did not clearly identify β-tricalcium phosphate (β-TCP) bands, albeit they were revealed by XRD. A possible reason for that could be the sample region covered by each technique, i.e., XRD is a bulk technique whereas diffusion reflectance FTIR obtains information especially from the surface of samples [9].

![Figure 3: Infrared spectroscopy spectra: (a) HA-granules; and (b) Zn-granules [9].](image)

5. **SOL-GEL METHOD THEORY**

In Sol-Gel technique, calcium nitrate and triethyl phosphite were used as starting materials. These chemicals were dissolved in distilled water, separately, under vigorous stirring. As-prepared calcium nitrate sol was added drop wise into the hydrolyzed phosphate sol and then aged and dried. Dried gel was then crushed into fine white powders with the help of mortar and pestle and a measured amount of metal oxide powders were added to the crushed amorphous powders, separately. Calcination was carried out at 250-500°C. These green structures were sintered at 1300°C for 6 hours in a muffle furnace for densification. Highest sintered density of 3.29 g/cc was measured for metal-doped powder.

The advantages of sol-gel technique are numerous; it results in a stoichiometric, homogeneous and pure coating due to mixing on the molecular scale; reduced firing temperatures due to small particles sizes with high surface areas; it has the ability to produce uniform fine-grained structures; the use of different chemical routes (alkoxide or aqueous based); and their ease of application to complex shapes with a range of coating techniques those being dip, spin, and spray coating. The lower processing temperature has another advantage; it avoids the phase transition (~1156 K) observed in titanium based alloys used for biomedical devices.
6. EXPERIMENTAL PROGRAM

6.1 Flow of Zinc doped HA preparation via sol-gel method

6.2 Materials and Procedures

For preparation of zinc doped hydroxapatite powders, there were several materials used in sol gel technique such as calcium nitrate tetrahydrate (Ca(NO₃)₂·4H₂O) from Merck, di-ammonium hydrogen phosphate ((NH₄)₂HPO₄) also from Merck, zinc nitrate hexahydrate from UNI-CHEM, urea from DULAB, EDTA (titriplex) from Merck and ammonium solution (NH₄OH) from B&M Chemical. Calcium nitrate tetrahydrate and di-ammonium hydrogen phosphate (reagent grade) were used as calcium and phosphorus precursors, respectively. Zinc nitrate hexahydrate was used as the dopant source which is Zinc. Urea (analytical grade) was used as gelling and ammonium donor and EDTA was used as chelating agent to prevent an immediate precipitate formation of calcium ions in the course of gel formation. The reaction had been conducted in basic solution using ammonium solution as solvent.

To produce 25 grams of Zn-doped HA, the first step is synthesis; 250 ml of ammonium solution (11%) was prepared and heated to the temperature of 60°C. Then, in a heated ammonium, 91 grams EDTA as gelling agent was added while stirring until it dissolves. The clear solution was obtained and the aqueous solution of 65 grams calcium nitrate tetrahydrate and zinc nitrate hexahydrate were poured into it, followed by 20 grams di-ammonium hydrogen phosphate and 22.6 grams urea subsequently added. The mixture was then heated at
100°C while stirring and synthesized for 3 to 4 hours. In this solution, under stirring, urea was solubilized too. In this way urea decomposition occurs in a very homogeneous system in term of ions concentration, and pH. This was reflux process at temperature of 100°C until a while gel was obtained. Nitrate ions removal involved a white gel phase formation passing through a transparent sol phase in one reactor only, to set at zero possible contamination. The obtained gel was then dried at +340°C for 3 hours under ambient static air and the black gel was obtained. The gel was subsequently subjected to 900°C calcinations for 3 hours under flowing air. The resultant solid of white powder was crushed using a mortar and pestle into a fine powder. All of these steps were then repeated for 2%, 5%, 10% and 15% Zinc doped Hydroxyapatite with different calcination temperature; 500°C, 600°C, 700°C and 800°C. Then, these fines Zinc HA powders had been assessed by the characterization examination involving the testing with X-ray diffraction, Fourier Transformed InfraRed Spectrometer (FTIR), Differential Thermal Analysis (DTA), Particle size nanosizer and Field Emission Scanning Electron Microscopy (FESEM).

7. EXPERIMENTAL PROGRAM

7.1 Experimental Results of phase analysis by XRD

XRD can be used to determine the particle or grain size of the powders. The grain size can be calculated using the Scherrer’s equation below:

\[
W_{\text{FWHM}} = \frac{k\lambda}{D \cos \theta} \cdot \frac{180}{\pi} \quad \text{or} \quad D = \frac{k\lambda}{W \cos \theta} \cdot \frac{180}{\pi} \quad (7.1)
\]

where, \(W_{\text{FWHM}}\) is the full width as half maximum of the peak (in degrees 2\(\theta\)) calculated automatically by the XRD 6000 software, \(D\) is the grain size, \(K\) is a constant generally equated as 0.94, and \(\lambda\) is the radiation wavelength. Table 1 below shows the values of particle size of Zn-BCP nano powders calculated from the Equation 7.1 at various percentages of zinc doped and temperature.

<table>
<thead>
<tr>
<th>Particle size of powders (nm)</th>
<th>5% Zn-doped</th>
<th>10% Zn-doped</th>
<th>15% Zn-doped</th>
</tr>
</thead>
<tbody>
<tr>
<td>500°C</td>
<td>10.52</td>
<td>10.21</td>
<td>11.24</td>
</tr>
<tr>
<td>600°C</td>
<td>11.52</td>
<td>12.10</td>
<td>11.87</td>
</tr>
<tr>
<td>700°C</td>
<td>25.70</td>
<td>24.83</td>
<td>23.44</td>
</tr>
<tr>
<td>800°C</td>
<td>37.97</td>
<td>38.31</td>
<td>36.94</td>
</tr>
<tr>
<td>900°C</td>
<td>50.10</td>
<td>49.24</td>
<td>44.13</td>
</tr>
</tbody>
</table>

From the results of particle size of zinc doped HA powder; the calculated values for Zn-BCP powders nanosize at different calcinations temperature demonstrate the pattern that as increasing the temperature, the size of particle increases. Larger particle size indicates that the
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particle is more crystalline as derived in the Scherrer’s equation theoretically, narrow width of well-defined XRD peak of $W_{FWHM}$ illustrate the crystallinity in powder particle. For the effect of percentage of zinc doped to the particle size, these results show in Table 2 are not in synchronize pattern, however, the particle size of powder is likely to decrease as the zinc doped increases particularly at high calcination temperature. These results of particle size are compared to the result obtained in XRD pattern Figure 5 show the XRD pattern of 5%, 10% and 15% Zn-doped BCP at different calcination temperature. According to the XRD patterns the TCP phase was identified at the peak about 31 degree. This is showing the BCP phase was produced which is the particle of HA and TCP rather than purely HA. The low temperature of calcination samples were characterized as low crystalline structure since the diffraction peaks have low intensity and are relatively broad compared to higher calcination temperature [11]. With increasing calcination temperature, crystal structure started appearing. It is observed that though that phase started appears at a fairly low temperature, but complete crystallization was achieved when the powder was calcined at 800ºC as seen in Figure 5. Increasing the calcination temperature will increase the ratio of TCP phase over the HA as the TCP peaks stated appearing at the calcination temperature of 800ºC for 15% Zn doped and at temperature of 700ºC for 5% and 10% Zn doped.
Theoretically, the phase of zinc oxide is located at the intensity of 36.3º (2θ). The Zinc Oxide element is observed to appear in the X-ray analysis of the Zn-BCP at the 10 and 15 mol% concentration of dopant (Zn) as shown in Figure 6.

7.2 Results of material evaluation and identification by FTIR

As for infrared spectroscopy, the comparison had been made between the Zn-BCP calcined at 900°C, black gel and pure BCP as illustrated in Figure 7. Obvious differences can be observed for black gel and powder samples spectra as the black gel present undefined bands because of its amorphous structure. Meanwhile, the spectra of pure BCP and Zn-BCP present similar bands though, with some different relative intensities. These results can be attributed to small variations in the sample surface compositions. The bands of HPO₄²⁻, O-H, and P-O were identified. The analysis showed that carbonate apatite present in the sample composition and CO₃ contributes the OH and PO₄ band groups.
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Figure 7: Infrared spectroscopy spectra of samples at 900°C calcination temperature of BCP gel, 0, 2, 5, 10 & 15 mol% Zn-BCP

The FTIR analysis of 5%, and 10% and 15% Zn doped HA with different calcination temperature were obtained as Figure 8. The first and small peaks related to C-O vibration band of CO$_3^{2-}$ suggest that some carbonate was incorporated during low temperature processing. Therefore, when the samples were calcined at 900°C, these vibration bands decreased and nearly disappeared. Figure 8 also indicates that as the temperature increase, the bands of HPO$_4$ at 840 cm$^{-1}$ and 1400 cm$^{-1}$ decrease and O-H at 620 cm$^{-1}$ increase. This is because HA materials show more crystallinity as the HPO$_4$ band disappears and O-H band appear in the FTIR analysis.
Figure 8: Infrared spectroscopy spectra a) 5 mol% Zn doped; b) 10 mol% Zn doped and c) 15 mol% Zn doped calcined at 500ºC-900ºC

7.3 Results of particle size by particle nanosizer

The analysis of the nanoparticle size of the zinc doped BCP structure for different calcination temperature and different percentage of zinc doped had been examined by considering particle distribution in the powder.

Figure 9(a) shows the particle size analysis for 15%, 500ºC Zn-BCP. The large portion of size distribution with the function of intensity is at diameter of 2238 nm and of 86.2% of the particle size distribution. Meanwhile in Figure 9(b), the particle size distribution is 100% at the diameter of 190.3 nm for 15% Zn-BCP at 900ºC. Furthermore, 94.1% of 2% Zn-BCP particle had distributed at particle size of 1322 nm and the remains is at particle size of 83.76 nm diameter as illustrated in Figure 9(c).

From all of the plots, it can be examined that, as the temperature increase, the plot is shifted to the left indicate the large mean size average in small value of nano size range of the particle distribution. We conclude that the particle size become larger as increasing the calcination temperature. Figure 9(c) shows that as decreasing the percentage of zinc dopant, the plot is also shifted to the left. Therefore, decrease in percentage of zinc will increase the particle size.
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Figure 9: particle size distribution of a) 15 mol%, 500ºC; b) 15 mol%, 900º; and c) 2 mol%, 900ºC of Zn-BCP

7.4 Results of morphological evaluation by FESEM

Figure 10 shows surface morphology of 15% Zn-BCP with 500ºC of calcination, 15% and 2% Zn-BCP which were calcined at 900ºC. FESEM observations of powders reveal that the powders were the agglomerate of fine particle. The average particle dimension are 70.16nm, 106.58nm and 121.6nm for 15% Zn-BCP subjected to 500ºC and 900ºC and 2% Zn-BCP subjected to 900ºC calcination respectively. This indicates that the particle size become larger as the calcination temperature is increased and as the percentage of zinc doped is decreased. These measured dimensions of nanoparticle powders of Zn-BCP can be compared to the analysis made in the XRD characterization and particle size calculation that had been discussed in the previous results.
8. CONCLUSIONS

Various concentration of zinc doping in Zn-BCP was successfully synthesized by using Sol-gel method which produce the biphasic structure of HA and TCP. XRD analysis had been performed by particle size calculation and peak patterns which explained that crystallinity of powder increases upon the increasing calcination temperature. Moreover, the ratio of TCP over HA had been increased by increasing the calcination temperature. From the FTIR analysis of the calcined powders, we analyzed a typical structure containing sharp O-H, P-O, HPO$_4$ and CO which was some of the structures present in the Zn-BCP structure. As increasing temperature, O-H band appeared and HPO$_4$ disappeared due to the increasing of the crystallization. These results can be compared to the previous results of XRD and FTIR which the decomposition of HPO$_4^{2-}$ led to the more crystallinity of the sample. The size distribution of the Zn-BCP was successfully determined by using zeta nanosizer. In FESEM evaluation, it is shown that the particles of Zn-BCP agglomerates are globular in shape while the individual particles have an average size of 70-130 nm in diameter. Furthermore, an increase in the mol fraction of Zn ions doped into HA made the agglomerated particles smaller and increasing the calcination temperature will make the particle larger. From these result, we can conclude that sol-gel method has the ability to generate nano-size Zn-doped HA powder at low cost of raw materials and low processing temperature. Most of the powders produced exhibited highly crystalline characteristics. Therefore, we can conclude that the objectives of this research to produce and characterize zinc doped hydroxyapatite have been successfully achieved.
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