Synthesis and characterization of sol-gel derived Hydroxyapatite-Bioglass composite nanopowders for biomedical applications

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Abstract

The main purpose of this study is to prepare and characterize hydroxyapatite (HA)–10%wt bioglass (BG) composite nanopowders and its bioactivity. Composites of hydroxyapatite with synthesized bioglass are prepared at various temperatures. Suitable calcination temperature is chosen by evaluating of the phase composition. X-ray diffraction (XRD), Transmission electron microscopy (TEM) and Scanning electron microscopy (SEM) techniques are utilized to characterize the prepared nanopowders. The bioactivity of the prepared composite samples is evaluated in an in vitro study by immersion of samples in simulated body fluid (SBF) for predicted time. Fourier transformed infrared (FTIR) spectroscopy and inductively coupled plasma (ICP) are used for evaluation of apatite formation and the bioactivity properties. Results show that HA-BG composite nanopowders are successfully prepared without any decomposition of hydroxyapatite. The suitable temperature for calcination is 600°C and the particle size of hydroxyapatite is about 40-70 nm. The apatite phase forms after 14 days immersing of the samples in SBF. It could be concluded that this process can be used to synthesize HA-BG composite nanopowders with improved bioactivity which is much needed for hard tissue repair and biomedical applications.

1. Introduction

Bioactive ceramics, such as bioglass and hydroxyapatite (HA), have been developed recently. A great deal of research has been focused on hydroxyapatite owing to its chemical and structural analogy to bone and teeth [1]. As a matter of fact, HA shows good biocompatibility with human body but its applications are limited to non-load bearing areas due to its low mechanical properties [2]. The rate of HA resorption also is very low. Therefore, composites could be a subject of interest in order to achieve a combination of properties and overcome the main limitation of hydroxyapatite [3]. Recently, many of the researchers have been focused on adding other ceramics to hydroxyapatite [4]. One of the ceramic additions could be bioactive glasses [5]. They are highly bioactive ceramics and their bioactivity has been demonstrated with the formation of a strong bonding with the adjacent bone. It is proposed that the presence of the bioactive glassy phase can provide faster response in terms of bone healing and bonding processes [6]. Along with hydroxyapatite, bioactive glasses could potentially be used as an additive due to considerable bioactivity performance [7]. It is also observed that glass-reinforced HA composites exhibit greater biological activities than HA [6].
In order to obtain a HA-BG composite with fine grains, it is necessary to prepare nanoparticles with a low sintering temperature. In addition, nanostructured HA is expected to have better mechanical and bioactivity properties in comparison with conventional HA used in implant systems. Also, the presence of the nano grains and high volume fractions of grain boundaries in HA has been found to increase osteoblast adhesion, proliferation, and mineralization [8-10].

Recently, the sol–gel process has been used in the preparation of ceramics because of its intrinsic advantages over other conventional processes [10]. The high surface area of dried gels results in high reactivity which permits a low process temperature. Finally, the process allows the preparation of a homogeneous mixture of HA and BG nanoparticles [11].

The aim of this research was preparation and characterization of HA–BG composite nanopowder and evaluation of the bioactivity. In this study, the effects of bioglass addition into HA on bioactivity properties were also investigated. Moreover, it was expected that addition of these ceramics with reduced grain size lower than 100 nm could improve the properties of the composite.

2. Material and Methods:

2.1. Preparation of composite nanopowders

At first, bioactive glass was synthesized. Starting materials used in this preparation were analytical grade Tetraethyl orthosilicate (TEOS, Merck), Triethyl phosphate (TEP, Merck), Ca(NO$_3$)$_2$.4H$_2$O (Merck), ethanol and hydrochloric acid (Merck).

The composition of prepared bioactive glass belongs to the system SiO$_2$-CaO-P$_2$O$_5$ with the composition 45S. Bioactive-glass nanopowders were prepared by sol-gel method described by Mehdikhani et al. [12]. Briefly, Ethanol was used as a solvent to obtain nanopowders. The chosen volume ratio of ethanol to TEOS was two. Proper amounts of 2N hydrochloric acid, deionized water, and TEOS were dissolved in ethanol, and were stirred at room temperature for 30 min. TEP was then dissolved into the provided acid silica sol. After 20 min of stirring, the calcium precursor was added into the acid sol. The solution was then stirred for an additional hour. The obtained gel was dried at 50°C for 48 hours and 100°C for 48 hours. Finally, the dried gel nanopowder was calcined at 600°C for 2 hours.

At the stage of composite nanopowder Hydroxyapatite-bioactive glass preparation, a designed amount of phosphorus pentoxide (P$_2$O$_5$, Merck) and calcium nitrate tetrahydrate Ca(NO$_3$)$_2$.4H$_2$O, Merck) was dissolved in absolute ethanol to form a Ca/P molar ratio 1.67 [13]. 10%wt prepared bioglass powder was added to HA sol. Prepared sol was stirred by magnetic stirrer at room temperature for 24 hr to transform homogenous sol to gel. The provided gel is aged and composite gel is oven dried at 80°C. Finally, the dried gel nanopowder was calcined in furnace at different temperatures 500, 600, 700, and 800°C.

2.2. Composite nanopowder characterization

Phase characterization of HA-BG composite nanopowders was performed using the X-ray diffractometer (XRD) (Philips X’Pert-MPD, Cu K$_\alpha$ radiation at 30 mA and 40 kV, over the 20 range of 20–80 degree at a scan rate of 3°/min). The experimental patterns obtained were compared with the standards compiled by the Joint Committee on Powder Diffraction and Standards (JCPDS), which included card # 09-0432 [14] for HA.

The crystallite sizes of the composite nanopowders were determined using broadening of XRD pattern peaks and Scherer’s equation (Eq. (1)) [15].

$$D = \frac{0.89\lambda}{B\cos\theta}$$  \hspace{1cm} (1)

Where D is crystallite size (nm), $\lambda$ is the wavelength of the X-ray beam, B is the width of the peak in the middle of its height, and $\theta$ is Bragg’s angle (degree). The crystallinity of HA was estimated from the XRD data using Eq. (2) [16]:

$$C = \frac{100\times\frac{I_{200}}{I_{110}}}{1+\frac{I_{200}}{I_{110}}}$$  \hspace{1cm} (2)

Where C is crystallinity, I$_{200}$ and I$_{110}$ are the peak intensities of (200) and (110) diffraction peaks, respectively.
\[ X_c = 1 - \left( \frac{V_{112/300}}{I_{300}} \right) \]  

(2)

in which, \( X_c \) is the degree of HA crystallinity, \( I_{300} \) is the intensity of the (300) peak, and \( V_{112/300} \) is the intensity of the shoulder between the (112) and (300) diffraction peaks.

A scanning electron microscope (SEM: Philips XL30) and a transmission electron microscope (TEM: Philips CM200) were utilized to evaluate the morphology and particle size of the synthesized HA-BG composite nanopowders.

In order to evaluate the bioactivity behaviour, the prepared composite nanopowders were immersed in a simulated body fluid (SBF) at 37°C temperature for 14 and 28 days. After that, the samples were removed from the solution and the dissolved Ca\(^{2+}\) and P ion concentration were measured by means of inductively coupled plasma (ICP) and the functional groups of powder were analysed with Fourier transformed infrared (FTIR) spectroscopy (Tensor 27, Bruker). The spectra were recorded from 4000 to 400 cm\(^{-1}\) wave number with a resolution of 2 cm\(^{-1}\).

3. Results and discussion

3.1. Powder characterization

Figure 1 shows an XRD pattern of prepared HA-BG composite nanopowder with 10wt% of bioactive glass which was obtained by sol–gel method after sintering at 600°C. The presence of hydroxyapatite peaks in accordance with No: 09-0432 files could be seen. As clearly observed, it is free of additional phases such as CaO and \( \beta-\)TCP and constituent phase (HA) was stable. Figure 2 shows the effect of sintering temperatures on the formation of composite nanopowder. The sintering temperatures perform a considerable role on the formation of composite nanopowders. It was found that at calcination temperature of 600°C, the dominate phase of the powder was hydroxyapatite (HA) without any decomposition of hydroxyapatite (HA). The peak intensities corresponding to additional crystalline phases were increased as the temperature increased. Therefore, it causes the decomposition of hydroxyapatite into the undesirable phases such as \( \beta-\)TCP and CaO. Therefore, the desired temperature was determined to be 600°C.

![X-ray diffraction pattern of prepared composite nanopowder sintered at 600°C.](image-url)
Figure 2. XRD patterns of the composite nanopowders obtained after calcination at various temperatures.

The degree of crystallinity influences the dissolution and the biological behaviour of HA composites. The measured HA crystallinity of HA-BG composite nanopowders are shown as Table 1. The results also showed that the crystallite size of the HA is less than 100 nm which is in agreement with the TEM micrograph (Figure 3) observation.

Figure 3. TEM micrograph of HA-BG composite nanopowders calcined at 600°C.
Table 1. Calculated crystallite sizes and HA crystallinity of the prepared HA-BG composite nanopowders, after calcination at different temperatures.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Crystallite size (nm)</th>
<th>Crystallinity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T500</td>
<td>43nm</td>
<td>42</td>
</tr>
<tr>
<td>T600</td>
<td>51nm</td>
<td>71</td>
</tr>
<tr>
<td>T700</td>
<td>60nm</td>
<td>72</td>
</tr>
<tr>
<td>T800</td>
<td>75nm</td>
<td>71</td>
</tr>
</tbody>
</table>

Figure 4 shows the SEM micrograph of the powder particles after heat treatment at 600°C. Some of the factors that might affect the agglomeration of particles are the tendency of the system to minimize the total surface area and the size of particles produced. According to the TEM micrograph, the particle sizes of the composite powder are below the 100 nanometer which is resulted in its high surface area to volume ratio and agglomeration.

Figure 4. SEM micrograph of HA-BG composite nanopowders showing the morphology and particle size of the powders.

3.2. Bioactivity evaluation

Composite nanopowder also was immersed in the SBF under the physiological condition of pH 7.4 at 37°C for a period of 14 and 28 days for in vitro evaluation. Figure 5 shows the FTIR spectra of HA-BG composite sample sintered at 600°C after 14 and 28 days immersion in SBF. The FTIR spectrum shows all characteristic absorption peaks of composite nanopowder. The peaks at 550–2974 cm⁻¹ range can be recognized as peaks relative to PO₄³⁻ ions. The peaks assigned to hydroxyl groups (OH⁻) and CO₃²⁻ in the hydroxyapatite could be observed at 3458.25, 2013.61, 636.48, respectively. The bands at 500-1000 cm⁻¹ (740.078, 817.552, and 1049.22) correspond to Si–O bands in SiO₄. In addition to FTIR analysis, calcium and phosphorous concentration in the SBF solution were evaluated and determined by ICP method. The ion concentration variation of these
ions show the obtained composite nanopowder had bioresorption due to its high surface area to volume ratio. It was reported in the previous study [17] that the release of Si and Ca ions into SBF medium and exchange with H\(^+\) in the SBF cause to increase of pH. According to this phenomenon, the negatively charged surface was formed which was resulted to attracting Ca ions to the interface between the powder and solution. Presence of adequate ions on the composite nanopowder causes nucleation and growth of the apatite on the surface of composite nanopowder. In addition, the variation of calcium and phosphorous concentrations in the SBF confirm the formation of calcium-phosphate layers. Finally, it can be concluded that prepared composite nanopowder possess apatite-formation ability and is bioactive. Production of nanoparticles instead of microparticle in this composite could be beneficial for higher bioactivity with optimized mechanical strength [6]. In other words, it could be a potential candidate for hard tissue engineering applications. It is expected that this composite with regard to their desirable bioactivity might be a suitable candidate for fabrication of composite scaffold with specific features such as both enough mechanical and bioresorptioonal properties.

Figure 5. FTIR spectra of the synthesized composite nanopowders, after immersion in simulated body fluid for 14 and 28 days.

4. Conclusions
Homogeneous composite nanopowders of hydroxyapatite/10wt% Bioglasses were synthesized by adding bioglass to hydroxyapatite sol. Calcination temperature was 600°C, because the composite powders calcined at this temperature exhibited suitable crystallinity and phase properties. All the powders studied consisted of big agglomerates composed of nanoparticles. HA-BG composite nanopowders of ~40–70 nm in size were prepared. By applying this process, the HA-BG composite nanopowders for various biomedical applications were successfully synthesized.
References

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