Rapid microwave assisted synthesis and characterization of nanosized silver-doped hydroxyapatite with antibacterial properties

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A B S T R A C T

Synthesis of nanosized particles of silver-doped hydroxyapatite (Ag-HA) as an antibacterial agent is of potential importance in managing infections associated with surgical implants. However, the process of synthesizing this agent is time consuming and inefficient. To overcome this problem, a rapid method for synthesizing nano-sized Ag-doped hydroxyapatite (Ag-HA) [Ca10-xAg x(PO4)6(OH)2] (x = 0.03–0.5) using microwave irradiation for 10 min at 800 W is proposed. The materials were characterized using XRD, FTIR, FESEM and EDX. Antibacterial behavior of the Ag-HA samples were evaluated using disc diffusion technique and results demonstrated good activity against Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and Eschericha coli.

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1. Introduction

Hydroxyapatite is widely used as a synthetic bone graft, drug carrier and metal prosthesis coating [1–2]; however; it exhibits poor antibacterial activities [3]. Long-term in vivo performance of HA can be affected by the presence of various bacterial infections resulting in debilitating pain and often results in removal of implants [4,5]. To overcome these problems, the use of antimicrobial agents, including metal ions (Ag⁺, Cu²⁺, Zn²⁺ and Ti⁴⁺) in hydroxyapatite is proposed [6–8].

Among various ions, silver ions have long been recognized to have strong inhibitory and bactericidal effects as well as a broad range of antimicrobial activities. Silver-based antimicrobials capture much attention, because of the low toxicity of the active Ag ion to human cells as well as being a long lasting biocide with high thermal stability and low volatility [9,10]. In recent days silver ions are most commonly used to control bacterial growth in a variety of medical applications, including dental work, catheters, and healing burn wounds [11]. Although various methods to synthesize silver into HA such as sol–gel or co-precipitation have been reported [12,13], it has disadvantages: it is expensive, toxic, long reaction time and difficulty in controlling the reaction. All these factors have effects on HA [12,14]. Microwave assisted synthesis of bioceramics has gained attention due to the simplicity [15–18]. However, to our knowledge, there are no reports describing the use of microwave refluxing on synthesizing nanosized Ag-doped hydroxyapatite. In this study, HA powders composed of various Ag concentrations (0.3, 1 and 5 wt%) were prepared, characterized and examined for antibacterial activity.

2. Materials and methods

2.1. Preparation

The reagents for synthesis were purchased from Q.Rec™ and used without further purification.

Wet-chemical precipitation method for synthesis of Ag-HA was expressed as follows:

\[
(10 - x)\text{Ca}^{2+} + x\text{Ag}^+ + \text{PO}_4^{3-} + 2\text{OH}^- \rightarrow \text{Ca}_{10-x}\text{Ag}_x(\text{PO}_4)_6(\text{OH})_2 \quad (0 \leq x \leq 0.5)
\]

Calcium and silver nitrate solution was prepared in (Ca + Ag): P molar ratio ~ 1.67 (Table 1). pH adjusted to 10 using ammonium hydroxide and ultrasonicated for 1 h. Diammonium hydrogen phosphate (0.6 M, pH 10) was added drop wise and stirred for 30 min and refluxed in microwave oven (Samsung; MW71B) at 800 W, 2.45 GHz, for 10 min. The suspension was filtered, washed, dried at 80 °C for 24 h. All samples were heat treated at 900 °C.
2.2. Characterization

XRD patterns were recorded on Bruker D8 Advance X-ray diffractometer operated at 40 kV and 40 mA with Cu-Kα radiation. Data was collected from 2θ range of 20–60° with step size of 0.05.

The size and morphology of the sample were studied using JEOL JSM-6700F SEM. Elements in the sample were analyzed by EDX coupled with FESEM and the functional groups were identified by FTIR Bruker (optic GmbH) ALPHA-T, using classic KBr pellet technique. Spectra were recorded over the region 400–4000 cm⁻¹ with scan rate 4 cm⁻¹.

Table 1
Sample ID, corresponding wt(%) and amounts of reagents added.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Added to 100 ml deionized water (g)</th>
<th>Ag wt(%)</th>
<th>Concentration (M)</th>
<th>Amount (g)</th>
<th>Concentration (M)</th>
<th>Amount (g)</th>
<th>Added</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>23.618</td>
<td>0</td>
<td>0.3</td>
<td>23.548</td>
<td>0.000294</td>
<td>0.05</td>
<td>0.3</td>
</tr>
<tr>
<td>1Ag-HA</td>
<td>23.38</td>
<td>0.02</td>
<td>0.98</td>
<td>23.38</td>
<td>0.02</td>
<td>0.17</td>
<td>1</td>
</tr>
<tr>
<td>5Ag-HA</td>
<td>22.438</td>
<td>0.06</td>
<td>0.96</td>
<td>22.438</td>
<td>0.06</td>
<td>0.84</td>
<td>5</td>
</tr>
</tbody>
</table>

Fig. 1. SEM images of microwave processed samples (x 50,000 magnification, bar = 100 nm) and EDX spectra of samples. Note: (a) HA, (b) 0.3Ag-HA, (c) 1Ag-HA and (d) 5Ag-HA.
2.3. Antibacterial activity

The antibacterial activities of HA with and without Ag were evaluated against Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli using agar disc-diffusion technique. Bacteria were obtained from Institute of Medical Research (IMR), Kuala Lumpur. Nutrient agar media was swabbed with respective organisms \((n=4)\) \((1 \times 10^8\) CFU/ml). Four discs in each plate were fixed at equal distance and incubated at 37°C for 24 h. The antibacterial activity was measured as zone of inhibition (cm) around the disc.

2.4. Ion release test

A quantity of 0.1 g of silver substituted HA was suspended in 20 ml phosphate buffer saline (pH 7.2) and shaken for 6 and 12 h at 37°C. The Ag concentrations in the solution were measured by AAS (Perkin Elmer AAnalyst 400).

3. Results and discussion

FE-SEM (Fig. 1a) demonstrates the non-homogeneity of the nanosized HA and Ag-doped HA particles which varies with the amount of Ag. The HA and 0.3Ag-HA particles (Fig. 1a and b) are seen as non-uniform agglomerates of elongated spherical shapes. Particle size were 70 nm and 74 ± 20 nm, respectively, \((n=30)\). Particles of 1Ag-HA showed less aggregation and broader size distribution (particles: 80 ± 18 nm, \(n=30\), Fig. 3c) while samples of 5Ag-HA showed smaller agglomerates with the larger particle size of 85 ± 20 nm \((n=30)\). The elemental composition determined by EDX (Fig. 1a–d) confirms the presence of Ag (Silver), O (Oxygen), P (Phosphorous) and Ca (Calcium) in all preparations.

The XRD patterns for HA and Ag-HA samples (heat treated at 900°C for 2 h) showed the characteristic peaks of HA located at 25.91°, 28.94°, 31.78°, 32.19°, 32.93°, 34.1°, 39.80°, 46.71° and 49.49°. The observed XRD peaks of HA diffraction patterns are in accordance with the diffraction pattern ICDD 09-432 corresponding to crystalline HA (Fig. 2a). Only the samples containing...
Ag-HA preparations affect the growth of microorganisms (Fig. 4). The O–H peak at 3569 cm$^{-1}$ corresponds to the adsorbed water and a slight shift of the OH$^-$ peak at 3672 cm$^{-1}$ was observed in the peaks associated with OH$^-$ and PO$_4$$^2-$ bands in the FTIR spectra for 0.3Ag-HA (Fig. 3b) indicating that Ag substitution to HA resulted in the inhibition zone of 0.5, 0.9, 0.8 and 1 cm, respectively. 5Ag-HA showed inhibition of 1.1, 0.9, 0.9 and 1.2 cm towards S. aureus, B. subtilis, P. aeruginosa and E. coli, respectively. HA alone has no antibacterial activity [19]. Therefore we can conclude that the inhibition of bacterial growth around the disc is due to the release of silver ions from HA lattices into the surrounding medium. The mechanism consists of silver ion interaction with proteins and enzymes of bacteria. As a result, structural damages occurred within cell wall and bacterial membrane. Particularly, silver effects on bacterial DNA and RNA and thereby prevents bacterial reproduction [10,18].

The silver ion test showed the concentration of Ag in PBS at 0.14, 0.21 and 0.29 ppm for 0.3Ag-HA, 1Ag-Ha and 5Ag-HA respectively at 6 h, while at 12 h the concentration of Ag was measured at 0.18, 0.28 and 0.35 ppm for 0.3Ag-HA, 0.5Ag-Ha and 1Ag-HA, respectively. This demonstrates that Ag ions are released slowly from the Ag-HA nanoparticles over time but produces accumulative concentrations.

1 cm for S. aureus, B. subtilis, P. aeruginosa and E. coli and for 1Ag-HA resulted in the inhibition zone of 0.5, 0.9, 0.8 and 1 cm, respectively. 5Ag-HA showed inhibition of 1.1, 0.9, 0.9 and 1.2 cm towards S. aureus, B. subtilis, P. aeruginosa and E. coli, respectively. HA alone has no antibacterial activity [19]. Therefore we can conclude that the inhibition of bacterial growth around the disc is due to the release of silver ions from HA lattices into the surrounding medium. The mechanism consists of silver ion interaction with proteins and enzymes of bacteria. As a result, structural damages occurred within cell wall and bacterial membrane. Particularly, silver effects on bacterial DNA and RNA and thereby prevents bacterial reproduction [10,18].

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4. Conclusion

A rapid microwave assisted process for the preparation of nanosized silver-doped hydroxyapatite has been described. XRD, FTIR and FESEM-EDX techniques have shown the presence of silver preparations without a high degree of agglomeration. 0.3Ag-HA was found to be the best concentration without altering the HA structure and thermal stability. Antibacterial activity results show that silver doped hydroxyapatite is active against most common gram-positive (S. aureus, B. subtilis) and gram negative (P. aeruginosa and E. coli) bacteria making it useful for clinical applications.

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References