Facile and simple synthesis of silver-doped hydroxyapatite porous microspheres with good sphericity

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Abstract
Silver-doped hydroxyapatite (Ag-HA) porous microspheres were synthesized by one-step hydrothermal route at 180 °C for 10 h in the presence of L-glutamic acid (Glu) as a growth regulator. The effects of Glu concentration and silver ion doping on the phase and morphology of the products were thoroughly characterized by X-ray diffraction and scanning electron microscopy. Results show that the presence of Glu induces the growth of hydroxyapatite crystals from flower-like aggregates to porous microspheres, and the addition of silver ions can maintain the roundness of hydroxyapatite microspheres. The Brunauer–Emmett–Teller analysis, particle dispersity and antibacterial property were also conducted. The optimal parameters for porous silver-doped hydroxyapatite microspheres with uniform morphology was obtained with the atomic molar ratio \( n(\text{Ag})/n(\text{Ag} + \text{Ca}) = 0.01 \) and Glu concentration at 0.06 M.

1 | INTRODUCTION

Hydroxyapatite (HA) is a kind of biomedical ceramic material. As an important inorganic component of human skeleton and teeth, HA has good biological performance and it is the most widely used biomaterials [1–3]. Porous microspheric HA can be used as bone filling materials, drug carriers etc., because of its special morphology, which has larger specific surface area and better fluidity. Meanwhile, it can improve the survival rate of cells and reduce toxic and side effects of human body [4–6].

At present, the main preparation methods of HA microspheres include high temperature melting method, microemulsion method, spray drying method, template method and so on [7, 8]. However, single structure and single performance microspheres cannot meet the clinical needs, and the pore morphology is not ideal. Many organic substances are easily adsorbed on the surface of HA microspheres, favouring the adsorption of bacteria and thus leading to implant-related infections. In order to improve the antimicrobial properties of HA microspheres, Cu2+, Zn2+ and Ag+ ions have been used to replace Ca2+ in HA microspheres, by using various doping methods, such as electrostatic spraying, thermal decomposition, chemical decomposition etc. [9–11]. However, most of these methods are complex and costly. Therefore, simple, efficient, energy-saving and environmentally friendly processes to synthesize porous HA microspheres with certain antibacterial properties become a focus of materials research.

In this work, a facile and economical method is reported to prepare silver-doped HA (Ag-HA) porous microspheres.
The phase composition and morphology of the products synthesized by adding different concentrations of Glu macromolecule and different doping amounts of silver ions were observed under the condition of fixed reaction time and temperature, providing experimental basis for the preparation of ion-doped HA porous microspheres to meet different performance requirements. The synthesized Ag-HA porous microspheres are expected to be used in sustained-release drug delivery systems, tissue engineering, protein adsorption, separation and purification, and bone graft substitutes.

2 | MATERIALS AND METHODS

Primarily, Ca(NO$_3$)$_2$·4H$_2$O and (NH$_4$)$_2$HPO$_4$ solutions were prepared according to Ca/P = 1.67 and mixed with 1 mol/L urea solution. The molar ratio Ca/urea is kept at 0.2. The solution pH was adjusted to 3.5 by dilute nitric acid with the volume ratio of nitric acid and DI water 1:1 after adding different concentrations of Glu. Doped HA samples, Ca$_{10-x}$Ag$_x$(PO$_4$)$_6$(OH)$_2$, with $x$ = 0.1, 0.3 and 0.5 (Ag$_x$HA), were achieved by adding different concentrations of AgCl aqueous solutions under magnetic stirring and setting the atomic ratio of Ag/ [Ag + Ca] at 1%, 3% and 5%. Then the solutions were poured into 100 mL cylindrical stainless steel autoclave reactors lined with Teflon, followed by simultaneous heat-treatment at 180 $^\circ$C for 10 h. Finally, the resultant precipitates were washed with deionized water and absolute ethanol, and dried at 80 $^\circ$C. The names and experimental conditions of the samples synthesized are shown in Table 1. S0 is a blank sample without adding Glu and AgCl aqueous solutions.

X-ray powder diffraction (XRD, D8 Advance, 40 kV, CuK$\alpha$ radiation, $\lambda$ = 1.5404 Å) and scanning electron microscopy (SEM, JSM-6510LV, 5 kV) were performed to analyse the phase and the morphology of the products, respectively. The specific surface area and particle dispersity of S2, S4, S5 and S6 samples were determined by the Brunauer–Emmett–Teller (BET, Micromeritics ASAP2460) and laser diffraction particle size analyser (LS13320), respectively. Antibacterial property of S0 and S4 samples towards Escherichia coli ($E. coli$) were evaluated by disc diffusion method [12].

### TABLE 1 Experimental conditions of synthesized samples

| Sample ID | Concentration of Glu [M] | pH | $n$(Ag)/$n$(Ag + Ca) |
|-----------|--------------------------|----|---------------------|
| S0        | 0                        | 3.5| /                   |
| S1        | 0.03                     | 3.5| /                   |
| S2        | 0.06                     | 3.5| /                   |
| S3        | 0.12                     | 3.5| /                   |
| S4        | 0.06                     | 3.5| 0.01                |
| S5        | 0.06                     | 3.5| 0.03                |
| S6        | 0.06                     | 3.5| 0.05                |

3 | RESULTS AND DISCUSSION

3.1 | Effect of Glu concentration on phase and morphology of products

During the hydrothermal reaction, the solution pH slowly increased with the hydrolysis of urea above 80 $^\circ$C, leading to the nucleation and growth of HA crystals. Figure 1 shows the XRD patterns by comparison with the standard spectrum of HA (JCPDS No. 09–0432) in the lowest bar chart and a typical SEM image of S0. The obtained product is pure HA and the strongest diffraction peak is (300). The product contains a 3D structure formed by irregular fibre arrays.

Figure 2 displays the XRD spectrum of the samples prepared by 180 $^\circ$C hydrothermal treatment for 10 h after adding different concentrations of Glu (S1-S3) with the PDF card of standard HA. All the peaks can be readily indexed to HA and no impurity peaks such as CaCO$_3$ are found in the products, implying that the addition of different amounts of Glu has no adverse effect on the phase purity. Compared with the XRD patterns of the products without adding Glu (Figure 1), the strongest peak shifts from (300) to (211), indicating that the preferred orientation of the crystal has changed and the morphology of the samples will change significantly [13–15]. In addition, it is noted that the more amount of Glu, the weaker the (300) intensity and the stronger the (002) diffraction intensity.

Figure 3 is the SEM images of the products prepared by hydrothermal 180 $^\circ$C treatment for 10 h after adding different concentration of Glu (see samples S1–S3 in Table 1). Evidently, the addition of Glu causes changes in the morphology of the produced HA particles. With a small amount of Glu added, urchin-like crystals with irregular short fibres emitted from the centre are formed (shown in Figure 3). In continuation, the morphology evolved into homogeneous and regular-shaped porous microspheres, in consistent with the XRD results (Figure 3(b)). Some original porous microspheres grow much larger and eventually reach an average diameter of $\approx$50 μm. However, further increase of the Glu concentration to 0.12 M (Figure 3(c)), results in the formation of irregular flower-like aggregates. Therefore, the optimum Glu concentration for the HA porous microspheres with uniform rules was 0.06 M.

3.2 | Effect of Ag$^+$ addition on the phase and morphology of the products

Typical XRD patterns of the products prepared by hydrothermal 180 $^\circ$C treatment for 10 h after adding Ag$^+$ with different concentrations (see samples S4–S6 in Table 1) are shown in Figure 4(a), with enlarged $2\theta$ degrees from 25$^\circ$–35$^\circ$ in Figure 4(b). No silver compounds were detected except that a strong peak caused by CaCO$_3$ appeared with $2\theta$ = 29$^\circ$ in Ag$_{0.1}$HA and Ag$_{0.5}$HA samples, suggesting that Ag substitution into HA has taken place to produce a relatively pure material.
Since carbonate is produced with the hydrolysis of urea during the hydrothermal process, the incorporation of a certain amount of CO$_3^{2-}$ in Ag-HA crystallites is inevitable [16, 17]. As the doping amount of Ag ions increases, all the diffraction peaks slightly shift to lower 2θ values. It is because that the ionic radius of Ag (1.15 Å) is larger than that of Ca (0.99 Å), resulting in the expansion of the crystal lattice [18, 19]. This result is in accordance with previous studies, which reported that the incorporation of ions with larger ionic radii than Ca$^{2+}$ will increase the lattice parameters [20, 21]. Therefore, the XRD patterns illustrate that the Ag ions have successfully replaced the Ca ions to form Ag-doped HA, rather than just a surface modification.

Figure 5 is the SEM images of the products prepared by hydrothermal 180 °C treatment for 10 h after adding different amounts of Ag$^+$, named S4–S6. With the addition of a relatively low concentration of silver ions (Figure 5), regular spherical microspheres with good roundness and a large average size ($\approx$50 μm) were observed. In addition, the surface of the microspheres shows porous structure, and the holes are connected with each other. As the increased addition of Ag$^+$ doping was continued (Figure 5(b)), the obtained particles are not spherical and the size of the microspheres tends to decrease. The surface of the microspheres is compact, the pore size decreases, and the connectivity between the pore and the pore decreases. More flower-like aggregates appear and the size uniformity of the microspheres turn weaker with the diameter sizes range from 10 to 110 μm. Further increase the Ag$^+$ doping to $n$(Ag)/$n$(Ag + Ca) = 5%, the sample is no longer regular spherical, but petal and urchin-like (Figure 5(c)). Considering the preparation of silver ion doped HA porous microspheres having a predominance of uniform microspheres, the optimal parameter when $n$(Ag)/$n$(Ag + Ca) = 0.01. It is established that the optimal concentration of silver ions for preparing well-rounded Ag-HA doped HA microspheres with a uniform size in this work is when the atomic ratio of Ag/[Ag + Ca] is 1%.

### 3.3 BET analysis

The specific surface area, pore volume and pore size of the synthetic samples are summarized in Table 2. Correspondingly, the nitrogen adsorption–desorption isotherms and the pore size distributions are shown in Figure 6. According to the International Union of Pure and Applied Chemistry [22], the

### Table 2: The surface analysis data of synthesized samples

| Sample ID | BET surface area [m²/g] | Pore volume [cm³/g] | Pore size [nm] |
|-----------|-------------------------|---------------------|---------------|
| S2        | 35.4294                 | 0.1557              | 17.5841       |
| S4        | 21.9466                 | 0.0700              | 12.7668       |
| S5        | 6.5513                  | 0.0373              | 22.7900       |
| S6        | 13.0450                 | 0.0586              | 17.9854       |
data shown in Figure 6 suggests that the obtained micro-
spheres exhibit similar isotherms of type IV with type H3
hysteresis loop deriving from sheet and block nano-apatite
grains aggregate with slit-shaped pores, indicating a typical
mesoporous structure with good pore accessibility. In Table 2,
S2 has the highest specific surface area of 35.4294 m$^2$/g and
this value was reduced with the increased amount of silver ions.
Amongst the Ag doped samples, S4(Ag$_{0.1}$HA) has the highest
BET surface area (21.9466 m$^2$/g) and the lowest pore size
(12.7668 nm).

3.4  Particle dispersity

Particle size distribution results of the S2, S4, S5 and S6 micro-
spheres are shown in Figure 7. It is found that the main particle
sizes of the four samples range from 30 to 200 μm. Besides,
several peaks appear in all the final products which is in accor-
dance with the SEM results, indicating that the particle sizes of
the microspheres are not uniform. The optimal concentration
of Ag ions to obtain Ag-HA microspheres with a relatively uni-
form and large size in this work is when the atomic molar ratio
3.5 Antibacterial property

To testify the antibacterial property of the Ag doped samples, antibacterial properties against *E. coli* Gram negative strain of HA and Ag$_{0.1}$HA microspheres powder compressed disc samples are tested. As is shown Figure 8(a,c), especially their enlarged focused images in Figure 8(b,d) it is clear to see that Ag$_{0.1}$HA sample has better antibacterial activity against *E. coli* with an evident inhibition zone. However, pure HA sample has no inhibition zone as is expected. Therefore, Ag ions play a significant role in the antibacterial activity. Ag ions can release from HA lattices to its surrounding medium, in this way, an inhibition zone of bacterial growth occurs around the disc [23].

3.6 Formation mechanism

Based on the experimental data and above discussion, we propose a possible formation mechanism of the well-developed Ag-HA porous microspheres with a uniform size controlled by Glu under a one-step hydrothermal route. It is generally believed that the effect of acidic amino acids on crystal growth is related to their electrostatic and chelate interactions [24]. At the initial stage of Ag-HA nucleation, metastable octacalcium phosphate (OCP, Ca$_8$H$_2$(PO$_4$)$_6$·5H$_2$O) first nucleates, then hydrolyses into Ag-HA crystal [25]. The carboxyl groups in Glu are preferentially adsorbed on the (100) surface of OCP. When the (100) surface of OCP is transformed into the (100) surface of HA, the adsorption behaviour still exists, which hinders the growth of HA crystals on the *a* and *b* planes [16]. The strong chemical bonding between Glu and HA surface is another factor that hinders the growth of Ag-HA, leading to petal-shaped microspheres eventually. In addition, accompanied by the hydrothermal reaction, silver ions enter the crystal lattice. Urea decomposes and releases OH$^-$, then the pH value of the solution gradually increases to alkaline and the microspheres nucleate and grow. Compared to other methods of fabricating Ag-HA microspheres, this process is advantageous because it is simple and efficient. It is conducted without toxic organic solvents, making the recovery of spherical particles without sintering processes possible. Moreover, the obtained microspheres present a large and uniform size, and there is no need for subsequent fractionation by sieving. The prepared microspheres are porous with a large size, promising to be used as effective carriers in the field of biomedicine.

4 CONCLUSION

Porous Ag-HA microspheres with an average diameter of $\approx$50 µm are successfully synthesized through a facile and one-step hydrothermal route. Results indicate that a similar phase can be achieved with the addition of Ag dopant. However, the main
diffraction peaks uniformly shifted to the left a little bit because Ag ions incorporated into the HA crystals. Besides, Glu played a vital role in regulating the morphology of the prepared products during the formation process of porous Ag-HA microspheres. BET analysis and particle dispersity show that Ag0.1HA sample has the highest BET surface area (21.9466 m²/g), lowest pore size and best particle size distribution. Also Ag doped HA microspheres achieve good antibacterial activity against *E. coli* Gram negative bacteria. Overall, the optimal concentration of Glu and Ag for the well-developed microspheres with regular morphology is when the Glu concentration is around 0.06 M and the atomic ratio of Ag/[Ag + Ca] is kept at 1%. Such Ag-HA material with porous structure and high sphericity can be highly dispersed and easily operated when used as drug carriers, filling materials and separation media. In future work, the uniformity of size distribution will be improved and biological behaviour both in vitro and in vivo of such porous Ag-HA microspheres will be conducted.

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