Glutamic acid-assisted synthesis of zinc-doped hydroxyapatite porous microspheres

Mei-li Qi\textsuperscript{1,a}, Fengce Mei\textsuperscript{1,b}, Fengkun Cui\textsuperscript{1,c,*}, Mei-li Qi\textsuperscript{2,d}, Liang Qi\textsuperscript{3,e}

\textsuperscript{1}School of Traffic and Civil Engineering Shandong Jiaotong University Ji’nan 250357, China
\textsuperscript{2}School of Materials Science and Engineering, Shandong University, Ji’nan 250061, China
\textsuperscript{3}Shandong Chaoyue Data Control Electronics Co., LTD, Ji’nan 250100, China
\textsuperscript{a}qimeili@sdjtu.edu.cn, \textsuperscript{b}17864197198@163.com, \textsuperscript{c}204118@sdjtu.edu.cn, \textsuperscript{d}qiliang@chaoyue.com.cn

Abstract—Zinc plays a crucial role in human bone metabolism. In the present study, zinc-doped hydroxyapatite (Zn-HA) porous microspheres were environmentally synthesized through a hydrothermal homogeneous precipitation route by using glutamic acid as the growth regulator. FE-SEM results show that Zn-HA microspheres consist of nanosheets and exhibit relatively uniform spherical morphology with porous and petal-like structure. EDS mapping indicates a homogeneous Zn distribution on the microsphere. The one-step process employed to realize zinc doping in the HA porous microspheres offers a functional route to obtain a green Zn-HA product of considerable potential in multiple fields, such as drug-controlled release matrix, injectable bone defect fillers, delivery carriers for proteins and genes.

1. INTRODUCTION
Hydroxyapatite (Ca\textsubscript{10}(PO\textsubscript{4})\textsubscript{6}(OH)\textsubscript{2}, HA) is similar to the inorganic component of human bones and tooth, and has excellent biological properties, such as bioactivity, biocompatibility and nontoxicity. All these advantages make it the most widely used biomaterials in recent years. Among various morphologies of HA, three-dimensional HA materials, especially porous HA microspheres are preferable to be used in drug-delivery systems, bone graft substitutes, and protein adsorption, separation and purification. It is because of the superior flowability, good mechanical and physical properties, high protein absorption capacity and additional specific surface area of HA microspheres [1-3].

However, low reactivity with existing bone limits the wider application of porous HA-based biomaterials. Inspired by the osteoconductivity and biocompatibility presented of HA microspheres in dental and orthopedic applications, the introduction of trace elements into HA microspheres can theoretically improve their osteoinductive potential [4-6]. Till now, many ions have been incorporated to HA, and the substitution affects the biological performance and the mechanical properties of stoichiometric HA [7-10]. For example, Si is essential to normal bone and connective tissue growth [7-8]. Na plays a key role on osteoporosis and bone metabolism. Mg is vital to calcification process and mineral metabolism [11, 12]. Sr plays special roles in bone re-modeling because of the relation to a reduction of bone resorption [13]. Therefore, synthesizing HA with ion-substitutions is significant to improve its properties and widen its applications [14-15].
Among various metal ions that HA structure allows to substitute Ca\(^{2+}\) ions in its crystal lattice, such as Cu\(^{2+}\), Ag\(^{+}\), and Zn\(^{2+}\), zinc is known to stimulate bone formation and enhance osteogenic response in osteoblasts by increasing cell proliferation, osteogenesis-related gene expression, and extracellular matrix synthesis [16, 17]. It is reported that compared with HA, zinc-containing HA microspheres showed enhanced absorption potential and long-term biocompatibility [18, 19]. Therefore, it would be desirable to prepare HA microspheres with a certain amount of zinc to improve the performance of HA materials.

Herein, a green synthesis method of Zn-HA porous microspheres through the hydrolysis of urea containing a nontoxic modifier, glutamic acid, is presented.

![Fig. 1](image-url) Characterization results of the as-synthesized products. (a) FE-SEM image of a single microsphere, (b) a magnified image of the microsphere in (a), and (c) corresponding EDS elemental analysis.

The structures of Zn-HA microspheres and corresponding element distribution are observed. The technique employed offers a new way to obtain Zn-HA porous microspheres with multiple biomedical applications. The formatter will need to create these components, incorporating the applicable criteria that follow.

2. EXPERIMENTAL

The hydrothermal homogeneous precipitation route for the synthesis of Zn-HA microspheres is described as follows:

\[(10-x)\text{Ca}^{2+} + x\text{Zn}^{2+} + 6\text{PO}_4^{3-} + 2\text{OH}^{-} \rightarrow \text{Ca}_{10-x}\text{Zn}_x(\text{PO}_4)_6(\text{OH})_2 \quad (x=0.3)\]

Briefly, the aqueous solution of CaCl\(_2\), (NH\(_4\))\(_2\)HPO\(_4\), 1 M urea were mixed, by setting the molar ratio of Ca/P at 1.67.

Initial pH values of the reactions were kept at 3.5 by using diluted HNO\(_3\) solution. After adding 1 M glutamic acid and ZnCl\(_2\) powers (the molar ratio n(Zn)/n[Zn+Ca]=3%) under magnetic stirring, the solutions were treated hydrothermally at 180°C for 10 h. Then the resultants were centrifugal cleaned with DI water and anhydrous ethanol, and finally dried at 80°C in an oven. Field emission scanning electron microscope (FE-SEM, 5 kV) equipped with an energy dispersive spectrometer (EDS) was used to observe the morphology and elements of the as-synthesized products. Before the test, samples were coated with gold to induce the conductivity.
3. RESULTS AND DISCUSSION

Fig. 1 shows the FE-SEM images of the obtained products regulated by glutamic acid. We can see that the products (Fig. 1a) consists of relatively uniform spherical crystallites with petal-like morphology. The diameter of a well-defined microsphere can reach about 30 μm. In addition, the microsphere has a porous structure. Fig. 1b demonstrates the morphology of a typical sphere, in which it is clear to see that the porous structures of the microsphere consist of flat flakes. A striking point is that the flakes are highly flexible and the pores are interconnected without any cracks, making the products a good candidate for drug delivery/cell carriers. After roughly estimated, the average interconnected pore size of the microsphere is around 1.5 μm.

The corresponding EDS spectrum and the content of all the elements are shown in Fig. 1c. From which we can see the presence of the elements Ca, P, O and a small amount of Zn in the final products. (Al and Pt elements are attributed to the aluminium foil used for FE-SEM test to increase the conductivity of the samples). As calculated, the weight ratio of Ca and P can be indexed to HA. Zn\(^{2+}\) can replace a small amount of Ca\(^{2+}\) in HA crystal and thus is a dopant element.

Element distribution results are shown in Fig. 2. Fig. 2 a-d presents the EDS map of Ca, P, O and Zn, respectively. It is indicated that all the elements detected are distributed homogeneously in the microsphere. Obviously, a homogeneous distribution of the elements Ca, P, O, as well as Zn on the porous microsphere crystal are observed, implying that the element Zn has been doped to the HA crystals, rather than simply adhered to the HA porous microspheres.

Based on the experimental results, there are two key points accounting for the Zn-HA porous microspheres. For one thing, urea decomposes and releases NH\(_4^+\) ions gradually after heating above 80°C, leading to a relatively homogeneous pH field to form HA crystals [20]. For another, carboxyl groups of glutamic acid interact with Ca\(^{2+}\) and Zn\(^{2+}\) ions on the surfaces of HA crystals, thus a soluble complex appears and then hydrolyzes slowly to release Ca\(^{2+}\) and Zn\(^{2+}\) ions. As the reaction goes forward, the
driving force of the nucleation increases, therefore the interface grows continuously. Accompanied by the hydrolysis process, OH\(^{-}\), Ca\(^{2+}\) and Zn\(^{2+}\) ions in the former nuclei combine with the free PO\(_4^{3-}\) ions in the reaction system. Then the Zn-HA crystals start to grow into porous microspheres.

4. SUMMARY
In summary, Zn-HA porous microspheres were green synthesized through a hydrothermal homogeneous precipitation route using glutamic acid as the growth regulator of HA crystals. Noticeably, the porous structures of the microspheres consist of interconnected pores without any cracks, and flat and flexible flakes. The hydrolysis of urea and chelation of glutamic acid are two key points attributed to the formation of Zn-HA porous microspheres. Such HA microspheres with zinc doping and porous structures are a promising candidate for separation and purification mediums, filling materials, and drug/cell carriers.

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