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Hydrothermal fabrication of porous hollow hydroxyapatite microspheres for a drug delivery system

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ABSTRACT

Porous hollow hydroxyapatite microspheres (PHHMs) are the promising biomaterials, owing to their excellent biocompatibility, biodegradability and bioactivity. PHHMs have been used as drug controlled carriers due to their advantages such as large drug loading capacity, nanochannels for drug loading and release and high specific surface area. In this study, PHHMs were prepared successfully in Na_2HPO_4 solution by an anion-exchange process using vaterite $CaCO_3$ through a hydrothermal method. The previous vaterite $CaCO_3$ was synthesized by a polymer-templated method in the poly(styrene sulfonic acid) sodium salt (PSS) aqueous solutions. The PHHMs have a size distribution from 0.8 to 2.0 μm, with an average pore size of about 24.3 nm. The wall of PHHMs is constructed with building units of hydroxyapatite nanofibers with an average length of 300 nm and an average width of 20 nm. The PHHMs displayed a high drug loading capacity and pH-responsive sustained–controlled drug release behavior when we used doxorubicin hydrochloride (DOX) as a loading drug. Moreover, the controlled drug release system showed a high ability to kill cancer cells and less damage to normal cells. These results indicated that PHHMs are promising for applications in various biomedical fields such as drug delivery system and oncotherapy.

1. Introduction

An important technique to treat disease would be to use controlled-drug-release vectors to ensure that drugs are specifically released at a constant rate. Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAp) is the most important inorganic constituent of human teeth and bones [1,2], and has attracted considerable attention in various biomedical areas such as gene and drug delivery [3,4], bone repair and tissue engineering owing to its excellent biocompatibility, biodegradability and bioactivity [5–7]. Recently, many studies have been carried out to investigate using hydroxyapatite materials as a controlled drug release system [8–10]. Among various morphologies and sizes of hydroxyapatite materials, PHHMs have great potential as candidate carriers for drug controlled release because of their highly specific surface area and large capacity for drug loading.

Porous hollow hydroxyapatite can serve as an ideal controlled drug release system due to the following advantages: (i) it possesses excellent biocompatibility, biodegradability and bioactivity [11,12], (ii) it exhibits good physic–chemical properties such as a large surface area, uniform pore size and high pore volume, which make it possible to load a large number of drugs and release at a constant, slow and controlled rate [13–15], and (iii) the OH groups in hydroxyapatite can adsorb the drug molecules which contain OH groups by the hydrogen bonding interactions, and thus improve the drug loading capacity and release property.

Up to now, various methods have been developed for the fabrication of hollow hydroxyapatite microspheres, such as a hydrothermal method [16,17], microwave-assistant method [18–21], template method [22–25], biopolymer-assisted synthesis method [26,27], spray drying method [28–30], solvothermal methods [31], and others [32]. Among of them, template-hydrothermal synthesis is the most common and simple method because the morphology of as-synthesized particles can be easily regulated by changing the morphology of sacrificial template. So far, template-hydrothermal synthesis has been proven as a versatile method to prepare hydroxyapatite with hollow structures. In spite of the considerable progress that has been made, the development of simple, rapid, energy-efficient and pollution-free methods for the preparation of PHHMs remains a challenge, especially to providing molecules that have a small diameter, are well dispersive and have a uniform size distribution.

In this paper, the PHHMs were fabricated in Na_2HPO_4 solution by a hydrothermal method using as-prepared vaterite $CaCO_3$ as the sacrificial template. The vaterite $CaCO_3$ could be easily transformed to PHHMs

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because the anion-exchange process of replacement of CO_3^{2-} by PO_4^{3-} has took place under the hydrothermal condition. This synthesis strategy is rapid, highly efficient, energy-saving and environmentally friendly. This approach to synthesize PHHMs has four advantages: firstly, the spherical structures of the sacrificial template are well retained in the PHHMs and a hollow urchin-like structure is formed after hydrothermal reaction process; secondly, The PHHMs are similar to biological apatite in chemical composition, and have excellent biocompatibility; thirdly, any surfactant will not be added during the reaction process and the PHHMs have good bioactivity; and finally, the PHHMs exhibit the porous hollow structures which make them have high drug loading efficiency and sustained drug release property. The PHHMs were explored for potential application in drug loading capacity and controlled drug release. The loaded drug is doxorubicin hydrochloride (DOX), a famous broad-spectrum anti-cancer clinical drug that has excellent therapeutic effect on oncotherapy. The PHHMs drug delivery system showed a high drug loading content, drug entrapment efficiency, sustained drug release property and obvious killing capacity to tumor cells. The experiments indicated that the PHHMs have great potentials for application in controlled drug release system and oncotherapy.

2. Materials and methods

2.1. Materials

Poly(styrene sulfonic acid) sodium salt (PSS, Powder, $M_w = 70{,}000$, Alfa Aesar, China), doxorubicin hydrochloride (DOX, Shanghai Shifeng Biological Technology Co., Ltd., China), calcium chloride ($CaCl_2$, 99%, Sigma-Aldrich), sodium carbonate (Na_2CO_3, 99.8%, Sigma-Aldrich), disodium hydrogen phosphate (Na_2HPO_4, 99.99%, Sigma-Aldrich), calcium carbonate ($CaCO_3$, vaterite, ACS reagent, ≥99%, Sigma-Aldrich), hydroxyapatite (HAp, synthetic, ≥99.995%, Sigma-Aldrich), sodium hydroxide (NaOH, 99%, Sigma-Aldrich), hydrochloric acid (HCl, Shanghai Sanying Chemical Reagent Co., Ltd.), ethanol (≥99.7%, Hangzhou Gaojing Fine Chemical Industry Co., Ltd.), and phosphate buffer saline ($pH = 7.4 \pm 0.1$, 1X) were used as received without further purification.

2.2. Preparation of $CaCO_3$

The hard-template of vaterite $CaCO_3$ was synthesized by a polymer-templated method using a PSS as a regulation template in an aqueous solution at room temperature. Briefly, the $CaCl_2$ solution (10 mL, 0.2 M) was poured into PSS (100 mL, 10 mg mL^{-1}) solution under vigorous magnetic agitation, and then the Na_2CO_3 solution (10 mL, 0.2 M) was added dropwise into the above mixed solution under vigorous magnetic stirring to obtain a white suspension. After the complete addition, the white suspension was kept at room temperature for 1 h followed by centrifugation (8000 rpm, 3 min), washed three times with distilled water and absolute ethanol respectively, and then dried at 60 °C for 24 h.

2.3. Preparation of PHHMs from $CaCO_3$ template

The PHHMs were prepared by an anion-exchange process using the previously prepared $CaCO_3$ as the sacrificial template in a hydrothermal method. In a typical experiment, 100 mL of 0.1 M of Na_2HPO_4 was added into 0.2 g of the $CaCO_3$. The pH of the resulting suspension was adjusted to 11.0 using a sodium hydroxide solution. The suspension was transferred into a Teflon-lined stainless steel autoclave, sealed, and heated by a hydrothermal method at 120 °C for 1 h. After the hydrothermal reaction was completed, the product was collected by centrifugation, washed three times with distilled water and absolute ethanol respectively, and dried at 60 °C for 24 h.

2.4. Characterization

The morphology and diameter of the as-synthesized samples were characterized by field emission scanning electron microscopy (FESEM) (ZEISS-ULTRA55, Japan), transmission electron microscopy (TEM) (JEM-2100, Japan). The samples were ultrasonically dispersed in absolute ethanol (0.1 mg mL^{-1}) and the size of the samples was characterized by dynamic light scattering (DLS) (ZetasizerZEN3600, UK). The crystalline phases of samples were examined with X-ray powder diffraction (XRD) (ARL X'TRA, Thermo Electron) using a monochromatic CuKα radiation ($\lambda = 1.54056$ Å) in the range of $2\theta = 20°$–60° and a scanning rate of 5° min^{-1}. Fourier transform infrared spectra (FTIR) (Nicolet 5700, Thermo Electron) were collected at room temperature by using the KBr disk technique, working in the range of wave numbers 4000–400 cm^{-1} at a resolution of 4 cm^{-1} (number of scans ~32). N_2 adsorption–desorption isotherms were measured with an automatic surface area and porosity analyzer (3 H-2000PS1, BeiShiDe Instrument) at 293.15 K. The pore size distributions were derived from the adsorption branches of the isotherms using density functional theory (DFT). Thermogravimetric analysis (TGA) was carried out on a thermogravimetric analyzer (Pyris 1 TGA, USA) with the heating rate of 10 °C min^{-1} under nitrogen atmosphere.

2.5. In vitro PHHMs degradation

For the *in vitro* PHHMs degradation experiment, 50 mg PHHMs were immersed in 10 mL phosphate buffered saline (PBS) with different pH values of 4.5, 5.6, 6.5 or 7.4 under stirring in a shaking bath at 37 °C. The supernatant was periodically withdrawn by centrifugation and replaced by the same volume of fresh medium. The concentration of Ca^{2+} of the clear supernatant was measured by UV–vis spectrophotometer (HITACHI U2800, Hitachi) at a wavelength of 612 nm and calculated by employing a calibration curve. All the tests were carried out in triplicate and the average values were shown in this study.

2.6. Drug loading and in vitro drug release

The drug loading and *in vitro* drug release experiments were performed using the PHHMs. The procedure of DOX loading was performed as follows: 50 mg of PHHMs was transferred into DOX solution (5 mL, 1 mg mL^{-1}) over 48 h under gentle shaking at 37 °C. Then, the DOX-loaded particles were rinsed by deionized water 3 times, collected by centrifugation and then vacuum dried at 60 °C for 24 h. The amount of DOX in the clear supernatant was measured by UV–vis spectrophotometer at a wavelength of 477 nm and calculated by employing a calibration curve. The DOX encapsulated in the PHHMs can be calculated by subtracting the amount of the DOX in the supernatant from the initial DOX amount. The drug entrapment efficiency and drug loading content of DOX were calculated according to the following equation, respectively:

$$\text{Drug entrapment efficiency} = (\text{DOX initial} - \text{DOX in the supernatant})/\text{DOX initial} \times 100\%$$

$$\text{Drug loading content} = (\text{DOX initial} - \text{DOX in the supernatant})/\text{microsphere amount} \times 100\%$$

For the *in vitro* drug release experiment, the DOX-loaded PHHMs (10 mg) were added to semipermeable bags (8–14 kDa porosity), then immersed in 30 mL phosphate buffered saline (PBS) with different pH values of 4.5, 5.6, 6.5 or 7.4 under magnetic stirring at a constant rate of 150 rpm at 37 °C. The sample (1 mL) was removed at given time intervals for UV–vis analysis and replaced by the same volume of fresh medium. The adsorption data were representative as the mean value of three parallel measurements.