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# Preparation, characterization and in vitro gentamicin release of porous HA microspheres

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ABSTRACT

Hydroxyapatite (HA) microspheres with high porosities were successfully obtained using an improved ice-templated spray drying (ITSD) technique for drug delivery applications. Pore structures and pore sizes of microspheres have great impact on drug loading and release kinetics. Therefore, solvent types, polyvinyl alcohol (PVA) contents and solid loadings of suspensions were adjusted to control the pore structures and pore sizes. Microspheres with interconnected pore networks and aligned pore structures were obtained using camphene-based and tert-butyl alcohol (TBA)-based suspensions, respectively. With the increase of PVA contents in suspensions, the growth of sintering neck became more obvious and the surface of HA particles became smoother. The inner pore structures of microspheres transformed from uniformly distributed cellular pores to three-dimensional interconnected pore networks, with the increase of solid loadings in suspensions. Gentamicin was successfully loaded into porous HA microspheres. The drug loading percentage increased from 40.59 to 49.82% with the increase of porosity of HA microspheres. The release percentage during the initial 18 h increased from 48.72 to 65.68% with the transformation of pore structures from independent cellular pores (main diameter ~ 3 μm) to three-dimensional interconnected pore networks (main diameter > 3 μm).



## 1. Introduction

Particulate systems for drug delivery have gained much attention in both research and industry, due to their predictable therapeutic response, prolonged drug release time and great efficacy [1–4]. Irregularly or densely packed particles often cause inflammatory reactions and impede the bone formation, while uniformly packed spherical particles contribute to bone in-growth and avoid inflammation [5]. Porous spherical particles have been generally utilized as fillers or packing materials in orthopedic and maxillofacial applications [6]. For nanospheres, their nano-sized dimension has restricted their application in cell-based therapy for bone-tissue engineering [7]. In contrary, porous microspheres can be well utilized for the delivery of macromolecules, drugs or cells, due to their micro-sized dimension, macro-pores and unique packing properties [8]. Packed microspheres form a matrix with uniform pores between particles, leading to efficient conduction of bone among particles [9]. For drug delivery systems, the pore structure and pore size of microspheres significantly influenced the drug release behavior [10]. The initial release rate depended much on the relation between hydrodynamic diameters and pore sizes of microspheres while the long-term release rate was predominantly controlled by the network of much smaller meshes [11]. Therefore, the pore structure and pore size are two key parameters for modulating drug loading and release behaviors.

Various methods have been applied for fabricating porous microspheres, including freeze-photocuring-casting (FPC) [12], thermal decomposition [13] and spray drying [14]. However, those methods generally have narrow application fields or show poor controllability of pore sizes and pore structures. Spray drying technique has a wide industrial application owing to its simple procedure. Ice-templating method has been widely utilized to fabricate porous materials with generally aligned pore structures and adjustable pore sizes [15,16]. In our previous work, a novel ice-templated spray drying (ITSD) method was developed to fabricate porous microspheres with controlled pore structures and pore sizes, taking advantages of ice-templating method and spray drying technique [17]. Compared to the spray freeze drying technique for producing irregular particles with low porosities [18,19], the improved ITSD technique provides a controllable temperature gradient to fabricate microspheres with adjustable pore structures and high porosities.

Bioceramics have been applied clinically as bone implants, including hydroxyapatite (HA) [20,21], tricalcium phosphate (TCP) [22] and bioglass [23]. Calcium phosphates have been considered as a potential material for drug delivery systems, due to its ability to release a therapeutic agent in situ for producing osteoconductivity of the material [24,25]. As the major component of skeletal tissues, hydroxyapatite (HA) has been applied clinically and experimentally in medical and

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bioengineering fields owing to its excellent biocompatibility, biodegradability and osteophony properties [26,27]. As a regular antibiotic, gentamicin can solve bone infection problems caused by the poor circulation of blood in osseous tissues to reach an adequate therapeutic level in the affected regions [28].

In this study, HA microspheres with controlled pore structures and pore sizes were obtained using an improved ITSD technique. Effects of solvent types, PVA contents and solid loadings of the suspensions on pore structures and particle size distributions of HA microspheres were investigated. The relationship between porosities and drug loadings of HA microspheres was investigated. Effects of pore structures and pore sizes of HA microspheres on in vitro release of gentamicin were presented and discussed.

## 2. Materials and methods

### 2.1. Fabrication of porous HA microspheres

Hydroxyapatite suspensions were prepared by adding hydroxyapatite powders with a mean particle size of 300 nm (Yipurui Co. Ltd., China) in the solvent of deionized water, camphene (Cuiechem Co. Ltd, China) or tert-butyl alcohol (TBA, Energy Chemical Co. Ltd, China). Ammonium polyacrylate (HydroDisper A160, Otise Co. Ltd, China) and polyvinyl alcohol (PVA, Kuraray Co. Ltd. Japan) were used as the dispersant and binder, respectively. Table 1 lists suspensions with different solvent types, solid loadings, and PVA contents. All suspensions were followed by ball milling for 48 h. Fig. 1 shows the schematic representation of the ITSD process for fabricating porous HA microspheres. Suspensions were sprayed directly into the small cylinder through a circular nozzle (Φ 0.3 mm). The cryogenic atmosphere in the small cylinder was provided by liquid nitrogen between the two steel cylinders, as shown in Fig. 1. The diameters of two cylinders were 12 mm and 8 mm, respectively. The liquid nitrogen volume and spray height were 25 120 mL and 840 mm, respectively. The frozen microspheres were collected and the ice in HA microspheres was subsequently removed in a freeze-drier device (FD-1A-50, Beijing Boyikang Medical Equipment Co. Ltd, China) at −60 °C. The dried HA microspheres were heated up to 600 °C at a heating rate of 1 °C/min and sintered at 1250 °C for 2 h.

### 2.2. Encapsulation of gentamicin sulfate (GS) in porous HA microspheres

Porous HA microspheres obtained from suspensions with different solid loadings were applied in the process of gentamicin encapsulation. For each batch, 0.2 g of porous HA microspheres was completely immersed in 6 mL of gentamicin sulfate solution (40 IU/mL) under vacuum at room temperature for 56 h and then dried at room temperature for 24 h.

Percentages of GS loadings were estimated through an indirect method, by calculating the difference of GS concentrations in the loading buffer solution, before and after loading. Percentage of drug loading was calculated using the formula [9]:

$$\text{Percentage drug loading} = \frac{X-Y}{X} \times 100\%$$

where X and Y represent the initial and final GS concentrations, respectively. Each test was performed in triplicate.

**Table 1**
A list of suspension compositions.

| Suspension series | Solvent type | Solid loading[a] | PVA content[b] | Ammonium polyacrylate content[b] |
|---|---|---|---|---|
| I | Camphene | 25.9 | 0 | 1.0 |
| II | TBA | 25.9 | 0 | 1.0 |
| III | Water | 25.9 | 0.5 | 1.0 |
| IV | Water | 25.9 | 1.0 | 1.0 |
| V | Water | 25.9 | 1.5 | 1.0 |
| VI | Water | 25.9 | 0 | 1.0 |
| VII | Water | 13.1 | 0 | 1.0 |
| VII | Water | 7.8 | 0 | 1.0 |

[a] wt.% in relation to the total amount of suspensions.
[b] wt.% in relation to the amount of HA powders.

### 2.3. In vitro release of gentamicin from GS-loaded HA microspheres

The in-vitro release of gentamicin from GS-loaded HA microspheres was performed in phosphate buffer saline (PBS, pH 7.4) at 37 °C. For each batch, 100 mg of GS-loaded HA microspheres was completely immersed in 10 mL of PBS solution. 3 mL of the release medium was collected at predetermined time intervals and then replaced by a fresh PBS solution (3 mL) each time. The collected release medium was measured at $\lambda = 248$ nm using a UV spectrophotometer (UV-6100S, METASH, China). Each test was performed in triplicate.

### 2.4. Characterization

The morphologies of HA microspheres were measured using a scanning electron microscope (SEM, Nova NanoSEM 230, USA) at an acceleration voltage of 10 kV and a distance of 7 mm. Each sample was coated with gold prior to imaging. The particle size distributions of HA microspheres were measured using a laser diffraction scattering particle sizer (Malvern Mastersizer, USA). The presence of carbon in GS-loaded HA microspheres was verified using the Energy Dispersive X-ray Spectrometer (EDS, Norman Instrument, USA) at an acceleration voltage of 20 kV and a distance of 7 mm. The pore size distributions of porous HA microspheres were measured using the mercury intrusion porosimetry (AutoPore IV 9500, Micromeritics, USA). The Hg-surface tension at 20 °C corresponds to 485 dyn/cm. The contact angle and the density of Hg were 130° and 13.5 g/cm$^3$, respectively. The pore diameter was calculated according to the Wasburn equation [29,30]:

$$D = \frac{-4\gamma\cos\theta}{P}$$

where D is the pore diameter (μm); γ is the surface tension of mercury (dynes/cm); θ and P are the contact angle (°) and the pressure (psia).

The element contents of carbon and nitrogen in GS-loaded HA microspheres were measured using the CS-444 infrared carbon–sulfur analyzer (LECO, USA) and the TC-436 oxygen–nitrogen analyzer (LECO, USA), respectively. The porosities of porous HA microspheres were measured based on Archimedes' principle. 1 g of dried HA microspheres for each sample was used to measure the porosity. The porosity (P) was calculated according to the following formula:

$$P = \frac{W_2 - W_1}{W_2 - W_1 + \rho_0 V_0} \times 100\%$$

where $W_1$ is the weight of sample in air, $W_2$ is the weight of sample with water, $\rho_0$ is the density of water (1.0 g/mL), $V_0$ is the increased volume of water caused by the immersed HA microspheres in water. In order to ensure the accuracy of $W_1$, HA microspheres were dried for 5 h in the vacuum drying chamber (P2F-6050, Jinghong Co. Ltd., China). The dried sample was immersed in water and then the increased volume of water was measured as $V_0$. Each test was performed in triplicate.