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Bioactive SiO_2-CaO-P_2O_5 hollow nanospheres for drug delivery

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ABSTRACT

Hollow nanospherical bioactive glasses have attracted much interest in the recent years because of their important applications in drug delivery and hard tissue repair. Herein, we developed a facile method to fabricate bioactive SiO_2-CaO-P_2O_5 (SCP) hollow nanospheres using polystyrene (PS) nanospheres as the hard template. The removal of PS templates by heating produced almost monodispersed hollow SCP nanospheres with an inner cavity of 210 nm, making them suitable for drug delivery. The impact of the glass composition on the formation of hollow SCP nanospheres was investigated in detail. High drug-loading capacity and sustained multistep release of theophylline in the SCP nanospheres were achieved. Furthermore, the SCP nanospheres exhibited apatite-forming ability when immersed in a simulated body fluid. Cytotoxicity of SCP nanospheres on live cells has been evaluated. The SCP hollow nanospheres with sustained drug release property and bioactivity are promising new-generation biomaterials.

1. Introduction

Since the discovery of bioactive glasses (BGs) by Hench et al. in 1971, BGs have attracted much attention because of important applications in the field of bone tissue regeneration [1–5]. BGs containing SiO_2, CaO, Na_2O, and P_2O_5 bond to the bone by forming an interlayer of hydroxyapatite (HA); this is regarded as the bioactivity of BGs [6]. The bioactivity of BGs strongly correlates to the particle size, morphology, surface characteristics, and microstructure of the material [7]. The BG prepared by the sol–gel method has superior properties than that prepared by the traditional melt-quenching method, e.g., a large porosity and surface area and high purity, endowing the sol–gel glass a higher bioactivity than the conventional BG [8,9].

Recently, organic/inorganic mesoporous nanomaterials with plenty of vacant sites and surface areas have been widely investigated because of their significant applications in diverse fields such as biomedicine and catalysis [6,10–12]. Among them, mesoporous spherical BG (MSBG) nanoparticles containing SiO_2-CaO or SiO_2-CaO-P_2O_5 (SCP) prepared by the sol–gel method showed high biocompatibility and therefore are attractive drug delivery vehicles [7,13–17]. Tsigkou et al. reported that monodispersed SiO_2-CaO nanospheres with a diameter of 215 nm could be internalized by human bone marrow and adipose-derived stem cells and located within cell vesicles and cytoplasm [16]. Moreover, the particle size significantly affected the bioactivity; the smaller particle size facilitated the cell proliferation and differentiation on the surface of MSBGs [18]. In particular, hollow BG nanospheres are ideal drug carriers because of their high drug-loading capacity and low particle weight [19–21]. Papas et al. synthesized SCP hollow nanospheres by the sol–gel method using polystyrene (PS) nanospheres as the hard template [19]; however, the bioactivity of SCP nanospheres and possibility as drug carriers have not been investigated.

In this study, hollow SCP nanospheres were prepared using PS nanospheres as the hard template via a modified Stöber method. The bioactivity of the material was investigated by immersing in a simulated body fluid (SBF) for several days. Moreover, the drug-loading and releasing behavior was studied using theophylline (TP) as the drug model. The effects of the SCP nanospheres on the cytotoxity were evaluated in vitro.

2. Experimental

2.1. Material synthesis

2.1.1. Preparation of PS nanospheres

The PS nanospheres were prepared by an emulsion polymerization method as reported previously [22]. In brief, styrene was first purified by washing with 40 mL NaOH (5 wt.%) aqueous solution thrice. Then, 13 mL purified styrene and 0.5 g PVP were mixed with 100 mL deionized water in a 250 mL three-neck round bottom flask under magnetic stirring for 15 min. After a solution of 0.3 g potassium persulfate ($K_2S_2O_8$) in 20 mL deionized water was added, the reaction mixture was deoxygenated by bubbling with nitrogen gas at room temperature for 30 min. The temperature of the mixture was then gradually increased to 70 °C and maintained at 70 °C for 24 h. The obtained precipitates were washed with anhydrous ethanol thrice and redispersed in ethanol for further use.

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2.1.2. Preparation of hollow SCP glass

Hollow SCP nanospheres were prepared by a modified Stöber method using PS nanospheres as the hard template as follows: First, 50 mg of PS nanospheres were dispersed in a solution of 25 mL deionized water and 25 mL anhydrous ethanol under stirring at 40 °C. Then, a solution of 80 mg cetyltrimethylammonium bromide (CTAB) in 5 mL deionized water was added, and the mixture was continuously stirred for 10 min. After the addition of 1 mL aqueous ammonia (35 wt.%), a solution of tetraethylorthosilicate and triethyl phosphate in 5 mL ethanol was added dropwise. The resulting mixture was stirred for 25 min, and a solution of calcium nitrate in a small amount of deionized water was added. The mixture was continuously stirred at 40 °C for 24 h. The obtained white precipitates were centrifuged, washed with ethanol and water thrice and dried at 60 °C. The dried powders were heated at 650 °C for 3 h in air to remove the organic templates, affording the final hollow SCP nanospheres.

2.2. Drug loading and controlled release assessment of SCP

TP was used as the drug model to investigate the drug-loading efficiency and release behaviors of the hollow SCP nanospheres. First, 100 mg TP was dissolved in 10 mL deionized water, achieving a concentration of 10 mg mL^{-1}. Then, 30 mg SCP hollow nanospheres were soaked in a 10 mL TP solution under ultrasonication. Then, the mixture was dried under vacuum under continuous ultrasonication until the deionized water was completely evaporated. The resulting powders were washed with deionized water to remove the unloaded TP. The loading amount of drug was determined by thermal analysis. The drug release behavior was evaluated via a previously reported procedure [17]. First, 50 mg of the TP-loaded sample was immersed in 50 mL SBF at 36.5 °C. The SBF with a similar composition as blood plasma was prepared according to a previous report [23]. At selected time intervals, aliquots of SBF (1 mL) were removed by centrifugation for testing, and the stock was replenished with 1 mL fresh SBF. The drug concentration was measured from the UV absorbance at 239 nm; this was determined from the absorption spectrum. The drug release behavior was expressed as the cumulative release percentage relative to the releasing time.

2.3. Bioactivity evaluation of SCP

To test the bioactivity of the nanospheres, the obtained hollow SCP nanospheres were immersed in SBF and kept at 37 °C for 7 and 14 days. The formation of the mineralized apatite was determined by XRD, FT-IR and SEM.

2.4. In vitro cytotoxicity of SCP

In vitro cytotoxicity of SCP nanospheres was evaluated using the Cell Counting Kit-8 (CCK-8) (YEASEN) in accordance with the manufacturer's instructions. FHC cell were used for the experiments. In detail, FHC cells were seeded at a concentration of 5×10^4 cells/mL in Dulbecco's modified Eagle's medium (DMEM) (HyClone) with 10% fetal bovine serum (Gibco) and 100 units/mL penicillin (HyClone) plus 100 g/mL streptomycin (Invitrogen) onto 96-well plates and cultured for 48 h at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Then, the culture medium was refreshed by 200 μL suspensions of hollow SCP nanospheres in DMEM at concentrations of 0, 12.5, 25, 50, 100, 200 μg/mL. After incubated for 24 h, the SCP suspension were removed and washed with SBF twice, then 2 μL CCK-8 reagent and 100 μL DMEM were added to each well and cells were incubated for 2 h. The cell proliferation was also evaluated by testing the cell viability using CCK-8 after culturing with the SCP nanospheres at the concentrations of 25 and 50 μg/mL for 1, 3 and 5 days. The OD450 nm value was measured by a microplate reader (Thermo). All assays were conducted in sextuplicate.

2.5. Material characterization

The microstructure of the obtained nanospheres was characterized using a JEOL-2100 transmission electron microscope (TEM) and an SN-3400 scanning electron microscope (SEM) equipped with energy dispersive spectroscopy (EDS). The specimens for TEM studies were prepared by depositing a drop of the suspension in ethanol onto a Cu grid coated with a layer of carbon film. For the SEM studies, the samples were adhered to the conducting resin. These samples were then conductively coated with gold by sputtering for 1 min. The infrared (IR) absorption spectra of the samples were obtained using a Varian 640-IR Fourier transform IR (FT-IR) spectrometer with a resolution of 4 cm^{-1} in the range 4000–400 cm^{-1} using the KBr method. The thermogravimetric (TG) and differential thermal analysis (DTA) of the sample was carried out using a TGA Instrument (TA instrument-waters LLC) at a heating rate of 10 °C min^{-1} in air. The X-ray diffraction analysis of the sample was carried out using a Persee XD-6 X-ray powder diffractometer operated at 36 kV and 20 mA using Cu Kα radiation ($\lambda = 0.154$ nm). The detector was scanned over a range of 2θ angles from 15° to 80° at a step size of 0.02° and dwell time of 1 s per step. The specific surface area, pore volume, and pore size distribution were determined by N_2 adsorption–desorption measurements (ASAP 2020). The specific surface area was determined according to the Brunauer–Emmett–Teller (BET) method. The pore size distribution was determined from the N_2 desorption branch of the obtained N_2 adsorption–

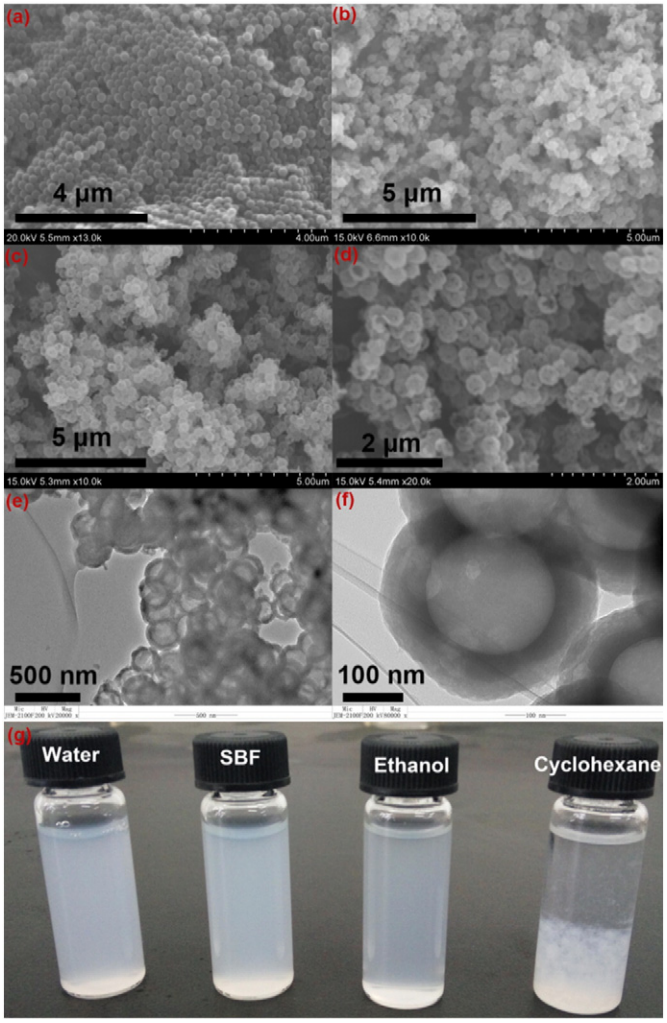

Fig. 1. SEM images of (a) PS nanospheres, (b) as-prepared PS@SCP; (c and d) SEM and (e and f) TEM images of PS@SCP annealed at 650 °C with different magnitudes; (g) photographs of hollow SCP nanospheres dispersed in various solvents.