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Silica nanoparticles with enlarged nanopore size for the loading and release of biological proteins

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ABSTRACT

Silica nanoparticles (SNs) with a nanoporous structure are attractive for the delivery of biomolecules. This study developed a SNs-based protein delivery system with nanopore sizes large enough to uptake protein molecules. The use of trioctylmethylammonium bromide (TOMAB) as an auxiliary chemical facilitated a dramatic increase in pore size from 2.6 nm to 17.4 nm. The surface was highly negatively-charged with a zeta potential of approximately −35 at pH 7. Positively-charged protein cytochrome C was encapsulated effectively within the large pore spaces of the SNs, with a protein loading capacity of almost 2-fold increase due to the pore size increase. The loaded protein exhibited sustained release for approximately one week with an initial burst in a day, suggesting the SNs tailored with enlarged nanopores should be useful for the delivery of large protein molecules for tissue regeneration.

1. Introduction

Silica nanoparticles (SNs) containing nanopores within the structure, which are more often known as mesoporous SNs, have been widely accepted as a potential delivery system for drugs, such as antibiotics, anticancer drugs, enzymes and nucleic acids [1–5]. Because of the nanopores inside the structure, SNs generally have an extremely high surface area (>1000 m^2/g) and surface volume (0.5–1 cm^3/g) to allow a high degree of surface reaction and to be capable of loading a large amount of drugs [5]. The chemically and structurally stable inorganic oxide framework of the SNs protects the drug molecules from exposure to a degrading environment and species, such as proteases and denaturation chemicals [3–5]. Furthermore, the surface of the SNs can be functionalized with different chemicals to adopt specific molecules and induce relevant biological reactions [5].

Therefore, the nanopore structure and surface properties of the SNs should be considered importantly in the design for drug delivery systems. MCM-41 and SBA-15 are the most widely studied nanoporous SNs, both of which are based on the precursor tetraethyl orthosilicate (TEOS) and are prepared using polymer templates, such as cetyltrimethylammonium bromide (CTAB) for the generation of pore arrays inside the nanoparticles [5]. In the conventional approach of using TEOS and CTAB in a water-based ammonia solution, the pore sizes of the SNs were at most a few nanometers. This range of pore sizes is sufficient only for encapsulating small-sized drugs, such as antibiotics, anticancers and proteins with a low molecular weight, limiting the applications for large-sized biomolecules, such as growth factors.

Some methodologies have increased the nanopore sizes of SNs [5–11]. Mostly, alkane groups, such as decane, octane and hexane, have been used to enlarge the pore size of the silica nanoparticles, where the hydrophobic alkane chain is placed at the core of the micelle and swells the structure [8,9]. Organic auxiliary chemicals, such as 3,5-trimethylbenzene and 1,3,5-triisopropylbenzene, have also been used to control the pore size of SNs [10,11]. In this case, the interaction of the surfactant with the auxiliary molecules determines the pore size.

Here we use trioctylmethylammonium bromide (TOMAB), which is considered to exert an auxiliary function for increasing the pore sizes, helping CTAB in the generation of a nanoporous structure within SNs. The processing routes to prepare SNs with enlarged pores are described, and the pore structure was examined. Moreover, the feasibility of the SNs in encapsulating proteins and subsequently releasing them were addressed using a model protein, cytochrome C. This study is considered to provide useful information on further developing biological protein delivery systems using inorganic nanoparticles.

2. Experimental

The preparation of SNs with small-sized nanopores was based on the method applied conventionally [2–4]. Typically, 6.123 g of cetyltrimethylammonium bromide (CTAB) used as a template was dissolved in 34.617 g of water, and 10.514 g of a 32% NH_4OH solution was added, which was followed by the addition of 3.5 g of tetraethyl

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orthosilicate (TEOS). The solution was ultrasonicated using a sonoreactor (20 Hz, 250 W) for 30 min and then stirred at 300 rpm for 20 h at room temperature to obtain a homogeneous suspension. For the preparation of SNs with large-sized nanopores, trioctylmethylammonium bromide (TOMAB) was added at different amounts in partial replacement of CTAB (molar ratio of TOMAB to CTAB was varied at 0.25, 0.5, 1, 2 and 4). Solid nanoparticles were recovered by centrifuging the suspensions at 10,000 rpm. The nanoparticles were washed three times with water and three times with 70% ethanol, and the samples were dried overnight at 60 °C to obtain white powders. The samples were placed in an alumina crucible, which was positioned in a box furnace, and then calcined by increasing the temperature to 650 °C for 10 h, holding at 650 °C for 6 h and air-cooling.

The morphology of the nanoparticles and the nanopores inside was observed by transmission electron microscopy (TEM; JEM-3010, JEOL). The samples for TEM were prepared by dispersing the nanoparticles in ethanol and placing a drop onto a holey Cu/Carbon grid. The particle size was analyzed by manually counting from the TEM images. The variation of the ζ-potentials of the nanoparticles was also measured at different pH (from 2 to 9 using buffer solution) using a Malvern Zetasizer (ZEN 3600, Malvern). For analysis, the nanoparticles were suspended in phosphate buffer saline solution (PBS; 10 mM phosphate and 75 mM sodium chloride) pH 7.4 at a concentration of 0.75 mg/ml and sonicated for 30 min. The pore structure of the nanoparticles was analyzed from the nitrogen gas adsorption and desorption isotherm at 77 K using a Quantachrome system (2SI-MP-9, Quantachrome). Fourier transform infrared (FT-IR; 640-IR, Varian) spectroscopy was used to confirm the chemical bonding status of the nanoparticles.

As a model protein, cytochrome C was used. The ζ-potentials of the protein were also confirmed using a Malvern Zetasizer. Before the protein tests, the calibration curve of the protein as obtained using the Beer–Lambert law: $A = acl$, where A is the absorbance, a is the proportionality constant (known as absorptivity), c is the concentration, and l is the path length, which is constant [12]. The protein concentration range was varied from 50 to 200 μg/ml, and the absorbance was read at 408 nm using a UV–vis spectrophotometer (Libra S22, Biochrom).

For the protein loading test, the loading time was first determined. Cytochrome C was dissolved at different concentrations (0.1, 0.25, 0.5, 1, 5 and 10 mg) in PBS. Within the protein solutions, the SNs (either normal SN or enlarged SN designated as 'SN' and 'E-SN', respectively) were added at 10 mg, ultrasonicated for 10 s and left for different times (up to 360 min) at 37 °C. At each incubation time, the nanoparticles were centrifuged and the upper clear solution was assessed for the remaining quantity of protein. The results were plotted as a function of the incubation time and the saturation point was determined at 60 min. Based on this, the protein loading capacity of the SNs was determined by the adsorption isotherm, i.e., plotting the protein quantity loaded within the SNs (either SN or E-SN) with respect to the concentration of protein initially added into the PBS (0.1, 0.25, 0.5, 1, 5 and 10 mg/ml). The entrapment of proteins was determined by characterizing the protein-loaded samples with FT-IR and TEM and comparing the results with protein-free ones.

The protein-loaded SNs ('SN' and 'E-SN') weighed at a quantity of 1 mg were immersed in 5 ml PBS and the samples were incubated at 37 °C for different periods (1, 3, 6, 12 and 24 h, and 3 and 7 days) under static conditions. At each incubation time, the nanoparticles were centrifuged and an aliquot of the supernatant (4 ml) was assayed using a UV–vis spectrophotometer to detect the proteins released from the nanoparticles. At each assay, 4 ml of fresh medium was refilled and the release test was continued for 7 days.

3. Results and discussion

The typical morphology of the SNs, which were synthesized to have normal small-sized nanopores (SN) and enlarged nanopores (E-SN), was observed by TEM (Fig. 1(a,b)). Compared to the mesoporous structure observed in the SNs (Fig. 1(a)), much larger sized pores were developed evenly within the nanoparticles (Fig. 1(b)). The sizes of the nanoparticles calculated from the TEM images were 330 (±65) nm and 280 (±29) nm, respectively, for SN and E-SN. The pore structures of the developed SNs, including the surface areas, pore volumes and pore diameters are summarized in Table 1. The measured pore sizes for SN and E-SN were 2.6 and 17.4 nm, respectively. Along with the dramatic increase in pore size,

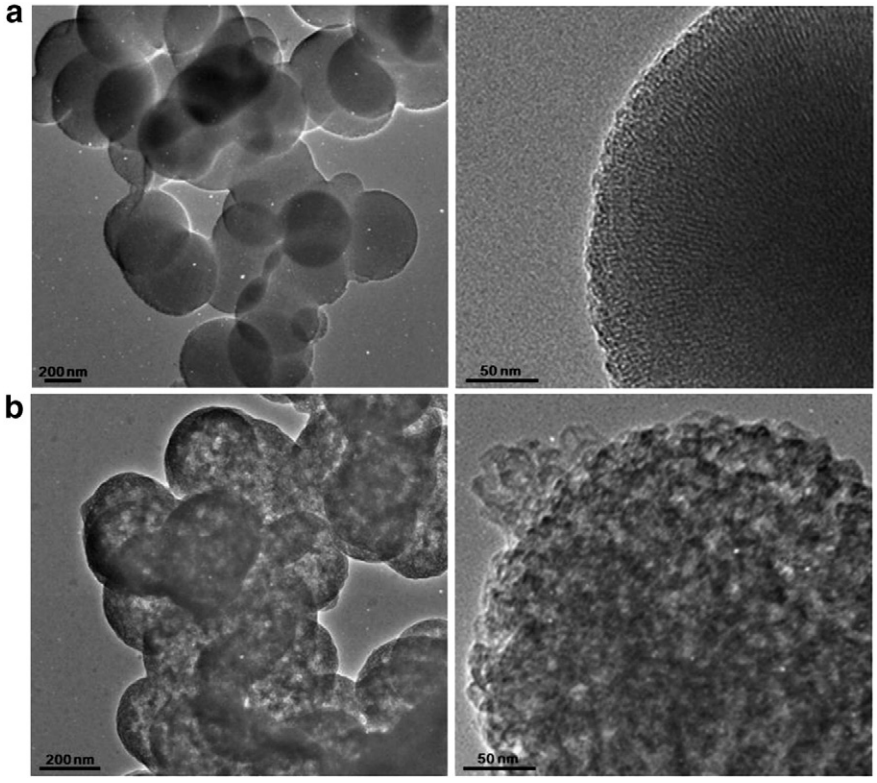

Fig. 1. TEM images of the silica nanoparticles with normal small-sized nanopores, SN (a) and the enlarged nanopores, E-SN (b), taken at different magnifications.