Microporous and Mesoporous Materials 181 (2013) 248–253

Contents lists available at ScienceDirect

Microporous and Mesoporous Materials

journal homepage: www.elsevier.com/locate/micromeso

Short Communication

Monodisperse mesoporous silica nanospheres with radially oriented mesochannels and their size effect on cell uptake

Yongjian Jiang [a], Chonglek Lee [a], Qiang Wang [a], Yonghui Deng [b], Deliang Fu [a,*]

[a] Department of Pancreatic Surgery, Huashan Hospital, Fudan University, Shanghai 200040, China
[b] Department of Chemistry & Laboratory of Advanced Materials, Fudan University, Shanghai 200433, China

ARTICLE INFO

Article history:
Received 8 June 2013
Received in revised form 1 August 2013
Accepted 2 August 2013
Available online 11 August 2013

Keywords:
Mesoporous silica nanospheres
Radially aligned mesopore
Synthesis
Cellular uptake
Cytotoxicity

ABSTRACT

A facile one-pot sol–gel approach was reported for the synthesis of monodisperse silica nanospheres (MSNs) with radially oriented mesochannels, tunable diameter ranging from 280 to 500 nm, by using cetyl trimethylammonium bromide (CTAB) as structure-directing agent, different amount of tetraethyl orthosilicate (TEOS) as the silica source and concentrated ammonia solution as the basic catalyst. Characterization and measurement using field-emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM), low angle X-ray powder diffraction (LA-XRD) and nitrogen adsorption–desorption reveal that the obtained monodisperse MSNs have uniform, radially aligned mesopore of about 2.3 nm, high surface area (about 960 m^2/g) and large pore volume of 0.58 cm^3/g. Cell experiments indicate that, they obtained MSNs possess excellent biocompatibility and the smaller MSNs have relatively lower cytotoxicity.

1. Introduction

Since the first discovery of MCM-41 by Mobil scientists, [1] mesoporous materials have sparked grand research interest in the past two decades [2]. As the most important members of these material families, mesoporous silica nanospheres (MSNs) have recently been intensively investigated for their remarkable properties such as high surface area, large pore volume, tunable pore architecture, and high physicochemical stability [3]. All these abovementioned outstanding capabilities endow mesoporous silica nanospheres with great potential in various biomedical applications, including controllable drug/gene/protein delivery, [4] gene transport and expression, [5] cell imaging, [6] diagnosis and bioanalysis [7].

After the first report showing that MSNs can be readily internalized by eukaryotic cells without detectable cytotoxicity in vitro [8], tremendous studies on their biomedical applications have been carried out to further understand the detailed mechanism of cell uptake of MSNs-based nanomaterials. As to practical biomedical-related applications, size monodispersity and porosity (especially, mesochannel orientation) are two most critical factors. Previously, Mou and co-workers probed the effect of sphere size on the cellular uptake of MSNs by Hela cells, which demonstrated the size-dependence of cellular uptake amount [9]. Also, Shi and co-workers found that the cytotoxicity of spherical MSNs is correlated with their particle sizes [10]. Though these two specific reports clearly favor the correlativity between particle size and biocompatibility or potential toxicological effects of MSNs, the exact relationship between them is still equivocal. On the other hand, mesoporous silica nanospheres with mesochannels perpendicular to their surface are rather desirable for their nearly unhindered access to the pore interiors. However, previous studies are most based on MCM-type mesoporous silica nanospheres, whose internal structures are actually composed of numerous domains of hexagonally ordered pore bundles with different mesochannel orientations. To date, there is no report but one concerning monodisperse silica nanospheres with solid core and mesoporous shell, which inevitably involve a typical two-step sol–gel synthesis [11]. Nonetheless, in the view of employing MSNs with unhindered access to the pore interiors for applications, the function of the relatively large solid silica core is negligible. To the best of our knowledge, the synthesis of monodisperse silica nanospheres (MSNs) with radially oriented mesochannels has been rarely reported. Additionally, little work has been done to investigate the relationship between particle size and biocompatibility or cytotoxicity of these kinds of mesoporous silica nanospheres, which is of crucial importance for their further applications in fields including biomedicine, and catalysis.

Herein, we report a facile one-pot sol–gel approach for the synthesis of monodisperse silica nanospheres (MSNs) with radially oriented mesochannels, tunable diameter ranging from 280 to 500 nm. The synthesis is accomplished in an ethanolic aqueous solution by using cetyl trimethylammonium bromide (CTAB) as structure-directing agent, different amount of tetraethyl

* Corresponding author. Tel.: +86 021 52887063; fax: +86 021 52888277.
E-mail address: surgeonfu@163.com (D. Fu).

1387-1811/$ - see front matter Crown Copyright © 2013 Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.micromeso.2013.08.002

orthosilicate (TEOS) as the silica source and concentrated ammonia solution as the basic catalyst. The physicochemical properties of thus obtained mesoporous silica nanospheres were characterized by Field-emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM), Low angle X-ray powder diffraction (LA-XRD) and nitrogen adsorption–desorption. Further study of the size effect on the cellular uptake of MSNs was performed. The effect of the internalized MSNs of different sizes with radially oriented mesochannels on the cytotoxicity was assessed. It was found that the smaller MSNs have lower cytotoxicity. The in vitro cell imaging test reveals that the obtained MSNs possess excellent biocompatibility.

2. Experimental

2.1. Chemicals

Tetraethylorthosilicate (TEOS), cetyltrimethylammonium bromide (CTAB), ethanol and concentrated ammonia solution (28 wt%) were of analytical grade and purchased from Shanghai Chemical Corp. Fluorescein isothiocyanate (FITC) was purchase from Aldrich. All commercially available reagents were used as received without further purification. Deionized water was utilized for all experiments. Cell culture medium DMEM (Dulbecco's Modified Eagle Media) and fetal bovine serum were purchased from Gibico. The Chinese Hamster Ovary cells (CHO) were obtained from the Shanghai Institutes for Biological Science (China). Cell counting kit (CCK-8) was purchased from Dojindo Molecular Technologies, Inc. (Japan).

2.1.1. Synthesis of MSNs

Typically, 20 mL of ethanol was mixed with 40 mL of H_2O, followed by the introduction of 1.0 mL of concentrated ammonia solution (28 wt%). Then, the solution was vigorously stirred for 0.5 h and 5 mL of CTAB surfactant solution (100 mM, dissolved in a 2:1 mixture of H_2O and ethanol) was subsequently added. After stirring the mixture for 1 h, a certain amount of TEOS was added. The composition of CTAB/TEOS/ethanol/water mole ratio is 1:2.7(or 4.5 or 9):744:4810. Then the solution was stirred at 25 °C overnight. The resulting suspension was subjected to centrifugation to collect the as-made product. After further washing with deionized water and drying at 313 K for 12 h. The resultant white powder was finally calcined at 823 K for 6 h in air to remove CTAB, resulting in mesoporous silica spheres. To obtain mesoporous silica spheres with different sizes, 0.3, 0.5 and 1.0 mL of TEOS were used in the above procedure, to produce MSN-1, MSN-2 and MSN-3, respectively.

2.1.2. Labeling mesoporous silica spheres with FITC probe molecules

The dried 400-nm sized mesoporous silica spheres (200 mg) and fluorescein isothiocyanate (FITC, 1.0 mg) were dispersed in anhydrous toluene (50 mL) by ultrasonication, and the resulting solution was stirred at 40 °C for 6 h. The product was separated by centrifugation and thoroughly washed with ethanol. Finally, the obtained FITC-mesoporous silica spheres were vacuum dried at 40 °C for further use.

2.1.3. Cell uptake and confocal imaging of FITC-mesoporous silica-incubated living cells

The FITC-mesoporous silica spheres could be readily dispersed in the serum-free media due to their high water dispersibility, and the obtained stock suspension was diluted to 30 μg/mL and sonicated for 30 min for further use. For single-label imaging, HeLa cells were stained with 30 μg/mL FITC-mesoporous silica nanospheres dispersion in a 5% CO_2 incubator at 37 °C for 2 h. Then, living cell imaging was carried out after washing the cells with PBS once more to remove the excess FITC-mesoporous silica spheres.

Confocal imaging of cells was performed with an Olympus FV1000 laser scanning confocal microscope (LSCM) and a 60× oil-immersion objective lens was used. Cells loaded with FITC-mesoporous silica spheres were excited at 488 nm by using a multiline argon ion laser. Emission was collected from 500 to 600 nm.

2.1.4. Cytotoxicity assay

For cytotoxicity study, the synthesized MSNs materials (MSN-1, MSN-2, MSN-3) with different sizes were dispersed in PBS at final concentration of 1 mg/ml respectively, and sonicated for 5 min before added to the cell cultures. 2×10^4 CHO cells/well grown in 100 μl DMEM containing 10% FBS and 1% penicillin/streptomycin were cultured in 96-well plates overnight before the relative volume of dispersed MSN were added. The concentration gradient as 10, 20, 40, 60, 80, and 100 μg/mL were respectively designed in the final cell cultures. Cells without treating were set as control and the cell-free medium as blank. Six replicates were done in every group in order to obtain the mean and standard deviation. Plates were then incubated at 37 °C with 5% CO_2 for 3 days followed by the addition of 10 μL of CCK-8. After another 3 h of incubation, the absorbance (Abs) was measured at 450 nm using a microplate reader. Cell viability was determined as $\{(Abs_{treated} - Abs_{blank})/(Abs_{control} - Abs_{blank})\} \times 100\%$.

2.1.5. Characterizations

Transmission electron microscopy (TEM) experiments were performed on a JEOL 2011 microscope (Japan) operated at 200 kV. The samples for the TEM measurements were firstly suspended in ethanol and dropped onto a holey carbon film on a copper grid. Then the grid was dried in air for further TEM observation. Field-emission scanning electron microscopy (FESEM) images were taken on the Hitachi S-4800 field emission scanning electron microscope. Low-angle XRD patterns were obtained on a Bruker D8 diffractometer (Germany) with Ni-filtered Cu Kα radiation (40 kV, 40 mA). Nitrogen sorption isotherms were measured with Micromeritics Tristar 3020 analyzer at 77 K. All samples were degassed at 180 for 6 h prior to the analysis. Specific surface areas were derived from the adsorption branch in a relative pressure range from 0.05 to 0.25, using the Brunauer–Emmett–Teller (BET) method. Barrett-Joyner-Halenda (BJH) model was utilized to calculate the pore size distributions (PSD) from the adsorption

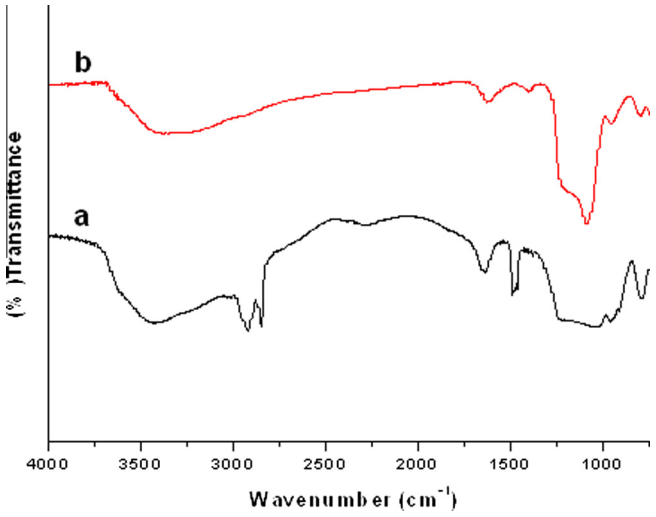

Fig. 1. Fourier transforms infrared spectra of (a) the as-made CTAB-silica composite and (b) MSN-1 after calcination in air at 550 °C.