Microporous and Mesoporous Materials 162 (2012) 14–23

Contents lists available at SciVerse ScienceDirect

Microporous and Mesoporous Materials

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

The shape effect of PEGylated mesoporous silica nanoparticles on cellular uptake pathway in Hela cells

Nanjing Hao [a,c], Linlin Li [a], Qiang Zhang [b], Xinglu Huang [a], Xianwei Meng [a], Yanqi Zhang [a], Dong Chen [a], Fangqiong Tang [a,*], Laifeng Li [a,*]

[a] Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing 100190, PR China
[b] The General Hospital of Chinese People's Liberation Army, Beijing 100853, PR China
[c] Graduate School of the Chinese Academy of Sciences, Beijing 100049, PR China

ARTICLE INFO

Article history:
Received 15 December 2011
Received in revised form 29 May 2012
Accepted 30 May 2012
Available online 8 June 2012

Keywords:
PEGylation
Shape
Serum protein
Mesoporous silica nanoparticle
Endocytotic mechanism

ABSTRACT

The shape of nanoparticles plays an important role in the interaction between cells and particles. However, the shape effect of mesoporous silica nanoparticles on the cellular uptake pathway and mechanism has not been reported. Herein, three different kinds of fluorescent mesoporous silica nanoparticles (FMSN, aspect ratio = 1, 2, and 4, ca. 100 nm in diameter) with similar fluorescent intensity at equivalent concentration were fabricated by a co-condensation strategy. In simulated body fluid (SBF), the PEGylated FMSN (FMSN-PEG) have higher dispersity and stability than the naked counterparts. So we chose the FMSN-PEG to research of shape effect on the cellular uptake pathway in Hela cells. We found that the uptake kinetics and pathway of three different shaped FMSN-PEG were obviously shape-dependent. The long-rod FMSN-PEG (NLR-PEG) showed higher intracellular retention amount than the short-rod FMSN-PEG (NSR-PEG) and the sphere FMSN-PEG (NS-PEG) almost over 8 h. The NSR-PEG showed the lowest intracellular amount especially with prolonged incubation time. We also found that the cellular entry pathway of NS-PEG, NSR-PEG, and NLR-PEG into Hela cells was regulated by particle shape. Spherical particles preferred to be internalized via the clathrin-mediated pathway, whereas MSN with larger aspect ratios (ARs) favored to be internalized via caveolae-mediated pathway, which could explain their different uptake kinetics. Our findings may provide useful information for optimizing the nano-based drug delivery and bio-imaging systems.

1. Introduction

Mesoporous silica nanoparticles (MSNs) with rigid structure, high specific surface area, large pore volume, tunable pore structure, great surface-modification capability and good biocompatibility have attracted great attentions for potential biomedical and biotechnological applications, such as protein/drug/gene delivery [1–3], cancer targeting therapy [4], and bio-imaging [5]. Particularly, previous studies including our own [6–9] have shown that MSN can be efficiently endocytosed and trafficked in mammalian cells. It is well known that cellular uptake and subsequent intracellular processing determine the final delivery efficiency of guest molecules by nanocarriers. Current evidences support that the nanoparticles could enter cells by means of endocytosis. It is distinguished that the different endocytotic pathways, such as clathrin-mediated endocytosis, caveolae-mediated endocytosis, and macropinocytosis, might affect the kinetics of intracellular complex processing and the final fate of nanoparticles with loaded guest molecules. For a sharpened understanding about intracellular fates of nanocarriers and efficiently delivering encapsulated guest molecules into given cells, the cellular uptake kinetics, the intracellular trafficking and the uptake mechanism, should be paid due considerations [10].

To date, it has be revealed that the uptake behaviors could be governed by cell types [11,12] and different chemophysical properties of nanoparticles, such as size [13,14], surface chemistry [15,16], and particle composition [17,18]. Recently, particle shape, which is an important physicochemical property of nanoparticles, has gained great attention and been demonstrated experimentally and theoretically to exert a great effect on cellular uptake behaviors [19–21]. Several studies have tried to explore the specific roles of particle shape on *in vitro* and *in vivo* biological behaviors of MSN. For example, Yu et al. [16] revealed that MSN with AR of 2 possessed higher hemolytic activity than that with AR of 1, 4, and 8, but the cellular toxicity on RAW 264.7 cells and A549 cells was not governed by particle shape. Our previous study showed that MSN with larger AR had a greater impact on cell proliferation, apoptosis, cytoskeleton formation, adhesion and migration, and

* Corresponding authors. Address: No. 29, Zhongguancun East Road, Beijing 100190, PR China. Tel./fax: +86 10 82543521.
E-mail addresses: tangfq@mail.ipc.ac.cn (F. Tang), lfli@mail.ipc.ac.cn (L. Li).

1387-1811/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.micromeso.2012.05.040

the cellular uptake rates of MSN with larger AR were faster than that of MSN with short AR at the 0.5 h time point [8]. We also found that the *in vivo* biodistribution, clearance, and biocompatibility of MSN were regulated by particle shape. Short-rod MSN (AR = 1.5) was easily trapped in the liver and had a more rapid clearance rate than long-rod MSN (AR = 5) in the excretion routes of urine and feces [22]. However, to the best of our knowledge, the shape effects of MSN on cellular uptake kinetics and endocytosis mechanism have still received little attention [16,21].

Recently, Meng et al. [23] showed that MSN with an AR of 2.1–2.5 were uptaken in higher quantities compared to shorter or longer length rods, and MSN with an AR of around 2 were internalized by macropinocytosis. However, the possible difference in uptake pathway of different shaped MSN was not been considered. Trewyn et al. [11] found that cellular uptake kinetics of spherical and tube-shaped FITC-doped MSN by CHO and fibroblast cells were regulated by particle shape and cell types. However, the possible reasons of different uptake kinetics induced by particle shape were not discussed. In addition, the stability of MSN in cellular incubation media was not considered. It has been reported that the components of incubation media, especially serum proteins, play significant roles in regulating dispersion status of nanoparticles and their interactions with cells [14,24]. The dispersity of nanoparticles also influences the fluorescent stability, which should be paid enough consideration to ensure the final accurate readout. Thus, a more controllable and stable system should be developed to explore the effect of shape on cellular uptake kinetics and mechanism. Polyethylene glycol (PEG), approved by FDA, has been widely used as a surface modifying agent to increase the dispersity of nanoparticles and can greatly increase the circulation half-life by inhibition of protein (opsonin) adsorption [25]. Therefore, PEGylation could provide great convenience for exploring the shape-mediated cellular uptake.

For researching the effect of shape on cellular uptake kinetics and pathway, herein, we fabricated different shaped FITC-doped MSN (FMSN) with similar particle diameter, chemical composition, surface charge, and especially similar fluorescent intensity for excluding the interference of other factors. To increase the dispersity and stability, the FMSN were PEGylated and the *in vitro* stability was studied. We then investigated the uptake kinetics and endocytosis pathway of FMSN-PEG with different shapes by Hela cells via fluorescent observation and flow cytometry. We also deeply discussed the cellular uptake mechanism of the differently shaped MSN.

2. Experimental

2.1. Reagents and materials

Cetyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS), aqueous ammonia, 3-aminopropyltrimethoxysilane (APTMS), anhydrous ethanol, and hydrochloric acid (HCl) were obtained from Beijing Chemical Reagents Company (China). Fluorescein isothiocyanate (FITC) and mPEG-SC (mPEG-succinimidyl carbonate, MW 5000) were purchased from Sigma and Beijing Kaizheng Biotech Development Co. Ltd. (China), respectively. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were from Gibco. Hoechst 33342 and Lysotracker Red were from Invitrogen.

2.2. Synthesis and characterization of FMSN

FMSN with different ARs were synthesized by two-step co-condensation strategy according to our previous report [22]. First, APTMS-FITC was prepared by stirring FITC in ethanolic APTMS solution (the amount of FITC was 4.6 mol% of APTMS) for 24 h. Separately, CTAB was dissolved in 70 mL H_2O, and $NH_3{\cdot}H_2O$ (28–30%) was added with magnetic stirring for 1 h. Two-thirds of the amount of TEOS was introduced to the solution and stirred for 1 h at room temperature. Then, APTMS-FITC and additional one-third of the amount of TEOS were added, respectively. After gently stirring for 5 h, the resultant samples were collected by centrifugation. The ARs of FMSN were controlled by changing the concentration of reaction reagents. For sphere, short rod, and long rod particles, the molar ratios of CTAB, H_2O, $NH_3{\cdot}H_2O$, TEOS, and APTMS-FITC in the reaction mixture were 0.07:1000:7:1:0.66, 0.14:1000:10:1:0.66, and 0.28:1000:13:2:1.32, respectively. To obtain the particles with mesoporous structures, CTAB as surfactant template was removed by extraction in acidic ethanol (1 mL of concentrated HCl in 50 mL ethanol) for 24 h. The particles were collected by centrifugation and then washed and redispersed several times with deionized water. All manipulations of the particles and solutions were performed in the dark. Morphology and structure of the resulting FMSN were observed with a JEM-2100 transmission electron microscope (TEM).

2.3. PEG modification of FMSN

In the FMSN fabrication process, the APTMS was excessive to FITC, which provides abundant free amino groups on the surface of FMSN. The PEGylation of FMSN was implemented by conjugating nanoparticles with mPEG-SC directly. In brief, 50 mg of the FMSN was dispersed in 10 mL of Tris–Cl buffer (50 mM, pH 8.0) and 25 mg mPEG-SC was added. The suspension was stirred for 4 h at room temperature, and the resulting PEGylated particles (FMSN-PEG) were collected by centrifugating and washing several times with deionic water. The nanoparticles were characterized using Varian Excalibur 3100 Fourier transform infrared spectrophotometer (FTIR) to detect the functional groups. The ζ-potential of naked and PEGylated FMSN was measured in a Malvern Zetasizer 3000HS.

2.4. In vitro fluorescent stability assay

The fluorescent intensity of different types of NPs was measured in physiological medium based on the previous methods [26,27]. In brief, a 100 $\mu g\ mL^{-1}$ solution of NPs was incubated with physiological saline solution containing 1.0% fetal bovine serum (FBS) for 0, 0.5, 1, 2, 4 and 8 h, respectively. Then, the fluorescent spectra of NPs in the presence or absence of FBS at different time intervals were measured with Varian Cary Eclipse Fluorescence Spectrophotometer. The resulting samples at 8 h time point were also observed by TEM.

2.5. Cell culture

HeLa cells were purchased from American Type Culture Collection and cultured in DMEM containing 10% fetal bovine serum, 2 mM L-glutamine, 100 U mL^{-1} penicillin, and 100 mg mL^{-1} streptomycin at 37 °C in 5% CO_2 and 95% air.

2.6. Fluorescent observation of cellular uptake

Hela cells (1.0×10^5) were seeded in 35-mm glass bottom dishes and were cultured in DMEM. After 24 h of cell attachment, the cells were treated with different shaped FMSN-PEG (100 mg mL^{-1}) for 2 h in serum-free media, and washed three times with PBS. Lysotracker Red, an acidotropic probe, was used for selective fluorescent labeling and tracking of acidic organelles in live cells using standard protocol (Invitrogen). The nucleus was stained with Hoechst 33342 (5 $\mu g\ mL^{-1}$) in serum-free DMEM