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The shape and size effects of polycation functionalized silica nanoparticles on gene transfection

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ABSTRACT

Silica nanoparticles are attractive candidates for the development of safe and efficient non-viral gene carriers, owing to their controlled morphologies, potential of facile surface modification and excellent biocompatibility as well as in vivo biodegradability. Conversely, the size and shape of nanoparticles are considered to have an intense influence on their interaction with cells and biological systems, but the effects of particle size and shape on gene transfection are poorly understood. In this work, a series of novel gene carriers were designed employing polycation modified silica nanoparticles with five different morphologies, while keeping uniform zeta potential and surface functionality. Then the effects of particle size and shape of these five different carriers on gene transfection were investigated. The morphology of silica nanoparticles is demonstrated to play an important role in gene transfection, especially when the amount of polycation is low. Chiral nanorods with larger aspect ratio were found to fabricate the most efficient gene carriers with compromised cytotoxicity. It was also noted that hollow nanosphere-based carriers exhibited better gene transfection performance than did solid counterparts. These results may provide new strategies to develop promising gene carriers and useful information for the application of nanoparticles in biomedical areas.

1. Introduction

Mesoporous silica nanoparticles demonstrate promising applications in many fields, such as catalysis, separation and biomedicine, owing to their controlled morphologies, high specific surface area and pore volume, potential for facile surface modification and excellent biocompatibility as well as in vivo biodegradability [1]. Monodispersed silica nanoparticles could form stable colloidal suspension and exhibit high movability in solution, so that they could be easily absorbed by cells, which makes them become promising candidates for drug and gene carriers [2–6]. Gene therapy plays an important role in the medical treatment of genetic disorders, while the key obstacle remains to explore an ideal gene carrier to transfer genes to the target cells efficiently and safely. Many non-viral carriers have been developed, including peptides, liposomes and polymers [7,8]. Compared with silica nanoparticles, they exhibited the drawbacks of high cost and lack of long-term stability. Moreover, silica nanoparticle-based gene carriers exhibit less cytotoxicity and could realize the delivery of drug/gene combinations, providing a more effective method of cancer therapy. Functionalized silica nanoparticles have attracted intense interests for gene delivery, owing to their advantages of protecting DNA from nuclease degradation and targeting cells successfully without immunogenicity or risk of cytotoxicity [9–11].

It has been put forward that physicochemical properties of silica nanoparticles, such as size, shape and surface charge can affect their non-specific uptake into cells and behaviors in biological systems, which might further influence their applications as gene carriers. Lu et al. [12] investigated silica nanoparticles with sizes ranging from 30 to 280 nm and reported that the maximum uptake by HeLa cells occurred at a nanoparticle size of 50 nm. Additionally, Zhu et al. [13] also suggested that cellular uptake efficiency of silica nanoparticles was highly size-dependent. Besides size, the shape, especially the aspect ratio [14], of silica nanoparticles is also considered to play an important role in cellular interactions. Huang et al. [15] revealed that silica nanoparticles with larger aspect ratios were taken up in larger numbers and had a greater impact on different aspects of cellular function, including cell

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proliferation, apoptosis, cytoskeleton formation, adhesion and migration. Furthermore, the shape of silica nanoparticles was proved to affect their interaction with human red blood cells [16] as well as in vivo biodistribution, clearance and biocompatibility [17]. The mechanism of the interaction between the nanoparticles and cell membranes affected by their morphologies was also widely investigated [18–21]. Therefore, the size and shape of silica nanoparticles have an intense impact on the interaction with cells and biological systems, which inspired the present authors to design safe and efficient gene carriers employing silica nanoparticles with different sizes and shapes.

The architecture of silica nanoparticles could be tailored by controlling the hydrolysis and polycondensation of tetraethyl orthosilicate (TEOS). It has been proposed that several factors, including catalyst, surfactant, temperature and pH, work synergically during the formation of silica nanoparticles [22,23]. Silica nanoparticles with a variety of morphologies, such as polyhedrons [24], nanorods [25], nanowires [26], helixes [27] and nanotubes [28], have been realized. The negative surface nature of silica nanoparticles significantly limited their application in biological systems. In order to carry maximal nucleic acid to the nucleus, surface modification through the rich hydroxyl groups is necessary to allow the positively charged surface to bind negatively charged DNA [29]. Moreover, to overcome the main problem of efficient gene transfection, silica nanoparticles must be engineered to escape from endolysosomal intracellular degradation to release cargo on the cytoplasm. Positively charged amino group [30] and polycations [11,31] could be used to functionalize silica nanoparticles to fabricate non-viral gene carriers with high efficiency and low cytotoxicity. Compared with small molecules, polycations endow the system with the characteristics of polymer, which could introduce target molecules through functional groups. Well-defined cationic poly((2-dimethylamino)ethyl methacrylate) (or PDMAEMA) could spontaneously condense DNA into compact complexes [32]; meanwhile, PDMAEMA could easily destabilize endosomes by the proton sponge effect and dissociate easily from the plasmid once delivered into the cytosol [8]. In general, high-molecular-weight PDMAEMA displays excellent gene transfer efficiency, but high cytotoxicity. In previous work, low-molecular-weight PDMAEMA was linked to layered double hydroxide nanoparticles [33] and hydroxyapatite nanoparticles [34] to achieve advanced gene delivery with compromised cytotoxicity. It is desirable that silica nanoparticles functionalized with moderate amount of PDMAEMA could produce high efficiency with reduced risk of cytotoxicity.

The present paper reports a series of novel gene carriers employing silica nanoparticles with different morphologies while uniform zeta potential and surface functionality. First, silica nanoparticles were synthesized with tailored morphologies and then modified with different amounts of PDMAEMA via atom transfer radical polymerization (ATRP). The effects of size and shape of silica nanoparticle carriers on gene transfection efficiency and cytotoxicity were investigated in detail to pick the most promising candidates for the development of safe and efficient gene carriers.

2. Materials and methods

2.1. Materials

TEOS (98%) and 3-aminopropyl triethoxysilane (APTES) were obtained from Energy Chemical Co., Ltd (Shanghai, China). Aqueous ammonia (NH_4OH, 25 wt.%, 99%), sodium hydroxide (NaOH, 96%), ethanol (99%) and dichloromethane (CH_2Cl_2, 99.5%) were obtained from Beijing Chemical Co. (China). Perfluorooctanoic acid (PFOA, 95%) and copper (I) bromide (CuBr, 98%) were purchased from Alfa Aesar Co. Cetyltrimethylammonium bromide (CTAB, 99%), Pluronic F-127 ($EO_{106}PO_{70}EO_{106}$), branched polyethylenimine (PEI; Mw ~25,000 Da), 2-bromoisobutyryl bromide (BIBB, 98%), triethylamine (TEA), *N*,*N*,*N*′,*N*′,*N*″-pentamethyl diethylenetriamine and 2-(dimethylamino) ethyl methacrylate (DMAEMA, 98%) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). DMAEMA was used after removal of the inhibitors in a ready-to-use disposable inhibitor-removal column (Sigma-Aldrich). 3-(4,5-Dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT), penicillin and streptomycin were purchased from Sigma Chemical Co. (St. Louis, MO). COS7 and HepG2 cell lines were purchased from the American Type Culture Collection (ATCC, Rockville, MD). The plasmid (encoding Renilla luciferase, pRL-CMV) was from Promega Co. (Cergy Pontoise, France), which was cloned originally from the marine organism *Renilla reniformis*. The plasmid DNA (pDNA) was amplified in *Escherichia coli* and purified according to the protocol of Qiagen GmbH (Hilden, Germany). All other chemicals were of analytical grade and used as received. The water used was deionized. The PDMAEMA homopolymer (M_n, $\sim 2.74 \times 10^4$ g mol^{-1}) with ~180 repeat units of DMAEMA was used as one control, as before [35].

2.2. Synthesis of silica nanoparticles with different morphologies

Silica nanoparticles with different sizes and shapes were synthesized by controlled hydrolysis and condensation of TEOS in the presence of surfactants and NH_4OH or NaOH as catalysts. Silica nanospheres with diameter ~100 nm (NS100) were obtained using the method described elsewhere [36], and 0.2 g of CTAB was first dissolved in 96 ml of water, and 0.7 ml of 2 M NaOH solution was added under stirring. The resultant mixture was then regulated at 80 °C for 30 min, followed by the addition of 1 ml of TEOS with vigorous stirring. The solution was regulated for another 2 h before centrifugation. The fabrication of hollow nanospheres with the diameter of ~100 nm (HNS100) was similar to that of nanospheres, except that 0.055 g of PFOA was introduced before the addition of TEOS [37]. In a typical synthesis of nanorods with diameter ~100 nm and length ~300 nm (NR300), 0.2 g of CTAB was dissolved in 70 ml of water, followed by the addition of 1.5 ml of ammonia under stirring. The obtained mixture was regulated at 26 °C for 1 h, and 1.2 ml of TEOS was then added with vigorous stirring, and the reaction was kept stirring for another 3 h. The synthesis of chiral nanorods with diameter ~100 nm and lengths ~200 nm and ~300 nm (CNR200, CNR300) was constructed by employing F127 and CTAB as a binary template [38]. The typical procedure was similar to the fabrication of silica nanorods (NR300) except that 0.2 g or 0.25 g of F127 was introduced in 100 ml water, and the amounts of reactants were 0.375 g or 0.4 g of CTAB, 1.3 ml or 1.2 ml of ammonia, and 1 ml or 1.2 ml of TEOS, respectively. All the silica solid products were collected by centrifuge, washed with water three times, and dried at 60 °C overnight. Calcination was carried out in air at 550 °C for 6 h to remove the surfactant template.

2.3. Immobilization of ATRP initiator on the surface of silica nanoparticles

The immobilization of the ATRP initiator on silica nanoparticles was carried out in two steps: (1) modification of the silica surface by APTES to produce the silica surface with terminal $-NH_2$ groups (SiO_2–NH_2); (2) reaction of the $-NH_2$ groups of silica nanoparticles with BIBB to produce the 2-bromoisobutyryl-immobilized nanoparticles (SiO_2–Br). The silanization process of SiO_2–NH_2 was performed by adopting similar procedures to those reported earlier [33,34,39]: 0.3 ml of APTES was added to the mixture of 18 ml of ethanol and 2 ml of water, and the solution was stirred for 30 min. Then, 0.2 g of SiO_2 nanoparticles was added, and the resultant reaction solution was stirred for 6 h at room temperature.