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Binding of CpG oligodeoxynucleotides to mesoporous silica nanoparticles for enhancing delivery efficiency

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ABSTRACT

We developed a potential cytosine-phosphate-guanosine oligodeoxynucleotides (CpG ODN) delivery system by binding of CpG ODN onto aminated mesoporous silica nanoparticles (MSNs) to form CpG/MSN-NH_2 complexes for Toll-like receptor 9 (TLR9)-mediated induction of cytokines. Serum stability, in vitro cytotoxicity, cellular uptake, and interleukin-6 (IL-6) induction of CpG/MSN-NH_2 complexes were investigated. The results showed that MSN-NH_2 nanoparticles had no cytotoxicity to Raw 264.7 cells, and binding of CpG ODN to MSN-NH_2 nanoparticles enhanced serum stability of CpG ODN due to the protection by nanoparticles. Furthermore, CpG/MSN-NH_2 complexes could be efficiently taken up by cells due to small particle size and good dispersity. Most importantly, CpG/MSN-NH_2 complexes significantly enhanced the level of IL-6 induction, stimulated by interaction between CpG ODN and TLR9 in endolysosomes. Therefore, MSNs would be a promising carrier for enhancing the delivery efficiency of CpG ODN.

1. Introduction

Cytosine-phosphate-guanosine (CpG) oligodeoxynucleotides (ODN) as a potent immunostimulator can induce innate and adaptive immune response through their recognition by human Toll-like receptor 9 (TLR9), a molecule located in the endolysosomes of B cells and antigen-presenting cells (APCs) [1–3]. For example, the interaction between TLR9 and CpG ODN in B cells induces proinflammatory cytokines-including interleukin (IL)-6, IL-10 and IL-12 through nuclear factor-κB and other signal transduction pathways, whereas in plasmacytoid dendritic cells, it induces type I interferons (IFNs), IL-6, IL-12 and tissue necrosis factor-α [4–6]. Therefore, CpG ODN can be used in immunotherapy for various illnesses including cancer, allergies/asthma, and infectious diseases [7–9].

To date, a variety of CpG ODN has been developed to stimulate immune response via the activation of TLR9. However, their clinical applications are hampered due to their nuclease degradation and rapid clearance in serum [10]. Recently, many efforts have been made to optimize the stability and activity of CpG ODN during CpG ODN delivery. Chemical modification of CpG ODN is an effective technique to protect against degradation by nucleases [11–13], but the modification of CpG ODN backbone could cause several side effects [14]. For example, the repeated administration of backbone-modified CpG ODN can result in the reduced immune response, lymphoid follicle destruction and organ enlargement [15]. Another strategy is to deliver CpG ODN using nanoparticles as carriers [16–27], which can not only efficiently protect CpG ODN from degradation through encapsulation of CpG ODN in their matrix or adsorption of CpG ODN on their surface, but also promote cellular uptake via endocytosis due to small particle size, and thereby enhance the cytokine induction and immune response. For example, Erikci et al. reported that the encapsulation of CpG OND in liposomes altered the release and delivery rates, and significantly increased Th1-biased cytokines and chemokines gene transcripts [17]. Sokolova et al. prepared multishell nanoparticles that cause CpG ODN to be adsorbed on calcium phosphate, which could protect CpG ODN from degradation by DNase. At the same time, calcium phosphate gradually dissolved in the acidic environment of the lysosome's interior, resulting in the slow release of CpG ODN [18]. Zhang et al. designed chitosan-coated boron nitride (BN) nanospheres to bind CpG ODN, and found that binding of CpG ODN on chitosan-coated BN nanospheres enhanced the production of IL-6 and TNF-α by peripheral blood mononuclear cells [19].

Mesoporous silica nanoparticles (MSNs) are a promising carrier for drug/gene delivery due to their mesoporous structure, biocompatibility and ease of surface functionalization. In particular, the mesoporous structure with high surface area, large pore volume

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and tunable mesopore size are beneficial for high drug/gene loading capacity through the storage of drug/gene in mesopores or adsorption of them on the external surface of MSNs [28,29]. Recent studies also confirmed the possibility of nucleic acid-based drug delivery using MSNs as carriers [29–38]. For example, Du et al. developed hydridized mesoporous silica nanocarriers by linking acetaldehyde cystine modified polyethylenimine (PEI) onto amino-functionalized dendrimer-like MSNs, and the hydridized nanocarriers not only showed low cytotoxicity and high gene loading capability, but also displayed high gene transfection efficiency [29]. Kim et al. reported the ability of aminated MSNs to deliver BMP2 plasmid DNA within rat mesenchymal stem cells, and the MSNs-based BMP2 pDNA delivery system showed a transfection efficiency of approximately 68% [30]. Most of the reported gene delivery systems are required to efficiently escape from endosomes after endocytosis, and thereby release genes to stimulate gene transfection. However, endosomal escape ability of MSNs is dependent on their particle size and surface chemistry, which could significantly influence the delivery efficiency [39–43].

For delivery of CpG ODN, the receptor TLR9 interact with CpG ODN in the endolysosome; therefore, endosomal escape of delivery system is not necessary, but high level of CpG ODN in the endolysosome could play an important role in enhancing the induction of cytokines and immune response. On one hand, high CpG ODN loading capacity on nanoparticles is thought to be crucial factor in the enhancement of delivery efficiency [25]. Studies demonstrated that MSNs can load high capacity of drug/DNA compared to conventional nanoparticles due to their high surface area and mesoporous structure [28,29]. On the other hand, cellular uptake of MSNs was also majorly dependent on the particle size, and in general, smaller MSNs exhibit high cellular uptake ability in vitro [39–43]. We reported SBA-15 mesoporous silica particles with an aspect ratio of 2 and a diameter of 500 nm as carrier for delivering CpG ODN to 293XL-hTLR9 cells, but the enhancement of delivery efficiency was limited due to the difficulty to be taken up by cells [26]. Therefore, high cellular uptake ability of small MSNs could contribute high level of CpG ODN in the endolysosome, and thereby stimulate cytokine induction and immune response.

In this study, we report a potential CpG ODN delivery system based on MSNs with a particle size of 60 nm. The small particle size of MSNs facilitates cellular uptake of CpG ODN delivery system, and mesoporous channels are beneficial for enhancing the CpG ODN loading capacity. After modifying with amino groups on MSNs, CpG ODN was bonded to MSNs through the electrostatic interaction to protect against degradation by nucleases. Furthermore, Raw 264.7 cells were used to culture with the CpG ODN delivery system, and in vitro cytotoxicity, cellular uptake and the TLR9-mediated induction of IL-6 were investigated in detail.

2. Experimental methods

2.1. Chemicals

Hexadecyltrimethylammonium p-toluenesulfonate (CTAT) and 3-aminopropyltriethoxysilane (APTES) were obtained from Sigma–Aldrich. Tetraethyl orthosilicate (TEOS), triethanolamine (TEA), ethanol were obtained from Sinopharm Chemical Reagent Co. Ltd. Agarose I™, 6 × sucrose DNA loading buffer II, 50 × TAE buffer, ethidium bromide (EB, 10 mg/mL), fetal bovine serum (FBS), CpG ODN (72 bases), ethylenediaminetetraacetic acid (EDTA) disodium salt dihydrate were obtained from Shanghai Sangon Biotech Co. Ltd. Ultrapure water was obtained from Millipore pure water system. All chemicals were analytical-reagent grade and used without further purification.

2.2. Synthesis of mesoporous silica nanoparticles (MSNs)

MSNs were prepared according to the previously reported method with some modification [44,45]. Briefly, CTAT (0.6836 g) and TEA (4 g) were dissolved in water (36 ml) under stirring and heated to 80 °C. After vigorous stirring for 1 h, 2 ml of TEOS was slowly added into the above solution, and continue to stir for another 2 h, resulting in the formation of a white colloidal precipitates. The white precipitates were collected by centrifugation, washed with water and ethanol for several times, and then dried in vacuum at 60 °C for 12 h. Finally, MSNs were obtained by calcining the white precipitates at 540 °C for 7 h to remove the organic templates.

2.3. Synthesis of aminated MSNs (MSN-NH_2)

Aminated MSNs (MSN-NH_2) nanoparticles were obtained by a reaction between MSNs and 3-aminopropyltriethoxysilane (APTES) in ethanol. Briefly, 300 mg of MSNs was homogeneously dispersed in 60 ml of ethanol by ultrasonication. And then, 0.9 ml of APTES was added to the suspension and slowly stirred the suspension for 24 h at the room temperature. The mixture was collected by centrifugation and extensively washed with ethanol, and the obtained white particles dried in vacuum at 60 °C for 24 h.

2.4. Characterization

Scanning electron microscopy (SEM) was carried out with an FEI Quanta 450 field emission scanning electron microscope. Transmission electron microscopy (TEM) images were obtained on a JEM-2100F transmission electron microscope. N_2 adsorption–desorption isotherms were obtained on a Micromeritics Tristar 3020 automated surface area and pore size analyzer at −196 °C under continuous adsorption condition. Brunauer–Emmett–Teller (BET) and Barrett–Joyner–Halenda (BJH) methods were used to determine the surface area and pore size distribution. Dynamic light scattering (DLS) measurements were performed on a Malvern zeta sizer Nano-ZS90. UV–vis analysis was conducted on a Nanodrop 2000C spectrophotometer.

2.5. Binding of CpG ODN to MSN-NH_2 nanoparticles (CpG/MSN-NH_2 complexes)

The sequence of CpG ODN molecule was 5′-TCAGAGAGTTAGA GAGTTAGAGAG-TCAGAGAGTTAGAGAGTTAGAGAGTCAGAGAGTTA GAGAGTTAGAGAG-3′ (72 bases), and CpG ODN was dissolved in ultrapure water at a concentration of 1 μg/μl and stored at −20 °C until use.

To bind CpG ODN to MSN-NH_2 nanoparticles, MSN-NH_2 nanoparticles were suspended in ultrapure water with a concentration of 1 μg/μl. Subsequently, the as-prepared MSN-NH_2 suspension dispersed in CpG ODN solution and the mixture with a weight ratio of MSN-NH_2/CpG ODN = 5, 10, 20, 50 or 100 was shaking at room temperature for 4 h. Then, the mixture was centrifugated at 12,000 rpm for 15 min to collect the CpG/MSN-NH_2 complexes. The supernatant was collected for UV–vis analysis to estimate the adsorbed amount of CpG ODN. The remained supernatants were analyzed with gel electrophoresis by loading onto 3% agarose gel with ethidium bromide and running with loading buffer at 120 V for 20 min.

2.6. Serum stability of CpG/MSN-NH_2 complexes

200 μg of CpG/MSN-NH_2 complexes containing 3 μg of CpG ODN was incubated in 25% FBS solution at 37 °C for 0, 2, 5 and 8 h, respectively. As a control, 3 μg of free CpG ODN was also