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# Mannosylated polyethylenimine coupled mesoporous silica nanoparticles for receptor-mediated gene delivery

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ABSTRACT

Organic–inorganic nanohybrids have been studied for their use as non-viral transfection agents. The purpose of this study was to examine the ability of mesoporous silica nanoparticles (MSN) coupled with mannosylated polyethylenimine (MP) to transfect plasmid DNA *in vitro*. Although MSN is biocompatible and has low cytotoxicity, it is not easily transfected into a variety of cell types. To overcome this barrier, MP was coupled to MSN (abbreviated as MPS) to target macrophage cells with mannose receptors and enhance transfection efficiency. The DNA conveyance ability of MPS was examined by evaluating properties such as particle size, zeta potential, complex formation, protection of plasmid DNA against DNase-I, and the release of DNA upon cell entry. Particle sizes of the MPS/DNA complexes decreased with increasing weight ratio of MPS to DNA, while the zeta potential increased. Complete MPS/DNA complexes were formed at a weight ratio of five, and their resistance to DNase-I was evaluated. Cytotoxicity studies showed that MPS/DNA complexes resulted in a high percentage of cell viability, compared with PEI 25K as a vector. The transfection efficiency of MPS/DNA complexes was evaluated on Raw 264.7 and HeLa cell lines. It was found that MPS/DNA complexes showed enhanced transfection efficiency through receptor-mediated endocytosis via mannose receptors. These results indicate that MPS can be employed in the future as a potential gene carrier to antigen presenting cells.



## 1. Introduction

In the past several years, gene therapy has been studied as a medical treatment due to its potential applications for the replacement of dysfunctional genes and cure of inherited and acquired diseases (Friedmann, 1996; Crystals, 1995a). Essentially, the term gene therapy refers to the transmission of DNA encoding a therapeutic gene of interest into targeted cells or organs with consequent expression of the transgene. However, a primary problem with gene therapy is how safely and efficiently the therapeutic genes are delivered to the targeted cells. In this context, many delivery systems have been developed that utilize a variety of materials. Systems currently under study are classified as either viral or non-viral vectors. Viral vectors are very effective in terms of transfection efficiency, but they have fatal drawbacks such as toxicity, immunogenicity, inflammatory response, and oncogenic effects when used *in vivo* (Crystals, 1995b; Tripathy et al., 1996). These limitations of viral vectors have led to the development of novel synthetic vectors which are non-viral in origin (Han, 2000).

In recent years, organic–inorganic hybrid materials, having properties both of inorganic and organic substances, have been extensively investigated as potential non-viral vectors for gene therapy applications (Chowdhury and Akaike, 2005). As an inorganic material, mesoporous silica nanoparticles (MSN), formed by polymerizing silica source in the presence of surfactants, have many advantages for intracellular delivery, such as large surface area, tunable pore sizes and volumes, and encapsulation of drugs, proteins and biogenic molecules. Moreover, they can be tailored with a variety of surface modifiers in order to increase biocompatibility and targetability. In addition, polyethylenimine (PEI) can be used as an organic modifier to generate efficient and versatile agents for gene delivery. PEI usage leads to high transfection efficiency due to its so-called 'proton sponge effect' allowing endosomal escape and transfer of DNA to the nucleus (Boussif et al., 1995; Yamazaki et al., 2000; Kirchis et al., 2001). However, the cytotoxicity and the transfection efficiency of PEI is affected by its various molecular weights and shapes (Fisher et al., 1999; Godbey et al., 1999). Some studies have demonstrated that high molecular weight PEI exhibits high transfection efficiency and cytotoxicity, while low molecular weight PEI shows reduced transfection efficiency and cytotoxicity (Thomas et al., 2005; Forrest et al., 2003; Tang et al., 2006).

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Macrophages play a major role as effector cells in the immune response to foreign antigens. These cells are specialized for the processing and presentation of antigens on the cell surface, which can lead to activation by T-cell recognition. The activated macrophages secrete various factors that regulate the development of the adaptive immune response and mediate inflammation, such as interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). For effective gene delivery and successful gene expression in macrophages, many strategies have been developed using selective targeting systems (Porgador et al., 1998; Akbari et al., 1999).

To enhance transfection efficiency and avoid non-specific interaction between plasma proteins and the cell membrane, receptor-mediated endocytosis of the DNA complexes is required. It is well known that mannose receptors are abundantly expressed on antigen presenting cells (APCs) such as macrophages (Sallusto et al., 1995; Ferkol et al., 1996; Jiang et al., 1995). The introduction of mannose to a gene carrier can provide selective macrophage targeting for a delivery system. Previously, Kim et al. reported that mannosylated chitosan induced receptor-mediated endocytosis and targeting into APCs, especially dendritic cells having mannose receptors (Kim et al., 2006).

This study describes the coupling of mannosylated polyethylenimine (MP) to the surface of MSN (abbreviated as MPS) as a method to lower cytotoxicity and enhance transfection efficiency through receptor-mediated endocytosis. The physicochemical properties of MPS/DNA complexes were evaluated by gel electrophoresis, particle size measurement and zeta potential measurement. The cytotoxicity and transfection efficiency of the MPS/DNA complexes *in vitro* were also evaluated with respect to the use of these nanohybrids as gene carriers.

## 2. Materials and methods

### 2.1. Materials

Tetraethyl orthosilicate (TEOS), hexadecyltrimethylammonium bromide (CTAB), 3-(triethoxysilyl)propylisocyanate, α-D-mannopyranosylphenyl isothiocyanate, and DNA (sodium salt, from calf thymus) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Branched polyethylenimine 1800 and 25 K were purchased from Wako (Osaka, Japan). Cell Titer 96 Aqueous One Solution Reagent for the cell viability assay, the Luciferase Reporter 1000 Assay System for the *in vitro* transfection assay and the Luciferase pGL3-control vector with SV-40 promoter were obtained from Promega (Madison, WI, USA).

### 2.2. Synthesis of mannosylated polyethylenimine (MP)

MP was synthesized according to a modified method of Diebold et al. (1999). Briefly, branched PEI 1800 dissolved in distilled water was mixed with various amounts of α-D-mannopyranosylphenylisothiocyanate in DMSO. The reaction was performed for 1 day at room temperature. Excess water was then added and lyophilized. This process was repeated several times. The content of mannose in MP was determined by a sulfuric acid micro-method (Monsigny et al., 1998).

### 2.3. Synthesis of MSN and MPS, PEI/MSN (PS)

MSN was prepared by the method of Radu et al. (2004). Briefly, CTAB (1.00 mg, 2.74 mmol) was first dissolved in 480 mL distilled water with NaOH (2.00 M, 3.5 mL). When the solution temperature was adjusted to 353 K, TEOS (5.00 mL, 22.4 mmol) was introduced dropwise to the solution. The mixture was stirred for 2 h to give rise to white precipitation. The solid product was spun down by centrifugation, washed with distilled water and methanol, and dried under vacuum. The resulting materials (1.50 g) were refluxed for 24 h in a solution of 9.00 mL of HCl (37.4%) in 160 mL methanol to remove the surfactant. The reflux was centrifuged, washed with water and methanol, and the surfactant-free MSN material was placed under high vacuum. MSN (1.00 g) was refluxed for 20 h in 80 mL anhydrous toluene with 3-(triethoxysilyl)propylisocyanate (0.25 mL, 1.00 mmol). The resulting material was mixed with MP in isopropyl alcohol. The product was centrifuged, washed with water and methanol, and dried under vacuum. The PEI was also coupled with MSN in isopropyl alcohol, and then centrifuged, washed and dried. The washing processes were repeated until no more PEI was detected in the supernatant solution. The synthetic scheme of MPS is shown in Fig. 1. The composition of MSP was evaluated by NMR spectroscopy (Bruker, 600 MHz, Germany).

### 2.4. Determination of grafting percentage

The amount of polymer grafted onto the silica surface was determined by weight loss by thermal gravimetric analysis (TGA Q5000-IR, TA instruments, USA). The polymer-grafted silica was heated at 10 °C/min to 800 °C under $N_2$. The percentage of grafting was determined by the following equation (Kaneko et al., 2006):

$$\text{Grafting}(\%) = \frac{A}{B}100$$

where *A* is the weight of polymer grafted onto the silica surface and *B* is the weight of MPS used in the reaction.

### 2.5. DNA retardation assay

The formation of plasmid DNA–nanoparticle complexes was examined by agarose gel electrophoresis. Complex formation was induced at various weight ratios from 1 to 50, and formation reactions were incubated at room temperature for 30 min. The complexes were loaded on 1% agarose gels, stained with ethidium bromide (0.2 μg/mL), and run with Tris–acetate (TAE) buffer at 100 V for 40 min.

### 2.6. Protection and release assay of DNA

MPS/DNA complexes and naked DNA (0.2 μg) were separately incubated with DNase-I (1 unit) in DNase/$Mg^{2+}$ digestion buffer (50 mM, Tris–Cl, pH 7.6, and 10 mM $MgCl_2$) at 37 °C for 30 min. All samples were subsequently treated with 4 μL 250 mM EDTA for 10 min to inactivate DNase-I digestion and mixed with sodium dodecyl sulfate (SDS) in 0.1 M NaOH (pH 7.2) at final concentration of 1.0%. Finally, all solutions were incubated at room temperature for 2 h and were run on 1% agarose gels in TAE running buffer at 50 V for 1 h.

### 2.7. Particle size measurement and zeta potential assay

The particle sizes and surface charges of the MPS/DNA complexes were determined according to weight ratios using an electrophoretic light scattering spectrophotometer (ELS 8000, Otsuka electronics, Osaka, Japan) with 90° and 20° scattering angles, respectively, at room temperature.

### 2.8. Transmission electron microscopy

The morphology of MPS/DNA complexes prepared at weight ratio 20 was observed using TEM (LIBRA 120, Carl Zeiss, Germany). One drop of MPS/DNA complexes was placed on a copper grid and