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Cationic methylcellulose derivative with serum-compatibility and endosome buffering ability for gene delivery systems

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ABSTRACT

In this work, methylcellulose was employed as a template polymer with graft of polyethylenimine 0.8 kDa (PEI0.8k) for gene delivery systems. Synthesized PEI-grafted oxidized methylcellulose (MC-PEI) could condense pDNA into positively charged and nano-sized particles, which could protect pDNA from serum nuclease. The cytotoxicity of MC-PEI was minimal in both serum-free and serum condition due to the biocompatibility of methylcellulose and low cytotoxicity of PEI0.8k. MC-PEI polyplex also showed low cytotoxicity in serum condition. In serum condition, MC-PEI showed less decreased transfection efficiency than PEI25k, meaning good serum-compatibility of MC-PEI. Bafilomycin A1-treated transfection results indicate that the transfection of MC-PEI is mediated via endosomal escape by endosome buffering ability. Flow cytometry results suggest that MC-PEI polyplex could be internalized into cells and efficiently deliver pDNA to cells due to its serum-compatibility. These results demonstrate that MC-PEI possesses a potential for efficient gene delivery systems.

1. Introduction

Gene delivery is to deliver genetic materials such as pDNA, oligonucleotides, or siRNA to cells for the induction, enhancement, or inhibition of gene expression. In order to deliver genetic materials to the targeted cells with high efficiency and low toxicity, tremendous gene delivery carriers have been developed, including viral vectors and non-viral vectors (Giacca & Zacchigna, 2012; Huang & Kamihira, 2013; Wang, Li, Ma, & Steinhoff, 2013).

Among them, polymeric gene delivery carriers have attracted a lot of attention due to their non-immunogenicity, low cytotoxicity, non-integration of exogenous genes into host chromosomes, ease of manufacturing, and the ability to transfer large size of genes (Luo & Saltzman, 2000). In addition, their capability to possess multiple bioactive functionalities via chemical modification makes them potential gene delivery carriers with high transfection efficiency (Jeong, Kim, & Park, 2007; Kang, Huh, & Bae, 2012; Liu & Huang, 2002).

Polysaccharides, long carbohydrate molecules of repeated monomer units joined together by glycosidic bonds, have been utilized as template polymers for gene delivery systems due to their advantages such as availability from replenishable resources, biocompatibility and biodegradability (Khan et al., 2012). In general, cationic moieties or other functionalities have been introduced to polysaccharides for gene delivery systems by chemical modifications of hydroxyl or carboxylate groups because they usually lack pDNA condensing ability except chitosan which has primary amines. For example, chitosan has been further modified to succinated chitosan (Toh, Chen, Lo, Huang, & Wang, 2011), chitosan lactate (Weecharangsan, Opanasopit, Ngawhirunpat, Rojanarata, & Apirakaramwong, 2006), poly(L-lysine)-grafted chitosan (Yu et al., 2007), or chitosan-graft-polyethylenimine (Jiang et al., 2007) for gene delivery systems. Cyclodextrin was also utilized for gene delivery systems as β-cyclodextrin containing polycations (Popielarski, Mishra, & Davis, 2003), cyclodextrin–polyethyleneimine conjugates (Forrest, Gabrielson, & Pack, 2005), or cationic polymers containing α-cyclodextrin and oligoethylenimine (Yang, Li, Goh, & Li, 2007). In the case of dextran, dextran–glycidyltrimethylammonium chloride conjugate (Thomas, Rekha, & Sharma, 2010), dextran-grafted polyethyleneimine (Tseng, Tang, & Fang, 2004), or

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dextran–spermine conjugate (Hosseinkhani, Azzam, Tabata, & Domb, 2004) has been developed for gene delivery systems. Other polysaccharides including pullulan (Rekha & Sharma, 2009), alginate (Krebs, Salter, Chen, Sutter, & Alsberg, 2010), or hyaluronan (Yun, Goetz, Yellen, & Chen, 2004) also has been utilized as template polymers for gene delivery.

Methylcellulose (MC) is one of the cellulose ether derivatives in which some hydroxyl groups of cellulose are methylated (1.4 < substitution degree < 2.5), which possesses an amphiphilic character. Its water solubility is known to depend on the distribution of methyl substituents (Hirrien, Desbrieres, & Rinaudo, 1996). MC has been used as a food additive or in cosmetics for thickening and emulsifier properties. Recently, applications of MC for hydrogel or drug delivery systems have been reported (Liang et al., 2004; Liu et al., 2004; Wang, Lapitsky, Kang, & Shoichet, 2009) due to its biocompatibility and unique sol–gel transition property (Hirrien, Chevillard, Desbrieres, Axelos, & Rinaudo, 1998). However, to our knowledge, chemical modification of MC itself has not been reported for gene delivery systems so far, although other cellulose derivatives such as hydroxypropyl cellulose (Xu et al., 2009) or carboxymethyl cellulose (Griesenbach et al., 2010) has been examined as gene delivery carriers and methylcellulose has been physically formulated for gene delivery (Griesenbach et al., 2010; Sinn, Burnight, Hickey, Blissard, & McCray, 2005).

In this study, methylcellulose was utilized as a template polymer for gene delivery systems due to its biocompatibility for the first time. MC-PEI was synthesized by grafting low molecular weight PEI (PEI0.8k) to periodate-oxidized MC (OXMC). PEI0.8k was employed to provide cationic property, maintaining low cytotoxicity. Then, the properties of MC-PEI for gene delivery systems were characterized to identify the potential as non-toxic and efficient gene delivery carriers.

2. Materials and methods

2.1. Materials

Methylcellulose (15 cP, 2% in H_2O), polyethylenimine (PEI, molecular weight 0.8k and 25 kDa), poly-L-lysine (PLL, molecular weight 70–150 kDa), agarose, ethylenediaminetetraacetic acid (EDTA), ethidium bromide, heparin sodium salt, and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma–Aldrich (St. Louis, MO). Sodium periodate and sodium tetrahydroborate were purchased from Junsei (Tokyo, Japan). The plasmid DNA, pCN-Luci containing a firefly luciferase reporter gene was amplified in *Escherichia coli* DH5α and isolated by PureLink HiPure Plasmid Filter Purification kit (Invitrogen, Carlsbad, CA). Luciferase assay system and reporter lysis buffer were purchased from Promega (Madison, WI). Fetal bovine serum (FBS), 0.25% Trypsin–EDTA, Dulbecco's phosphate buffered saline (DPBS), and Dulbecco's modified Eagle's medium (DMEM) were purchased from Invitrogen (Carlsbad, CA). BCA™ protein assay kit was purchased from PIERCE (Rockford, IL). All other chemicals were purchased and used without any further purification.

2.2. Synthesis and characterization of MC-PEI

First, MC and sodium periodate were dissolved in water, respectively. In the case of MC, MC powder was added to the about 1/3 of the required volume of hot water (80 °C) with agitation and the remainder of the water was added as cold water to lower the temperature. After cooling to 0–5 °C for 30 min, the solution was agitated further for at least 30 min at room temperature for complete solubilization of MC. Then, MC solution was added by dropwise to the sodium periodate solution while stirring, in order to oxidize MC. After 24 h of oxidation reaction (room temperature, dark condition, under nitrogen environment), the reaction mixture was dialyzed against ultrapure water with dialysis membrane (MWCO = 3.5k) for 2 days. The oxidized MC (OXMC) was obtained by following lyophilization. Secondly, PEI0.8k (5 molar equivalent to glucose units of OXMC) water solution was added to the OXMC solution for the conjugation of PEI to OXMC and the reaction was conducted for 24 h at room temperature (dark condition, under nitrogen environment). Sodium tetrahydroborate water solution was then subsequently mixed with the solution. After 24 h of further reaction (room temperature, dark condition, under nitrogen environment), the reaction mixture was dialyzed for 3 days. The final product, MC-PEI was obtained as a white solid after following lyophilization. The each step of polymer synthesis was confirmed by ^{1}H NMR and ^{13}C NMR (D_2O, 400 MHz JEOL JNM-LA400 and 600 MHz AVANCE 600). FT-IR spectra of the polymers were also recorded with a FT-IR spectrometer (Nicolet iS5, Thermo Scientific). The polymer samples were analyzed with the range of 600–4000 cm^{-1} using KBr pellets containing the prepared materials. The synthetic scheme of MC-PEI was shown in Fig. 1. The molecular weights of polymers were determined by gel permeation chromatography (GPC: YL-9100, Young Lin Instrument, Korea). Polyethyleneglycols with various molecular weights were used as standards. The assay was run on Ultrahydrogel 250 column with 1% formic acid as an eluent. The concentration of the polymer solutions was set to 10 mg/mL and the flow rate to 1 mL/min.

2.3. Agarose gel electrophoresis

Agarose gel electrophoresis was performed to examine the pDNA condensation ability of polymers. Agarose gel (0.7%, w/v) containing ethidium bromide (0.5 μg/mL) was prepared in Tris–Acetate–EDTA (TAE) buffer. The polyplexes (0.5 μg pDNA) were prepared in Hepes buffer (pH 7.4) at various weight ratios (polymer/pDNA) for 30 min of incubation at room temperature. After loading of samples, the electrophoresis was run for 15 min at 100 V (Mupid-2plus, Takara Bio Inc., Japan). The locations of pDNA bands were observed by UV illuminator (ChemiDoc XRS+ gel documentation system, Bio-Rad, Hercules, CA).

2.4. Protection ability of polyplex from serum

The protection ability of polyplex from serum was investigated by agarose gel electrophoresis. Polyplex solutions (0.5 μg pDNA) prepared at various weight ratios were incubated with 50% FBS for 30 min at 37 °C. Then, heparin sodium salt solutions were added to the polyplex solutions to 10 mg/mL of final concentration for the dissociation of pDNA from polyplex. After 30 min of further incubations, the polyplex solutions were electrophoresed and the locations of released pDNA bands were identified by UV illuminator. PEI25k polyplex was used as a control.

2.5. Average particle size and Zeta-potential measurements

Average particle sizes and Zeta-potential values of polyplexes were measured by Zeta-sizer Nano ZS (Malvern Instruments, UK) with He–Ne laser beam (633 nm) at 25 °C. The polyplex solutions (5 μg pDNA in 0.5 mL) were prepared in ultrapure water at various weight ratios ranging from 0.5 to 100. After 30 min of incubation, the polyplex solutions were diluted to 1 mL before measurements. Average particle sized and Zeta-potential values were measured 3 times.