Biomaterials 35 (2014) 4236–4246

Contents lists available at ScienceDirect

Biomaterials

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

A mannosylated cell-penetrating peptide-graft-polyethylenimine as a gene delivery vector

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ARTICLE INFO

Article history:
Received 23 November 2013
Accepted 27 January 2014
Available online 14 February 2014

Keywords:
Cell-penetrating peptides (CPPs)
Polyethylenimine (PEI)
Mannose
APCs-targeted gene delivery vector

ABSTRACT

Polyethylenimine (PEI) is widely applied in non-viral gene delivery vectors. PEI with high molecular weight is highly effective in gene transfection but is high cytotoxic. Conversely, PEI with low molecular weight displays lower cytotoxicity but less delivering efficiency. To overcome this issue, a novel copolymer with mannosylated, a cell-penetrating peptide (CPP), grafting into PEI with molecular weight of 1800 (Man-PEI_{1800}-CPP) were prepared in this study to target antigen-presenting cells (APCs) with mannose receptors and enhance transfection efficiency with grafting CPP. The copolymer was characterized by ^1H NMR and FTIR. Spherical nanoparticles were formed with diameters of about 80–250 nm by mixing the copolymer and DNA at various charge ratios of copolymer/DNA(N/P). Gel retardation assays indicated that Man-PEI_{1800}-CPP polymers efficiently condensed DNA at low N/P ratios. Cytotoxicity studies showed that Man-PEI_{1800}-CPP/DNA complexes maintained in a high percentage of cell viability compared to the PEI with molecular weight of 25 k (PEI_{25k}). Laser scan confocal microscopy and flow cytometry confirmed that Man-PEI_{1800}-CPP/DNA complexes resulted in higher cell uptake efficiency on DC2.4 cells than on Hela cells line. The transfection efficiency of Man-PEI_{1800}-CPP was significantly higher than that of PEI_{25k} on DC2.4 cells. More importantly, the complexes were mainly distributed in the epidermis and dermis of skin and targeted on splenocytes after percutaneous coating based on microneedles in vivo. These results indicated that Man-PEI_{1800}-CPP was a potential APCs targeted of non-virus vector for gene therapy.

1. Introduction

Gene therapy is a promising approach for the treatment of inherited and acquired diseases that are currently considered incurable [1,2]. However, the primary bottleneck of gene therapy is the safe and efficient delivery of therapeutic genes into the targeted cells. Over the past few decades, many non-viral delivery systems have been focused in research worldwide [3–5]. Polycationic polymers are extensively investigated as potential non-viral vectors, which can efficiently complex with negatively charged DNA, thereby increasing DNA stability. Polyethylenimine (PEI) is a promising candidate among polycationic polymers used for transfection [6,7]. Given these features such as effective DNA condensation, endocytotic pathway uptake, and endosomal escape capacity, PEI has superior transfection efficiency in vitro [8,9]. However, PEI/DNA complexes have two outstanding problems. Firstly, PEI with high molecular weight is highly effective in gene transfection but also shows high cytotoxic while PEI with low molecular weight displays lower cytotoxicity but limited delivering efficiency [10]. Secondly, PEI lacks satisfactory specificity toward targeted cells, which is another major drawback of gene delivery.

To overcome these issues, various modifications have been applied on PEI to reduce cytotoxicity but increase target specificity. One of the strategies is to couple macromolecules like polyethylene glycol [11,12] or combine with ligands for tissue-specific targeting [13,14].

Recently, cell-penetrating peptides (CPPs) have been widely used in gene delivery system [15]. They usually contain positively charged amino acid residues, such as arginine and lysine. These residues are capable of translocating various macromolecules

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http://dx.doi.org/10.1016/j.biomaterials.2014.01.065

across the plasma membrane and targeting the cell nucleus. Non-toxicity is one of the benefits of using CPPs for therapeutic delivery compared with other cytoplasmic delivery devices, such as liposomes and polymers. Moreover, CPPs can enhance the transfection efficiency of PEI with low molecular weight [16].

Specific ligands can bind to target cell-surface receptors, which can trigger receptor-mediated endocytosis [17]. Mannose is a most used ligand that binds mannose receptors on cells to induce receptor-mediated endocytosis, which increases the delivery efficiency and enhance the specificity to targeted cells [18]. As we known, the receptor is expressed on the surface of antigen-presenting cells (APCs; for example, dendritic cell and macrophages) in the immune system [19–21].

In this study, we synthesized a copolymer with mannosylated, a cell-penetrating peptide (CPP), grafting into PEI with molecular weight of 1800 (Man-PEI_{1800}-CPP) for gene-delivery vector to improve cell targeting, increase transfection efficiency, and reduce cytotoxicity. A schematic diagram depicting the formation of the complexes and in vivo cell targeting based on microneedle is shown in Fig. 1. We evaluated the physicochemical properties including of particle size and zeta-potential, DNA condensation and protection efficiency, in vitro cytotoxicity and transfection as well as cell uptake on DC2.4 cells with abundant mannose receptors and Hela cells with lacking of mannose receptors were all investigated in detail. Moreover, skin distribution of the complexes after percutaneous coating based on microneedle in vivo was observed and the effect of in vivo targeting on splenocytes was evaluated using BALB/c mice. Man-PEI_{1800}-CPP is expected to be an effective delivery system for plasmid DNA to improve transfection and target APCs in the immune system.

2. Materials and methods

2.1. Materials

Branched PEI_{25k}, α-D-mannopyranosylphenyl isothiocyanate (MPITC), 3-(4,5-dimethyl-thiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), agarose, and HEPES were purchased from Sigma–Aldrich (St Louis, MO, USA). PEI_{1800} (Alfa Aesar, Ward Hill, MA, USA) was used under vacuum drying oven at 70 °C for 1 h. Cell culture medium RPMI-1640 and fetal bovine serum were obtained from Gibco Co. (Life Technologies, Grand Island, NY, USA). DC2.4 and Hela cell lines were purchased from the Chinese Academy of Sciences Kunming cell library, China. The plasmid pEGFP-N2 encoding enhanced green fluorescent protein and the plasmid pGL3 encoding luciferase were obtained from BD Biosciences Clonetech Co. (Mountain View, CA USA). Prior to using, it was isolated from DH5-α *Escherichia coli* and purified using the Qiagen End-free Plasmid Purification Kit (Germantown, MD, USA), respectively. CY_3-labeled DNA was synthesized by Sangon Biotech Co. Ltd. (Shanghai, China). The luciferase assay system was purchased from Promega (Madison, WI, USA). The bicinchoninic acid (BCA) protein assay kit was purchased from the Beyotime Institute of Biotechnology (Haimen, Jiangsu, China). Cell-penetrating peptides (CPPs, RRRQRRKKRC-SH) was purchased from Shanghai Saiqi Trade Co., Ltd. (Shanghai, China). All the other chemicals and solvents were obtained commercially and used without further purification.

2.2. Synthesis of Man-PEI_{1800} and Man-PEI_{1800}-CPP

2.2.1. Synthesis of Man-PEI_{1800}

56 mg (or 180 μmol) MPITC was dissolved in 10 mL methanol and this solution was added dropwise into PEI_{1800} solution (108 mg or 60 μmol dissolved in 0.5 mL methanol). The reaction was allowed to proceed at room temperature for a while until no MPITC was detectable by thin liquid chromatogram (TLC) analysis. The reaction mixture was evaporated to remove methanol. The resulting sticky product was Man-PEI_{1800} as confirmed by IR and ^{1}H NMR.

2.2.2. Synthesis of Man-PEI_{1800}-CPP

9.0 mg (or 60 μmol) SPDP was dissolved in 1.0 mL methanol. This solution was added dropwise into the above obtained Man-PEI solution (dissolved in 10 mL methanol). After stirring for another 30 min at room temperature under N_2 until SPDP was absent in TLC, the reaction mixture was evaporated to remove the solvent. The residue was dissolved in 5 mL of PBS buffer (pH 7.5, 10 mM) to obtain Man-PEI-SPDP solution. 110 mg (or 60 μmol) CPP was dissolved in 1.0 mL PBS buffer and was added to the Man-PEI-SPDP solution. The mixture was stirred at room temperature under N_2 for 5 h and subsequently centrifuged at 12,000 rpm for 10 min. The supernatant was collected and purified by dialysis (molecular weight cut-off is 2 kDa) against de-ionized water for 2 days. After lyophilization, Man-PEI_{1800}-CPP, a solid white product, was finally obtained (Fig. 2.). This copolymer was confirmed by IR and ^{1}H NMR.

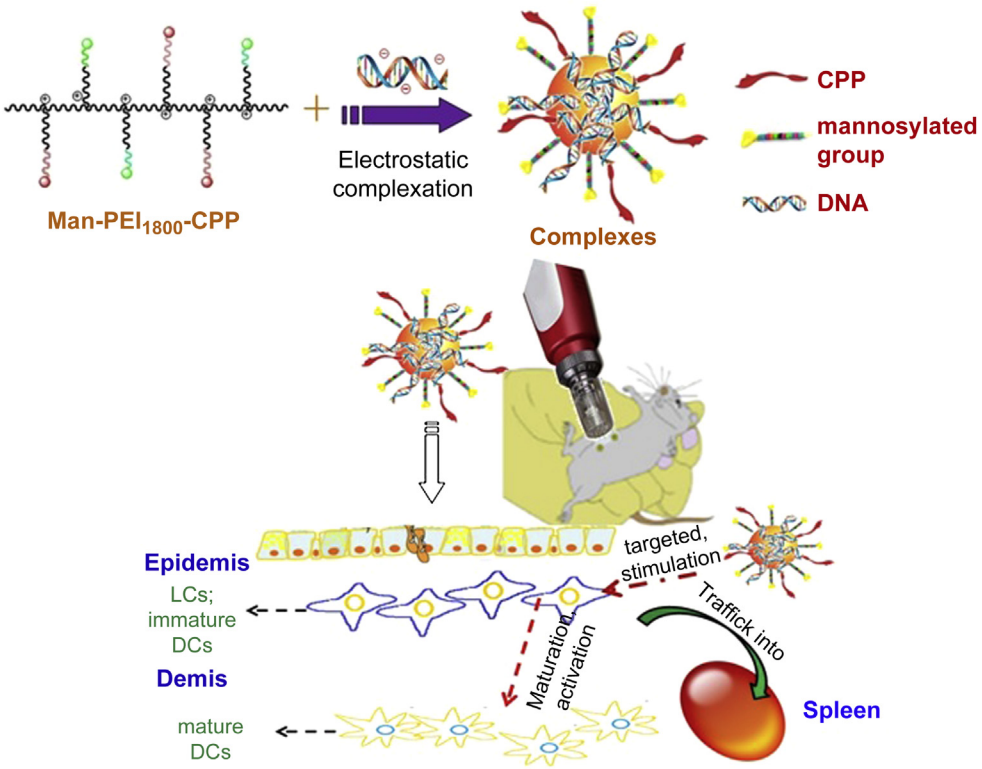

Fig. 1. A schematic diagram showing the formation of Man-PEI_{1800}-CPP/DNA complexes and the cell targeting of the complexes for microneedle-mediated transcutaneous delivery.