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A systemic gene vector constructed by zwitterionic polymer modified low molecular weight PEI

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ABSTRACT

Good blood compatibility and long-term circulation are very important to polycationic systemic gene vectors. In this work, polysulfobetaine-modified low molecular weight polyethyleneimine (LMW PEI, 1.8k) was synthesized and investigated as a vector for gene delivery in vitro and in vivo. PHEAA-b-PMPDSAH was synthesized via atomic transfer radical polymerization method, and then LMW PEI was grafted to PHEAA-b-PMPDSAH by an amido–hydroxy reaction. Incorporation of PMPDSAH into PEI was shown to retain the uncompromised ability to condense DNA into nanocomplexes. MTT assays revealed that the cytotoxicity of LMW PEI-PHEAA-b-PMPDSAH/DNA complexes was lower than that of PEI (25k)/DNA and LMW PEI-PHEAA/DNA complexes. LMW PEI-PHEAA-b-PMPDSAH$_{50}$ was much superior to PEI (25k) in mediating gene transfection in the presence of 10% serum. At higher serum contents, the transfection of LMW PEI-PHEAA and PEI (25k) was deteriorated, whereas LMW PEI-PHEAA-b-PMPDSAH$_{50}$ still retained better transfection efficiency, 8-fold more effective than PEI (25k). The expression of red fluorescence protein (RFP) was evaluated by small animal in vivo fluorescence imaging system and the results showed that the expression of RFP was much higher in the mice injected with LMW PEI-PHEAA-b-PMPDSAH$_{50}$/pDNA-RFP than with LMW PEI-HEAA/pDNA-RFP. Both in vitro and in vivo results suggested that LMW PEI-PHEAA-b-PMPDSAH$_X$ copolymer holds a great potential as a vector for systemic gene therapy.

1. Introduction

Non-viral vectors based on the cationic polymers appear to have a promising potential in gene therapy, given the problems of safety encountered with viral vectors [1]. The main obstacles in the use of polyplex nanoparticles via systemic delivery are their aggregation, instability, toxicity and their propensity to be captured by the mononuclear phagocyte system. The clearance rate of these vectors from the circulatory system in fact depends on their physicochemical surface characteristics. To be 'stealthy' (undetectable by macrophages), vectors have to be as small and neutral as possible [2–4]. PEG is currently the most used polymer in coating methods. With merits of this PEG shielding shell, non-specific interactions with biological components were minimized and allows for well dispersing in the blood fluid to stealthily circulate [5–9].

Recently, zwitterionic polymers arguably a promising alternative to conventional PEG, have been shown to exhibit ultra-high resistance to non-specific protein adsorption [10–12]. Zwitterionic polymers containing the pendant groups of phosphobetaine, sulfobetaine and carboxybetaine have attracted increasing attention due to their long term stability and high resistance to nonspecific protein adsorption. Whitesides et al. pointed out that self-assembled monolayers composed of sulfobetaine groups were effective in resisting adsorption of bovine serum albumin, fibrinogen and lysozyme than other hydrophilic groups [13]. Moreover, sulfobetaines were reported to be used in surface modification of biomaterials with good blood compatibility [11,14–16]. The nanoparticles modified with zwitterionic polymer possess a comparable serum stability and in vivo pharmacokinetic profile to their PEG-conjugated counterparts [17]. In our previous work, poly(N,N-dimethyl-N-(3-sulfopropyl) ammonium hydroxide)/polysulfobetaine (PDMAEMA-b-PMPDSAH) diblock copolymer was synthesized and explored as a gene delivery vector. The PDMAEMA-b-PMPDSAH exhibited increased gene transfection at higher serum contents compared to PDMAEMA homopolymer [18]. However, PDMAEMA-b-PMPDSAH diblock copolymer exhibited a lower gene transfection than that of PEI (25k).

Linear or branched polyethyleneimine (PEI) in a wide range of molecular weights have been extensively studied as DNA delivery vector both in vitro and in vivo [19,20]. Although high molecular weight (HMW) PEI (25k) showed high gene expression, its high toxicity becomes a major concern when using PEI to deliver DNA

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by systemic application [21,22]. On the other hand, LMW PEIs were less toxic but showed rather poor transfection activity. To overcome this dilemma, one effective approach is to crosslink LMW PEI to form HMW conjugates. These cross-linked conjugates showed considerably high transfection efficiency, meanwhile exhibiting low cytotoxicity. In the previous research, poly(2-(2-methoxyethoxy) ethyl methacrylate)-b-poly(2-hydroxyethyl methacrylate) ($PMEO_2MA$-b-PHEMA) grafted to PEI (1.2k) was synthesized and studied as a gene vector, which showed a low cytotoxicity and equivalent transfection efficiency comparing with PEI (25k) [23].

In this research, a new cationic polymer constructed by MPDSAH modified LMW PEI (1.8k) was used as a systemic gene vector. LMW PEI was grafted to linear polymer backbone to give comb-shaped copolymers and MPDSAH would provide the polymer with serum resistance. The physicochemical properties cytotoxicity, in vitro transfection, and gene transfection in different serum contents of this MPDSAH modified PEI/pDNA complexes were investigated. A plasmid DNA encoding red fluorescence protein (pDNA-RFP) and nude mice model were employed to evaluate the in vivo gene transfection.

2. Materials and methods

2.1. Materials

N-(3-(methacryloylamino) propyl)-N,N-dimethyl-N-(3-sulfopropyl) ammonium hydroxide (MPDSAH), N,N,N,N,N-pentamethyldiethylenetriamine (PMDETA), ethyl α-bromoisobutyrate(EbiB) copper chloride (CuCl), LMW PEI and HMW PEI, N-hydroxyethylacrylamide(HEAA) and N,N′-carbonyldiimidazole (CDI) were purchased from Sigma-Aldrich Chemical Co. Plasmid pGL3-control with SV40 promoter and enhancer sequences encoding luciferase was obtained from Promega Co. pDNA-RFP with COX-2 promoter was donated by Dr. Jun Liu, China Institute of Metrology. All other reagents used were analytical grade.

2.2. Synthesis of LMW PEI-PHEAA-PMPDSAH copolymers

The PHEAA-b-PMPDSAH copolymer was synthesized by atomic transfer radical polymerization method as previously reported [18]. Briefly, HEAA (3.2 mmol), NaCl (0.08 mmol), CuCl (0.04 mmol) and EbiB (0.04 mmol) dissolved in 5 mL ultra pure water were placed in a reaction tube, and degassed via three freeze thaw cycles. PMDETA (0.04 mmol) dissolved in 1 mL ultra pure water was then added via a syringe under nitrogen and the reaction mixture was degassed via another three freeze thaw cycles. The reaction was carried out at room temperature for 3 h. Then degassed MPDSAH (2 mmol or 5 mmol) and 2 mL ultra pure water were added into the reaction system under nitrogen. The reactor was placed again at room temperature for another 3 h. After that, the copolymer was dissolved in deionized water followed by dialysis (MWCO3500) to remove the impurities and unreacted monomers. Finally, the block copolymer was collected by freeze drying overnight. PHEAA was synthesized without the injection of MPDSAH. LMW PEI (1.8 kDa) was separately grafted to PHEAA and the block copolymer PHEAA-b-PMPDSAH activated by CDI [23]. After the reaction, the mixture was dialyzed (MWCO of 3500) in ultrapure water for 3 days to remove unreacted LMW PEI. Finally, the polymer was collected by freeze drying overnight. ^{1}H NMR spectra of the polymer solutions in D_2O were measured on Bruker Advance 300 MHz spectrometer. In this research, the molecular weight of PHEAA was fixed and the copolymer was described as LMW PEI-PHEAA-b-$PMPDSAH_x$; x indicated the real chain length of MPDSAH.

2.3. Preparation and evaluation of polymer/DNA complexes

The polymer/DNA complexes with varied weight ratios were prepared by adding a predetermined amount of LMW PEI-PHEAA-b-PMPDSAH or LMW PEI-PHEAA to an equal volume of pDNA solution. The actual experiments used for preparation polymer/DNA complexes, transmission electron microscope (TEM), electrophoresis assay and zeta potential were performed as reported previously [18]. The polymer/DNA complexes were formed at a fixed weight ratio of 20:1, and the particle size was measured by laser particle size analyzer both in water and in 0.9% NaCl solution.

2.4. Cytotoxicity assay and in vitro gene transfection

COS-7 cells (African green monkey kidney cells), plasmid pGL3-control and PEI (25k) were respectively used as the targeted cells, report gene and control to evaluate cytotoxicity. COS-7 cells were seeded into a 96-well plate at a density of 5×10^4 cells per well in 180 μL of growth medium and incubated for 24 h. The concentration of pDNA was fixed at 1 μg/mL and that of the cationic polymer was varied according to the mass ratio of polymer/DNA complexes. The polymer/pDNA complexes formed as mentioned above were incubated for 30 min before adding into the wells. After co-culture time of 24 h, the medium was removed and replaced with a mixture of 180 μL of growth medium and 20 μL MTT (5 mg/mL in PBS). The plate was further incubated for 4 h. Then all media were discarded carefully and 150 μL of DMSO was added to each well. The plate was gently shaken for 10 min to dissolve the blue formazan crystals. The absorbance was measured at 570 nm. The results were expressed as the mean percentage of cell viability relative to untreated cells.

COS-7 cells at a density of 1×10^5 were plated into a 24-well plate and incubated for 24 h. Then the medium was replaced with fresh DMEM at various concentrations of FBS. A sample of complex containing 1 μg of DNA was applied to each well. After incubating for 4 h, the complex-containing medium was replaced with cell-growth medium. Following 20 h of additional incubation, the growth medium was removed, and each well was washed with PBS twice. The cells in each well were treated with 150 μL of reporter lysis buffer for 15 min (Promega) followed by freeze thaw cycles to ensure complete lysis. The lysate was centrifuged for 2 min at 12,000 g at room temperature. Finally, 50 μL supernatant of each sample was put into a 96-well plate, and diluted with 50 ml of luciferase reagent (Bright-GloTM luciferase assay system, Promega, Madison, WI, USA). The amount of protein was determined by BCA protein assay kit. All transfection were done in triplicate. Values were given as mean relative light units (RLUs) per milligram of cell protein. PEI (25k) was used as a positive control. PEI/DNA complex with weight ratio of 2/1 was prepared referring to the reported protocols based on the comprehensive performance.

2.5. In vivo gene transfection and biodistribution

The experiment was carried out on six-week-old (20–25 g) male nude mice (Department of Laboratory Animal Science of Peking University Health Science Center, Beijing, China) housed at 24–26 °C. Protocols used were in conformance with the guidelines of the committee on animal experimentation of Peking University Health Science Center. The mice were divided into three groups randomly and 200 μL of solution were injected intravenously in the tail vein of mice. In Group B and C, 10 μg pDNA (within the complexes) was injected into the vein and the polymer/DNA complex with a ratio of 20:1 was used, which was in accordance with in vitro protocol. Herein, normal saline was used as a control (Group