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Synthesis and characterization of low molecular weight polyethyleneimine-terminated Poly(β-amino ester) for highly efficient gene delivery of minicircle DNA

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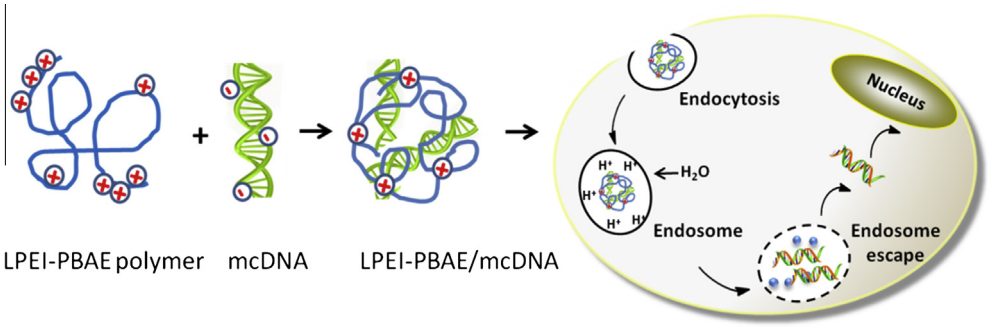
GRAPHICAL ABSTRACT



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ABSTRACT

Gene therapy has held great promise for treating specific acquired and inherited diseases. However, the lack of safe and efficient gene delivery systems remains as the major challenge. Poly(β-amino ester)s (PBAEs) have attracted much attention due to their outstanding properties in biosafety, DNA delivery efficiency and convenience in synthesis. In this paper, we reported the further enhancement of the PBAE functions by increasing its positive charge through conjugating with low molecular weight polyethylenimine (LPEI). The resulted LPEI–PBAE polymer was able to condense minicircle DNA (mcDNA) forming nanoparticles with a diameter of 50–200 nm. Furthermore, as compared to parental PBAE and a commercial transfection reagent very common in laboratory application, the LPEI–PBAE demonstrated significantly higher transfection efficiency with little cytotoxicity. These results suggested LPEI–PBAEs are worthy of further optimization for gene therapy applications.

1. Introduction

Gene therapy is one of the most promising therapeutics for the treatment of cancers [1,2] and many inherited and infectious diseases. Nevertheless, the lack of safe and efficient gene delivery systems still hinders its clinical application [3,4]. Although viral

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vectors are able to mediate gene transfer highly efficiently [5], their immunogenicity, potential genetic toxicity and high production cost severely hamper their applications [6]. Plasmid DNAs, the standard non-viral gene vectors, have the merits in safety and low production cost. However, their bacterial backbones pose multiple detrimental effects such as shutting down-transgene expression, the so-called transcriptional silencing effect [7,8]. Recently, minicircle DNAs (mcDNAs), a class of enhanced non-viral vectors encoding almost solely the transgene expression cassette, have attracted a lot of attentions [9]. Because of being free of plasmid backbone DNAs, minicircles are devoid of the associated detrimental effects and are able to express transgene product persistently [10]. However, they are still waiting for the delivery technology for wide clinical applications.

As the attempts to break down the bottleneck of non-viral vector-mediated human gene therapy, various DNA delivery techniques have been tried vigorously, including lipids [11], cationic polymers [12,13], dendrimers [14,15] and so on. Herein, a class of cationic gene vectors, Poly(β-amino ester)s (PBAE) have shown great potential with high transfection efficiency. They contain multiple amine groups which are able to interact with the negative-charged DNAs, resulting in their condensation effectively [16,17]. In addition, the mild Michael synthesis condition endows PBAEs with a variety of chemical diversity for further modification [18,19], and the hydrolysis degradable ester group reduces the cytotoxicity [20].

Polyethyleneimine (PEI) is one of the most commonly used gene transfection cationic polymers. Since the report of high transfection ability of PEI in 1995 [21], various derivatives of PEI were synthesized for gene transfection studies. It has been reported that only high molecular weight PEIs could bind nucleic acids to form stable and compact complexes [22], capable of entering cells through endocytosis and leading to a high gene transfection efficiency [23,24]. However, high molecular weight PEIs are not a good choice for human gene therapy due to their severe cytotoxicity. In this study, we used low molecular weight PEI (LPEI, MW 600 Da) to modify PBAE in order to increase its water solubility and positive charge density. Conjugation of LPEI to PBAE molecular chain was mediated by the reaction between the amines of PEI and acrylate groups of PBAE. We found that the resulted LPEI–PABE polymer could effectively condense mcDNA, and that the resulted nanoparticles demonstrated a high transfection efficacy and low toxicity when tested using pig iliac endothelial cells (PIEC) and human hepatocarcinoma cell line (Huh7).

2. Materials and methods

2.1. Materials

4-Amino-1-butanol and 1,4-butanediol diacrylate and dimethyl sulfoxide (DMSO) were purchased from Sigma–Aldrich. Branched PEI (MW 600 Da) was obtained from Aladdin Reagent Co., China. Diethyl ether, ethanol, and chloroform were purchased Sinopharm Chemical Reagent Co., Ltd, China. Trypsin (0.25%) was obtained from Sigma–Aldrich, USA. Penicillin and streptomycin were from Invitrogen-BRL, Carlsbad, USA. X-tremeGENE HP DNA Transfection Reagent was from Roche, Sweden. Cell Counting Kit-8 assay was from Dojindo, Kumamoto, Japan. All the reagents and chemicals were of analytical or HPLC grade unless otherwise specified and were used without further purification. DMEM, RPMI-1640 media and fetal bovine serum (FBS) were obtained from Shanghai Biotch Co., Ltd. (HyClone® Shanghai, China). The mcDNA expressing enhanced green fluorescent protein (eGFP) was made through the previous reported method [9,10]. PIEC cell (swine endothelial cell line) and Huh7 cell (human hepatocarcinoma cell line) were purchased from cell bank of Chinese Academy of Science, Shanghai.

2.2. Cell culture

All cell lines were cultivated at 37 °C in a 5% CO_2 incubator. PIEC cell was cultured in RPMI-1640 and Huh7 were cultured in DMEM, supplemented with 10% heat-inactivated FBS, 100 IU/ml penicillin and 100 IU/ml streptomycin.

2.3. Synthesis of PBAE polymers

Acrylate-terminated Poly(β-amino ester) (Acrylate-PBAE) was synthesized by a typical Michael addition reaction [25]. Briefly, 1.09 g 1,4-butanediol diacrylate and 0.446 g 4-amino butanol were separately added to a round-bottom flask. The reaction was performed at 90 °C for 24 h with stirring under the protection of nitrogen. Then, the flask was cooled down to room temperature. The obtained polymer was dissolved in $CHCl_3$ and precipitated by diethyl ether twice for purification.

LPEI–PBAE was obtained by dissolved 0.168 g Acrylate-PBAE and 0.056 g LPEI in $CHCl_3$, and then maintained the reaction stirring overnight at room temperature. The extra LPEI was removed by size exclusion chromatography on a Sephadex G-10 column using absolute ethanol as the mobile phase. The polymer was stored at −20 °C in DMSO (100 mg/ml).

2.4. Characterization of PBAEs

The confirmation of synthesis and the composition of the prepared PBAEs were determined by ^{1}H nuclear magnetic resonance (^{1}H NMR, AVANCE III 400, Bruker, Switzerland) and Fourier transform infrared spectroscopy (FTIR, FTIR-7600, Lambda, Australia). The molecular weight of PBAE copolymers were measured by using Gel permeation chromatography (GPC, Viscotek 270max, Malvern, USA). NaAc/HAc buffer solution (0.1 M, pH 5.0) was used as eluent at a flow rate of 1.0 ml/min.

2.5. Preparation and characterization of PBAE/mcDNA nanocomplexes

The LPEI–PBAE polymer in MDSO at 100 mg/ml was diluted with NaAc/HAc buffer (pH 5.0) to 10 mg/ml. Then, the PBAE solutions were rapidly added to mcDNA solution with equivalent volume to obtain a LPEI–PBAE/mcDNA complex. After incubation for 30 min at room temperature, the hydrodynamic particle sizes and zeta potential of the LPEI–PBAE/mcDNA nanocomplexes were measured by dynamic light scattering (DLS, Malvern Zetasizer Nano ZS, USA). The morphology of LPEI–PBAE/mcDNA nanocomplexes at N/P ratio of 80 were observed by transmission electron microscope (TEM, FEI Tecnai G2 F20 S-Twin, USA) at a low accelerating voltage after staining with phosphotungstic acid water solution (2%, w/w). The nanocomplexes were electrophoresed on the 1% (W/V) agarose gel containing EB and with Tris-acetate (TAE) running buffer at 110 V for 30 min. DNA was visualized with a UV lamp using a Bio-Rad Universal Hood II. For the DNase stability assay, the Acrylate-PBAE/mcDNA and LPEI–PBAE/mcDNA nanocomplexes (N/P ratio of 80) were incubated with DNase I at 0.1 U (1 μl) for 2 min at 37 °C, then added the stop solution. The resulting samples were treated with heparin and then subjected to electrophoresis as described above.

2.6. In vitro transfection and cytotoxicity

Firstly, PIEC and Huh7 cells were seeded in 96-well plates at 1.0×10^4 cells per well and incubated at 37 °C overnight. Then, the culture medium was replaced with blank medium containing PBAE/DNA nanocomplexes with 0.5 μg mcDNA at N/P ratio 30, 50 and 80 were added into the well. Cells were incubated for an additional 24 and 48 h. Transfection phenomenon was observed by