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The characteristics and transfection efficiency of PEI modified by biodegradable poly(β-amino ester)

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Abstract To improve the cytotoxicity of PEI25k and the transfection efficiency of poly(β-amino ester) with DNA, we synthesized a poly(β-amino ester), PEDP, bearing ester linkages in the backbone and tertiary amines in the backbone and side chain and prepared a binary mixture, PEDP–PEI25k, using physical blending meyhod. Both poly (β-amino ester) PEDP and binary mixture PEDP–PEI25k, readily self-assembled with plasmid DNA (pCMV-β gal) in a HEPES buffer, were characterized by dynamic light scattering. The results reveal that PEDP–PEI25k was able to self-assemble plasmid DNA into PEDP–PEI25k/DNA nano-complexes small enough to enter a cell through endocytosis. Titration studies were performed to determine the buffering capacities of PEDP and PEDP–PEI25k. The COS-7 cell viabilities in the presence of PEDP and PEDP–PEI25k were studied. At low mass ratio of PEDP/PEI25k (1/1), it is found that the transfection curve of PEDP–PEI25k/DNA bearing a maximum peak is similar to that of PEI25k/DNA. In addition, the PEDP–PEI25k/DNA complexes were able to transfect COS-7 cells in vitro with a high efficiency comparable to a well-known gene carrier PEI25k/DNA. The results indicate that binary mixture PEDP–PEI25k is an attractive cationic carrier for gene delivery and an interesting candidate for further study.

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1 Introduction

Two major classes of non-viral systems are cationic polymers and cationic lipids. These cationic materials form complexes with negatively charged DNA by electrostatic interaction leading to polyplexes and lipoplexes [1], respectively. They can potentially provide major advantages over viral counterparts because of greater control of their molecular composition for the simplified method and analysis, relatively lower immunogenic response, the possibility of selected modifications and the capacity to carry large inserts [2]. The polyplexes not only can protect DNA from nuclease degradation but also are self-assemble to form a nanoscale size sufficient to enter the cell through endocytosis [3, 4]. Also, cationic polymers would provide a pH-buffering ability allowing them to behave as a "proton sponges" which assisting in the escape of complexes from lysosome and improving the transfection efficiency [5, 6]. Cationic polymers, such as poly(ethylenimine) (PEI) [7], chitosan [8], poly-L-lysine (PLL) [9], and polyurethane [10–12], are usually studied on gene delivery. Several problems such as toxicity, lack of biodegradability, and low transfection efficiency need to be solved before practical use features in shuttling genes into cells [13, 14]. There have been great efforts to synthesize biodegradable cationic polymers as gene delivery. Clearly, the potential advantages are their reduced toxicity and avoidance of build-up of the polymer in the cells [15, 16]. Water-soluble cationic polyesters may potentially be used for delivering DNA, since they are able to form polyion complexes with DNA and may degrade quite rapidly [17–20]. We also

systemically investigated the correlations between the structure of polymers and the physicochemical characteristics of polymer/DNA complexes. In this study, PEDP and PEDP–PEI25k were designed and their structures in correlating to DNA condensation capacity, buffering capacity, cytotoxicities, and transfection efficiency are discussed. However, it is found that PEDP has some significant limitations, namely, condensation ability with DNA and poor buffering capacity. One of the chief causes of poor gene delivery is the inefficient release of complexes from endosomes into the cytoplasm. Poly(ethylenimine) (PEI) has been revealed to be the most effective non-viral carrier because of its high pH buffering capacity that is believed to enhance the escape of the complexes from the endosomal compartment. The binary mixture of PEDP and PEI25k prepared using physical method was used as the DNA carrier and studied on cellular delivery of plasmid DNA in this article.

2 Materials and methods

2.1 Materials

1,4-Butandiol diacrylate was obtained from Merck, Germany. Butyl amine and 3-(dimethylamino)-1-propylamine were obtained from Fluka, Switzerland. 1-(3-Aminopropyl)imidazole and Polyethylenimine (Branched PEI, Mw = 2500) were obtained from Sigma-Aldrich, Germany. The solvent of *N,N*-dimethylformamide (DMF, Tedia Co., USA) was dried over calcium hydride and distilled just before use. *N*-[2-hydroxyethyl] piperazine-*N*′-[2-ethanesulfonic acid] (HEPES) were obtained from Sigma Co. (USA). *N*-methyldibenzopyrazine methyl sulfate (electron-coupling reagent) and sodium (2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-2*H*-tetrazolium-5-carboxanilide) (XTT) were purchased from Roche Co. (USA). The plasmid pCMV-LacZ (pCMV-βgal) contained a CMV promoter to drive the β-galactosidase (LacZ) gene expression. The plasmid DNA was amplified in *Escherichia coli* (DH5α strain) and purified using column chromatography (Qiagen® Plasmid Mega kit, Germany). The purified plasmid DNA was dissolved in a tris(hydroxymethyl) methylamine-ethyldiaminetetraacetic acid (Tris-EDTA) buffer (pH 8.0) and determined using the ratio of UV absorbance at 260 nm/280 nm. Monkey SV40 transformed kidney fibroblast COS-7 cells were obtained from American Type Culture Collection (ATCC, CRL-1651). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM, GibcoBRL Co., Ltd.) supplemented with 10% FBS, 4.5 g/l glucose, 1.5 g/l sodium bicarbonate, and 4 mM L-glutamine, and maintained at 37°C in a humidified 5% CO_2-containing atmosphere.

2.2 Synthesis of Poly(β-amino ester) (PEDP)

The 1,4-Butandiol diacrylate with amino monomers molar ratio of 1/1 were mixed in anhydrous CH_2Cl_2 solvent within the double-necked reaction flask under dry nitrogen purge. Then heated ranged were from room temperature to 80°C and reacted for 0.5–6.5 h. The polymer was precipitated in ethyl ether and then dried at 40°C under vacuum. Yield was 86%. The polymer was characterized by FT-IR, ^{1}H NMR, and ^{13}C NMR.

2.3 Poly(β-amino ester) characterization

The poly(β-amino ester)s were characterized by nuclear magnetic resonance (NMR, Bruker Avancetm DPX-200 spectrometer) and Fourier transform infrared spectroscopy (FT-IR, Mattson Galaxy 5000 series spectrophotometer). All of the chemical shifts in ^{1}H NMR and ^{13}C NMR spectra were reported in parts per million (ppm). A 99.8% pure DMSO-d_6 was used as the solvent to characterize polymers. IR spectra were recorded on a spectrometer as KBr pellets. The molecular weights of polymers were determined by gel permeation chromatography (GPC) analysis (VISCOTEK, Refractive Index Detector, Model: 504). THF was used as the eluent and polystyrene as the reference. The weight- and number-average molecular weights (Mw and Mn, respectively) were calibrated with standard polystyrene samples. The sample concentration in the THF was 2.5 mg/ml, and the flow rate was 1.0 ml/min.

2.4 Buffering capacity of polymers

Acid-base titration was used to evaluate the buffering capacity of PEI25k, PEDP, and PEDP-PEI25k. In this assay, 10 mg of polymer was dissolved in 10 ml of 150 mM NaCl, and then 100 µl of 1 N NaOH was added to the solution to adjust the pH to the alkaline range at 11.6. HCl (0.1 N) was used as the titratant to lower the pH to acidic conditions at around 2.0–2.5. Titration increment size = 100 µl.

2.5 Hydrolytic degradation of polymers

PEDP was dissolved in a buffer solution (pH 7.4) with a concentration of 10 mg/ml, and then incubated in a water bath at 37°C for various durations. After hydrolysis for various durations, the solution was dried in a vacuum for several hours to remove water. The molecular weight of the polymer was determined using gel permeation chromatography (GPC).

2.6 Preparation and characterization of Polymer/DNA Complexes

5.0 mg/ml of the polymer was dissolved in 20 mM HEPES buffer solution (pH 7.4), and its serial dilutions were made. The polymer serial dilutions were rapidly added into the DNA solutions to obtain polymer/DNA complexes. Then, the complexes were allowed to self-assemble in the HEPES buffer solution and incubated at room temperature for 30 min before measuring. The hydrodynamic sizes of the polymer/DNA complexes were determined by dynamic light scatter (Nicomp 380 system, USA).

2.7 Gel electrophoresis of Polymer/DNA complexes

The electrophoretic mobility of polymer/DNA complexes were measured with 0.7% agarose gel in Tris-Acetate-EDTA (TAE) buffer containing ethidium bromide (0.6 μg/ml). After electrophoresis at 100 V for 90 min, the DNA band was visualized by UV irradiation and photographed.

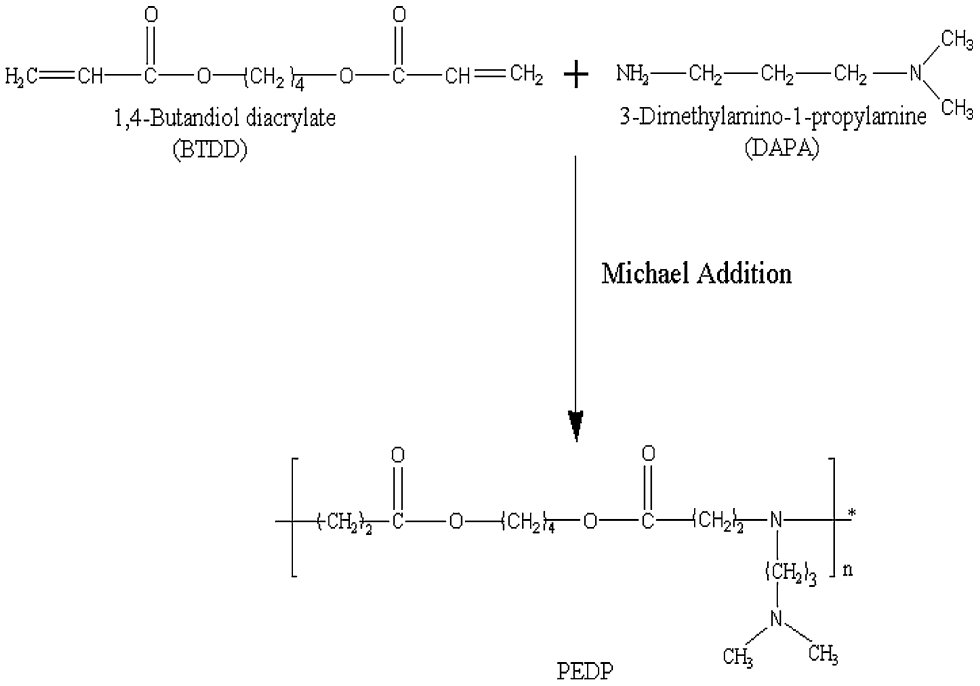
2.8 XTT assay

The influence of the polymer concentration on the cell viability was evaluated in a cell culture for the various polymers. The cytotoxicities of PEDP–PEI25k/DNA and PEDP/DNA for comparison with that of PEI25k/DNA were evaluated using the XTT assay. In a 96-well plate, COS-7 cells were cultured in complete DMEM and then seeded at a density of 1.0×10^4 cells/well. The cells were incubated at 37°C and 5% CO_2 in a humidified atmosphere for 24 h. Subsequently, the cells were incubated for 1 h in 200 μl FBS-free DMEM containing polymer with various concentrations. The cells were incubated in DMEM as a negative control. After 1 h, the cells were washed with 200 μl PBS solution and replaced by complete DMEM for a further 48 h of incubation. Then, 50 μl of XTT labeling mixture was added to each well and the cells were further incubated at 37°C for 1 h. Results are expressed as the relative cell viability (%) with respect to control wells containing culture medium.

2.9 Transfection protocol and ONPG assay

COS-7 cells were used to evaluate the transfection efficiency of polymer/DNA complexes. The cells were seed in a 96-well plate (1.0×10^4 cells per well) in complete DMEM and incubated for 24 h before transfection trials. The DNA concentration was kept constant at 5 μg/ml (1.0 μg/well) and the amounts of polymers were varied. 200 μl solutions of polymer/DNA complexes was taken and incubated with cells for 1 h at 37°C. The medium was replaced afterwards with complete DMEM and the cells were incubated for another 48 h. For evaluating transfection efficiency, the cells were washed with 0.3 ml PBS and then permeabilized with 20 μl cell lysis buffer at 4°C for 20 min. An ONPG solution (180 μg/well) was added after lysis treatment and the cells were incubated at 37°C for 1 h. The expression of pCMV-βgal gene was measured spectrometrically using an ELISA reader at a wavelength of 405 nm.

Scheme 1 Synthesis of PEDP



3 Results and discussion

3.1 Structural characterizations of synthesized Poly(β-amino ester)s

Poly(β-amino ester) (PEDP) was successfully synthesized through the reaction of 1,4-butandiol diacrylate with 3-(dimethylamino)-1-propylamine as Scheme 1. Figure 1 showed the FT-IR spectra of the reactants and products. The disappearance of peaks at 1650 cm^{-1}(C=C stretching, alkene) and 3320 cm^{-1} (NH stretching, amine) were found to represent the success of Michael addition reaction. The chemical shifts of the characterized protons and carbons are shown in Figs. 2 and 3 and listed in Tables 1 and 2, respectively. These shifts are based on the assigned labels of protons and carbons in the chemical structures of the PEDP as indicated. The FT-IR, ^{1}H NMR, and ^{13}C NMR characterizations of the synthesized polymers provided clear evidence that the PEDP was successfully synthesized. In addition, the GPC data of PEDP show that the weight-averaged molecular weight was 20500 with polydispersity index of 1.85.

3.2 Buffering capacity of polymers

Titration studies were performed to determine the buffering capacities of the various polymers regarding a proton buffering effect within the endosomal/lysosomal compartments of the cell (Fig. 4). All of the polycation solutions had a pH of 11.5–11.8 after the addition of 1.0 N NaOH. The non-viral vector PEI25k showed a buffering capacity over a wide pH range, which is probably due to the high amount of amine functions present in the polymeric chain. The physical incorporation of PEI25k content changed the buffering region of the PEDP alone. It was found that the slop of titration curves of PEDP–PEI25k was similar with PEI25k.The data showed the more PEI25k content the more buffering capacity was observed.

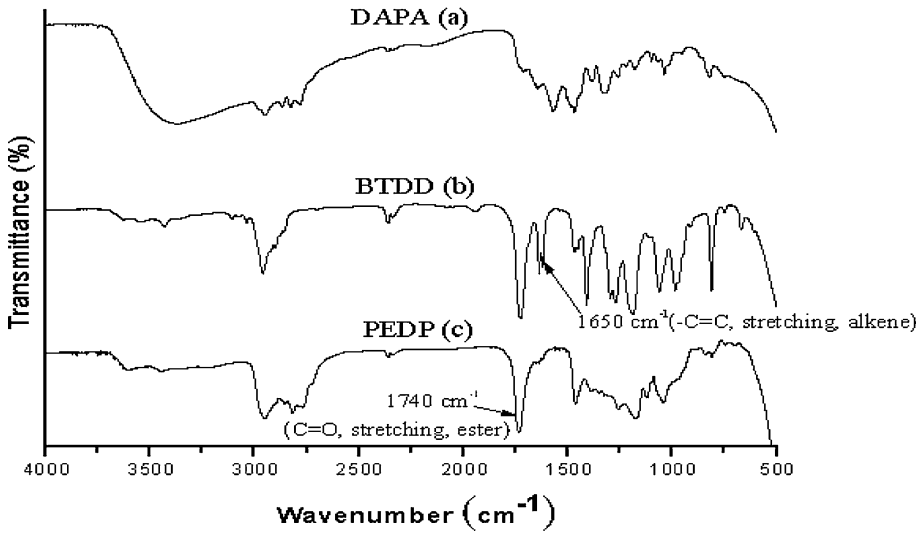


Fig. 1 FT-IR spectra of (*a*) DAPA, (*b*) BTDD, and (*c*) PEDP

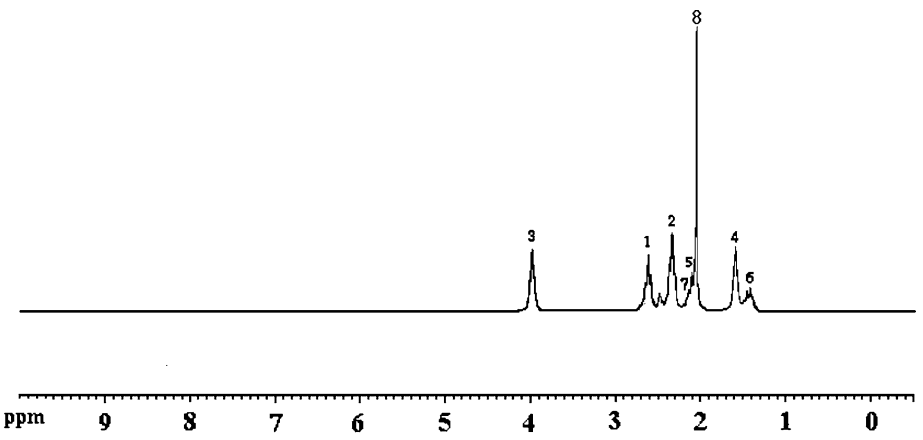


Fig. 2 ^{1}H-NMR spectrum of PEDP

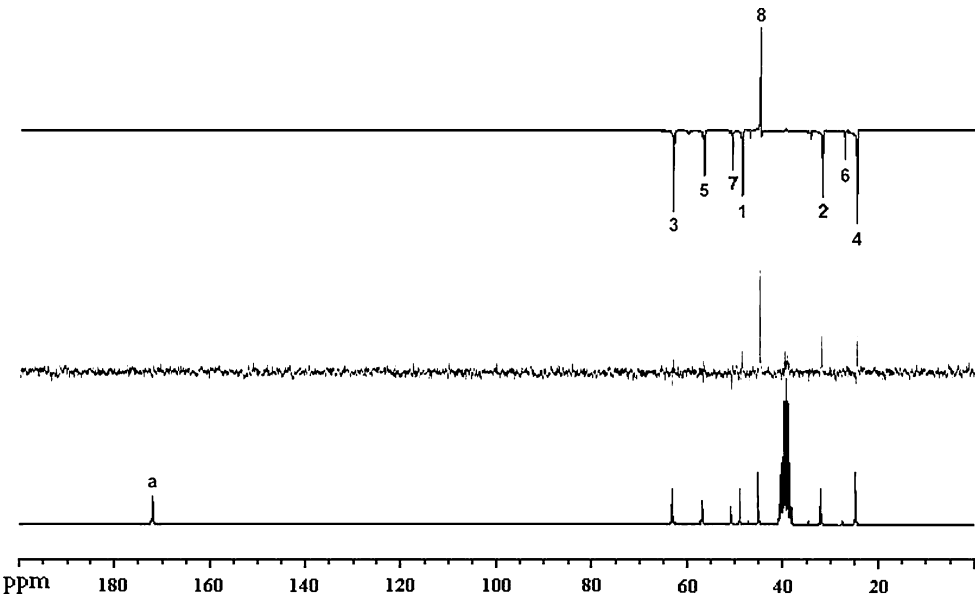
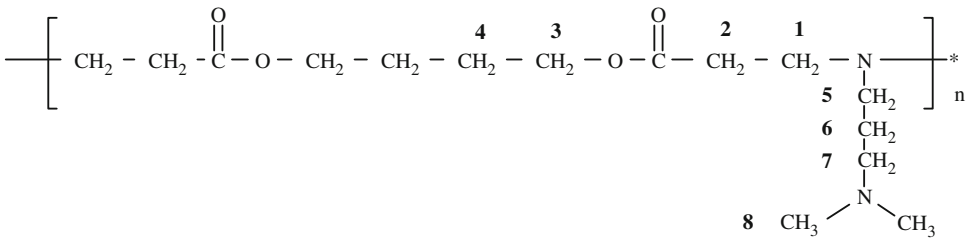


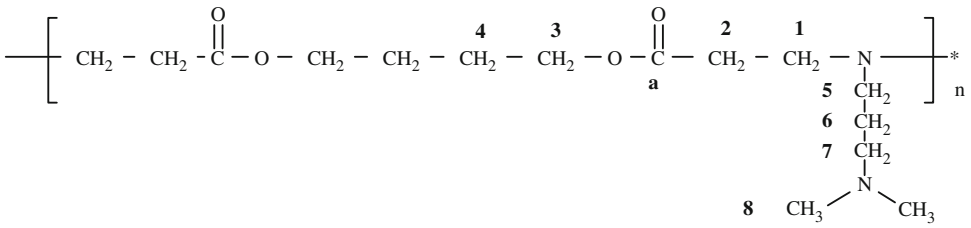
Fig. 3 ^{13}C-NMR spectrum of PEDP

Table 1 ^{1}H NMR data of PEDP



Types of proton	1	2	3	4	5	6	7	8
Chemical shift (ppm)	2.59–2.72	2.31–2.37	3.99	1.60	2.11–2.15	1.38–1.49	2.17–2.21	2.06

Table 2 ^{13}C NMR data of PEDP



Types of carbon	Aliphatic carbons								Carbonyl carbon
	1	2	3	4	5	6	7	8	a
Chemical shift (ppm)	48.9	32.1	65.3	24.8	56.9	27.4	50.9	45.1	172.0

3.3 In vitro hydrolysis of polymers

Figure 5 shows the degradation profile of PEDP which was monitored at 37°C at buffer pH values of 7.4 in order to approximate the environments within endosmal vesicles. There is a continuous decrease of molecular weight of these PEDP with time. The degradation studies indicate that the half-life of PEDP in the HEPES buffer was about

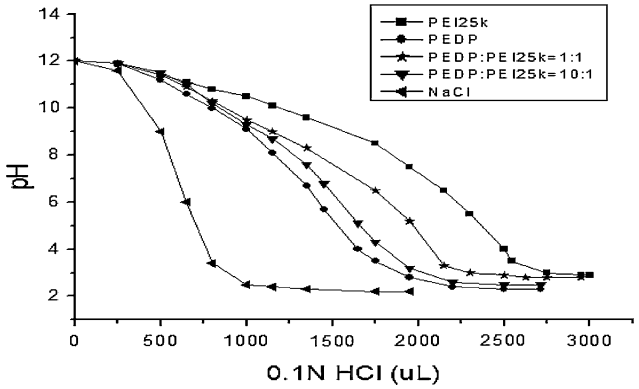


Fig. 4 Acid-base titration profile of various polymers with 0.1 N HCl

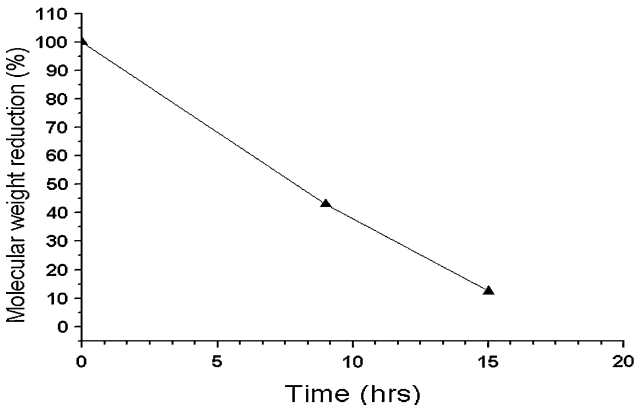


Fig. 5 Hydrolytic degradation of PEDP

8 h at pH 7.4. These results show that PEDP polymer exhibited higher hydrolytic degradation rate at pH7.4.

3.4 Particle size of polymer/DNA complexe and Zeta-potential of polymers

Figure 6 showed the sizes of PEDP and the binary mixture, (PEDP–PEI25k)/DNA, with various mass ratios (PEDP/PEI25k) ranging from 1/1 to 50/1, as determined by the dynamic light scattering (DLS). The particle size of PEDP/DNA complexes had an average diameter to exceeding 800 nm at all mass ratios. At the low mass ratio of (PEDP–PEI25k)/DNA, the particle size of the (PEDP–PEI25k)/DNA complexes had an average diameters from 50 to 200 nm, the size required for cellular endocytosis [21, 22]. The structure (linear, branched or dendritic) and molecular weight of polymers also affect the transfection efficiency in delivering DNA via endocytosis. The results showed that adding PEI25k into PEDP condense DNA better than PEDP alone. In Fig. 7, it shows the Zeta-potential of these carriers. It was found that the zeta-potential of PEDP–PEI25k increased as the PEI25k content increased. We also found that the Zeta-potential of the resulting complex (polymer/DNA) changed from negative charge to positive charge when the amounts of PEDP–PEI25k increased (data not shown). Complexes with extra positive charges on their surfaces had better interaction with the target cell membrane, resulting in an enhanced uptake.

3.5 DNA gel retardation assay

The electrophoretic mobility behavior of free DNA, PEDP–PEI25k/DNA, and PEDP/DNA is shown in Fig. 8. Increasing amounts of PEDP–PEI25k and PEDP led to the neutralization of DNA negative charges, as shown by gel retardation. The DNA mobility on agarose gel was influenced by the presence of PEDP–PEI25k and PEDP. Figure 8a–f shows the PEDP/DNA and PEDP–PEI25k can all condense DNA into complexes. Plasmid DNA was totally retained by PEDP at a mass ratio of 7/1 as shown in Fig. 8a. At the low mass ratio of 1/1 of PEDP–PEI25k/DNA, plasmid DNA was totally retained as shown in Fig. 8b–f.

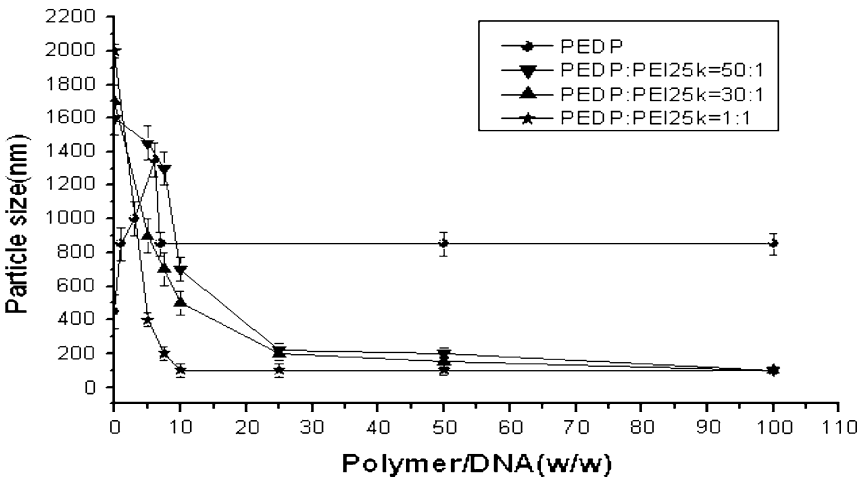


Fig. 6 The average effective diameter of polyplexes of various PEDP/PEI25k ratio (w/w) as a function of the PEDP-PEI25k/DNA ratio (w/w). Results are presented as mean ± SD ($n = 3$)

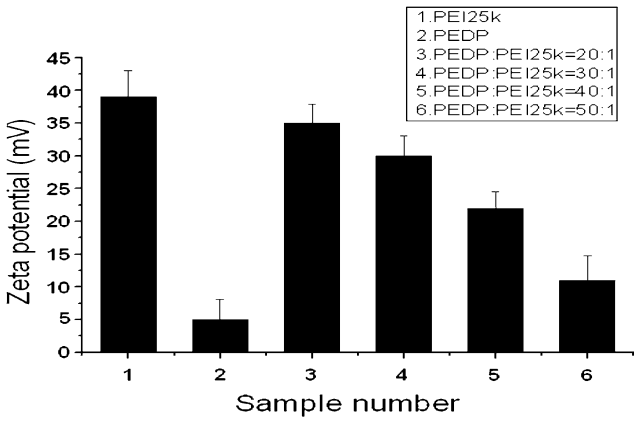


Fig. 7 Zeta potential of PEDP-PEI25K prepared at various mass ratios. Results are presented as mean ± SD ($n = 3$)

3.6 Cytotoxicity of polymer/DNA

A critical element for the gene delivery system is cytotoxicity. Cell damage resulting from a cytotoxic delivery system is deleterious because cells must be capable of supporting translation and transcription. To determine the cytotoxicity of PEDP–PEI25k/DNA and PEDP/DNA for comparison with that of PEI25k/DNA, we performed a XTT assay using the COS-7 cell line. Cells were incubated with increasing amounts of PEDP–PEI25k/DNA, PEU/DNA and PEI25k/DNA. Relative cell viabilities of PEDP–PEI25k/DNA, PEDP/DNA, and PEI25k/DNA were shown in Fig. 9. As can be seen, the cell viability of PEI25k/DNA complexes decreased rapidly with an increase in PEI25k concentration, whereas that of PEDP–PEI25k/DNA and PEDP/DNA did not change much with an increase in PEDP–PEI25k and PEDP, respectively. The results show that PEDP–PEI25k/DNA and PEDP/DNA exhibited substantially lower toxicity on COS-7 cells than did high molecule weight PEI25k/DNA. We infer that PEDP–PEI25k/DNA can provide better cytotoxicity profiles than these presently used for PEI25k/DNA due to the addition of Poly(β-amino ester)(PEDP), which reduces the high charge density of the polycation.

3.7 Cellular delivery of plasmid DNA viaPEDP and PEDP–PEI25k vectors

The transfection efficiency is expressed by the amount of β-galactosidase (equivalent to ONPG absorbance) measured in COS-7. The effect of weight ratios of PEDP/DNA, PEDP–PEI25k/DNA, and PEI25k/DNA complexes on transfection in COS-7 cells are shown in Fig. 10. As can be seen, PEDP–PEI25k containing branched PEI25k had the greater transfection efficiency than PEDP without branched PEI25k. The transfection efficiencies of PEDP–PEI25k/DNA and PEDP/DNA complexes increased with an increase in weight ratios, whereas that of PEI25k/DNA complexes did not observe. The best relative transfection efficiency of PEDP–PEI25k/DNA complexes was reached at a weight ratio 50/1(for weight ratio of PEDP/PEI25k = 1/1), which

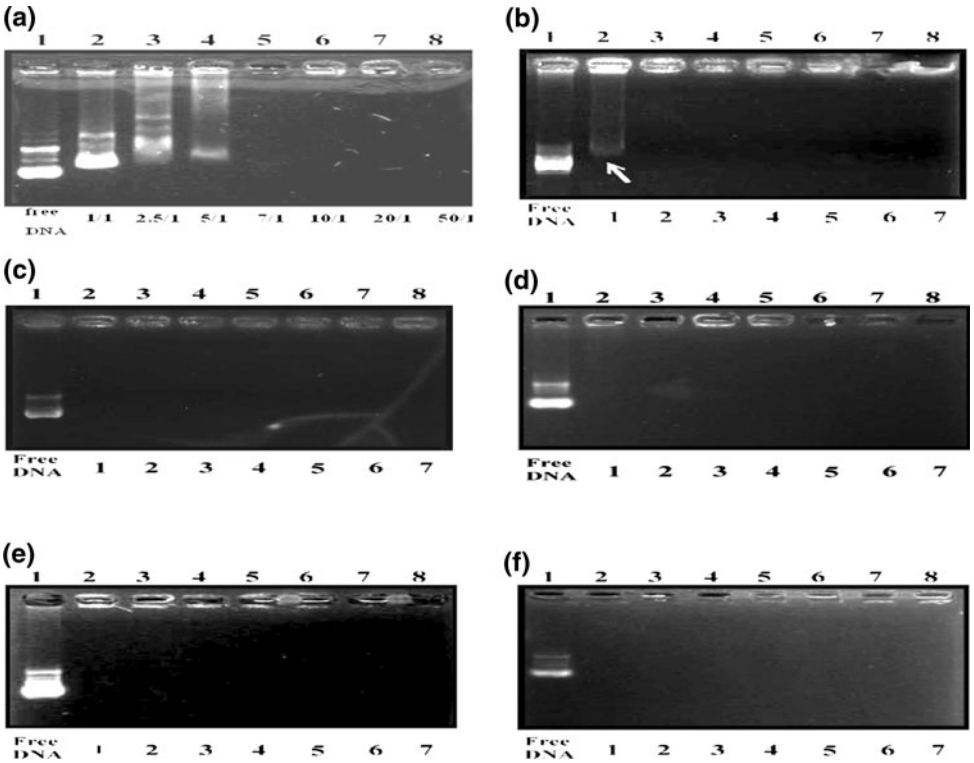


Fig. 8 Agarose gel electrophoresis of DNA condensed with the polymers of **a** PEDP, **b** PEDP/PEI25k = 50/1, **c** PEDP/PEI25k = 40/1, **d** PEDP/PEI25k = 30/1, **e** PEDP/PEI25k = 5/1, **f** PEDP/PEI25k = 1/1. Lanes 1 of **a–f**. 400 ng pCMV-βgal; Lanes 2–8 of **a:** PEDP/DNA (w/w): 1/1, 2.5/1, 5/1, 7/1, 10/1, 20/1, 50/1; Lanes 2–8 of **b–f:** PEDP-PEI25k/DNA (w/w): 1/1, 2/1, 3/1, 4/1, 5/1, 6/1, 7/1

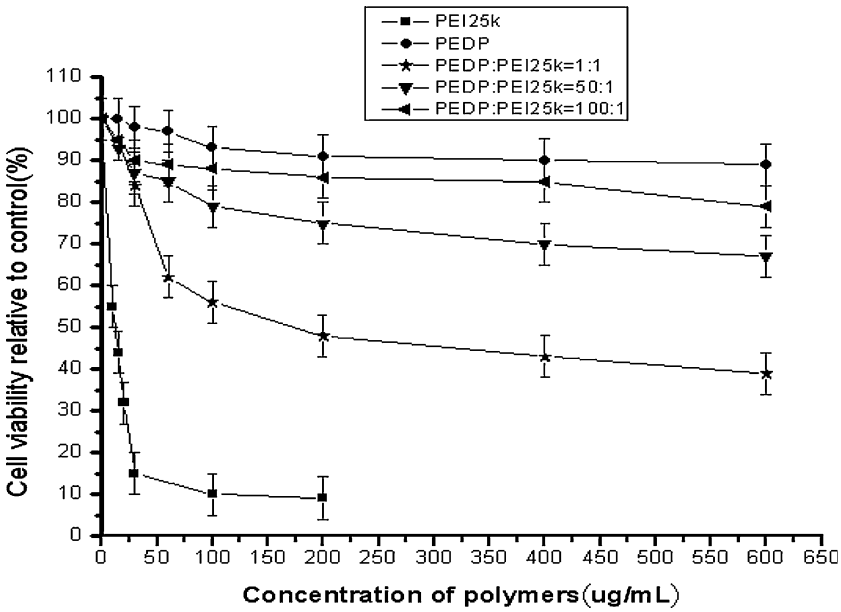


Fig. 9 Cytotoxicity of polymers in COS-7 cells. Results are presented as mean ± SD ($n = 3$)

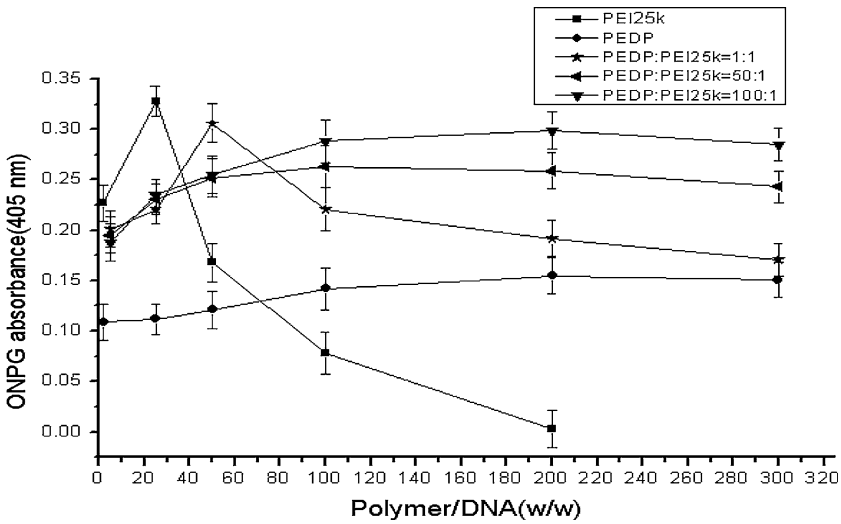


Fig. 10 Transfection efficiency of the polymer/DNA complexes into cultured COS-7 cells. Result are presented as mean ± SD ($n = 3$)

was higher than that of PEDP/DNA complexes. This discrepancy may be due to the difference in proton sponage effect and size distribution of relative complex. These results demonstrated that the physical introduction of branched PEI25k into the PEDP has a significant effect on the size distribution and transfection ability of relative complex.

4 Conclusion

Poly(β-amino ester) (PEDP–PEI25k) containing branched PEI25k with low cytotoxicity and high transfection efficiency was prepared and then characterized in this study. PEDP–PEI25k and DNA had a strong electrostatic interaction to self-assemble nano-particles. The positive charges of PEDP–PEI25k condense DNA to form DNA–polycation complexes. The transfection efficiency of PEDP–PEI25k into COS-7 cells was better than that of the PEDP without branched PEI25k, which resulted in numerous amine groups of PEDP–PEI25k with branched PEI25k in the binary mixture. PEDP–PEI25k could potentially be used in a non-viral gene delivery system.

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References

1. Zhang S, Xu Y, Wang B, Qiao W, Liu D, Li Z. Cationic compounds used in lipoplexes and polyplexes for gene delivery. J Control Release. 2004;100:165–80.

2. Kreiss P, Cameron B, Rangara R, Mailhe P, Aguerre-Charriol O, Airiau M, et al. Plasmid DNA size does not affect the physicochemical properties of lipoplexes but modulates gene transfer efficiency. Nucleic Acids Res. 1999;27:3792–8.
3. Lynn DM, Langer R. Degradable poly(β-amino esters): synthesis, characterization, and self-assembly with plasmid DNA. J Am Chem Soc. 2000;122:10761–8.
4. Anderson DG, Lynn DM, Langer R. Semi-automated synthesis and screening of a large library of degradable cationic polymers for gene delivery. Angew Chem Int Ed. 2003;42:3153–8.
5. Boussif O, Lezoualc'h F, Zanta MA, Mergny MD, Scherman D, Demeneix B, et al. A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. Proc Natl Acad Sci USA. 1995;92:7297–301.
6. Grabe M, Oster G. Regulation of organelle acidity. J Gen Physiol. 2001;117:329–44.
7. Gosselin MA, Guo W, Lee RJ. Efficient gene transfer using reversibly cross-linked low molecular weight polyethylenimine. Bioconjugate Chem. 2001;12:989–94.
8. Weecharangsan W, Opanasopit P, Ngawhirunpat T, Apirakaramwong A, Rojanarata T, Ruktanonchai U, et al. Evaluation of chitosan salts as non-viral gene vectors in CHO-K1 cells. Inter J Pharm. 2008;348:161–8.
9. Wolfert MA, Dash PR, Nazarova O, Oupicky D, Seymour LW, Smart S, et al. Polyelectrolyte vectors for gene delivery: influence of cationic polymer on biophysical properties of complexes formed with DNA. Bioconjugate Chem. 1999;10:993–1004.
10. Hung WC, Shau MD, Kao HC, Shih MF, Cherng JY. The synthesis of cationic polyurethanes to study the effect of amines and structures on their DNA transfection potential. J Control Release. 2009;133:68–76.
11. Shau MD, Tseng SJ, Yang TF, Cherng JY, Chin WK. Effect of molecular weight on the transfection efficiency of novel polyurethane as a biodegradable gene vector. J Biomed Mater Res A. 2006;77A:736–46.
12. Tseng SJ, Tang SC, Shau MD, Zeng YF, Cherng JY, Shih MF. Structural characterization and buffering capacity in relation to the transfection efficiency of biodegradable polyurethane. Bioconjugate Chem. 2005;16:1375–81.
13. Lv H, Zhang S, Wang B, Cui S, Yan J. Toxicity of cationic lipids and cationic polymers in gene delivery. J Control Release. 2006;114:100–9.
14. Godbey WT, Wu KK, Mikos AG. Poly(ethylenimine) and its role in gene delivery. J Control Release. 1999;60:149–60.
15. Nam HY, Nam K, Hahn HJ, Kim BH, Lim HJ, Kim HJ, et al. Biodegradable PAMAM ester for enhanced transfection efficiency with low cytotoxicity. Biomaterials. 2009;30:674–81.
16. Han M, Bae Y, Nishiyama N, Miyata K, Oba M, Kataoka K. Transfection study using multicellular tumor spheroids for screening non-viral polymeric gene vectors with low cytotoxicity and high transfection efficiencies. J Control Release. 2007;121:38–48.
17. Zhong Z, Song Y, Engbersen JFJ, Lok MC, Hennink WE, Feijen J. A versatile family of degradable non-viral gene carriers based on hyperbranched poly(ester amine)s. J Control Release. 2005;109:317–29.
18. Yamanouchi D, Wu J, Lazar AN, Craig Kent K, Chu C-C, Liu B. Biodegradable arginine-based poly(ester-amide)s as non-viral gene delivery reagents. Biomaterials. 2008;29:3269–77.
19. Anderson DG, Akinc A, Hossain N, Langer R. Structure/property studies of polymeric gene delivery using a library of poly([beta]-amino esters). Mol Ther. 2005;11:426–34.
20. Kim HJ, Kwon MS, Choi JS, Kim BH, Yoon JK, Kim K, et al. Synthesis and characterization of poly(amino ester) for slow biodegradable gene delivery vector. Bioorg Med Chem. 2007;15:1708–15.
21. Yang Y, Xu Z, Chen S, Gao Y, Gu W, Chen L, et al. Histidylated cationic polyorganophosphazene/DNA self-assembled nanoparticles for gene delivery. Int J Pharm. 2008;353:277–82.
22. Reineke TM, Davis ME. Structural effects of carbohydrate-containing polycations on gene delivery. 1. Carbohydrate size and its distance from charge centers. Bioconjugate Chem. 2003;14:247–54.