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Synthesis and characterizations of new glycidyl-based cationic poly(aminoester) and study on gene delivery

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ABSTRACT

New glycidyl-based (epoxide-based) poly(aminoester) (EPAE) containing hydroxyl and amino groups in the backbone and side chain was synthesized. EPAE self-assembled readily with the plasmid DNA(pCMV-βgal) in HEPES buffer and was characterized by dynamic light scattering, Zeta-potential, fluorescence images, and XTT cell viability assays. To evaluate the effect of molecular weight of EPAE system on transfection, EPAE polymers with three different molecular weights (EPAE22k, EPAE18k, and EPAE8k) were also prepared. This study found that all EPAE polymers were able to bind plasmid DNA and yielded positively charged complexes with a nano-sized particles (200 nm). The EPAE22k/DNA and EPAE18k/DNA complexes were able to transfect COS-7 cell in vitro with higher transfection efficiency than other EPAE8k/DNA. These results demonstrated that molecular weight of EPAE system had a significant effect on transferring ability. Examination of the cytotoxicity of PEI25k and EPAEs system revealed that EPAEs system had lower cytotoxicity. In this article, EPAEs seemed to be a novel cationic poly(aminoester) for gene delivery and an interesting candidate for further study.

1. Introduction

Non-viral gene delivery systems based on complexes of condensed DNA with polycations have attracted great attention in recent years. The polycations/DNA complexes not only can protect DNA from nuclease degradation but also have a nanoscale size small enough to enter the cell through endocytosis (Reineke and Davis, 2003; Yang et al., 2008). Amine-containing polycations such as poly L-lysine (PLL) (Asayama et al., 1997), poly(2-(dimethylamino) ethylmethacrylate) (PDMAEMA) (Van de watering et al., 1997), and polyethyleneimine (PEI) (Boussif et al., 1995), polyamidoamine (Zhang et al., 2005) have been proposed as carrier for genetic materials because they readily form complexes with DNA. However, these polycations are nonbiodegradable materials associated with a considerable degree of cytotoxicity. These drawbacks prohibit their use in some gene delivery systems where a toxicity-free carrier is required. Consequently, have been great efforts to synthesize biodegradable polymers that can be used as gene and drug carriers. The most important factors affecting the biodegradability of polymer materials included the chemical structure, physical and physico-mechanical properties (Acemoglu, 2004). Polymer degradation is classified as surface erosion (seen in polyanhydrides) or bulk erosion (displayed by polyesters, where it is controlled by the hydrophobic character of the polymer backbone). In surface erosion, the biodegradation proceeds exclusively at the surface of polymer. Degradation proceeds throughout the polymer matrix and immediate drop of molecular weight is observed in the case of bulk erosion. Polyanhydrides and polyesters are well-known biodegradable biomaterials used in wide range of applications, including the controlled release of therapeutic agents (Park et al., 1996; Luten et al., 2008). A number of reported biodegradable gene carriers include poly(4-hydroxy-L-proline ester) (Putnam and Langer, 1999), poly(α-(4-aminobutyl)-L-glycolic acid) (Lim et al., 2000), crosslinked poly(aminoester) (Lim et al., 2002), linear poly(β-aminoester)s (Guo et al., 2009; Alkin et al., 2003; Lynn et al., 2001), polyphosphoester (Wang et al., 2003; Zhao et al., 2003), polyurethanes (Liu et al., 2009; Jian et al., 2009), and polyethylenimine derivatives (Ahn et al., 2002; Petersen et al., 2002). Typically, these biodegradable polycations contain both chargeable amino groups, to allow for ionic interaction with the negatively charged DNA phosphate, and a degradable region, such as hydrolyzed ester, amide, and urethane linkages. Poly(β-aminoester) shows particular promise as delivery agents, as they are highly biodegradable in vitro and easily synthesized via Michael addition of a primary amine to a diacrylate. In addition to polymer composition, the polymer molecular weight is critical for transfection efficiency (Shau et al., 2006; Bieber et al., 2002). The molecular weight of a polymer is proportional to the number of polymers cation-DNA anion inter-

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actions and therefore affects the affinity of the polymer to DNA. Short polymers do not effectively condense DNA, while polymers that are too long retard the unpacking of DNA required for transcription and translation (Schaffer et al., 2000). The polymer/DNA ratio reflects the charge ratio between the positively charged polymer and the negatively charged DNA. This impacts a number of important transfection properties, such as the stability, cellular uptake level, and cytotoxicity of the resulting complex. In this study, new cationic biodegradable poly(aminoester)s, EPAEs, bearing two secondary amines and one tertiary amine in the backbone and three hydroxyl groups in one repeated unit, were synthesized. To evaluate the effect of molecular weight of EPAE systems on transfection, three EPAE polymers with different molecular weights were prepared in this article. The effects of molecular weight and hydroxyl group of EPAEs on transfection ability have been investigated. It was found that a polymer with higher molecular weight not only has higher transfection efficiency, but also a higher cytotoxicity because of the high cationic charge density. We also observed that the introduction of hydroxyl group into the backbone and side chain could improve the transfection efficiency.

2. Materials and methods

2.1. Materials

Glycidyl methacrylate and 1,4-diaminobutane were purchased from Acros Co. (USA). N,N-Dimethylethylenediamine and *n*-hexane were obtained from Fluka Co. (Switzerland). The solvent of N,N-dimethylformamide (DMF, Tedia Co., USA) was dried over calcium hydride and distilled just before use. Polyethylenimine (Branched PEI, Mw = 25,000), 6-amino-1-hexanol, ethyl isocyanatoacetate, and 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)-2H-tetrazolium-5-carboxanilide) (XTT) were purchased from Roche Co. (USA).

2.2. Polymer characterizations

The structures of the polymers were characterized by nuclear magnetic resonance (NMR, Bruker AMX-400 spectrometer) and Fourier transform infrared (FT-IR, Mattson Galaxy Series 5000 spectroscope). The molecular weight and distribution of the polymer was determined by gel permeation chromatography analysis (GPC, Waters Model LC-2410) based on polystyrene standards in THF.

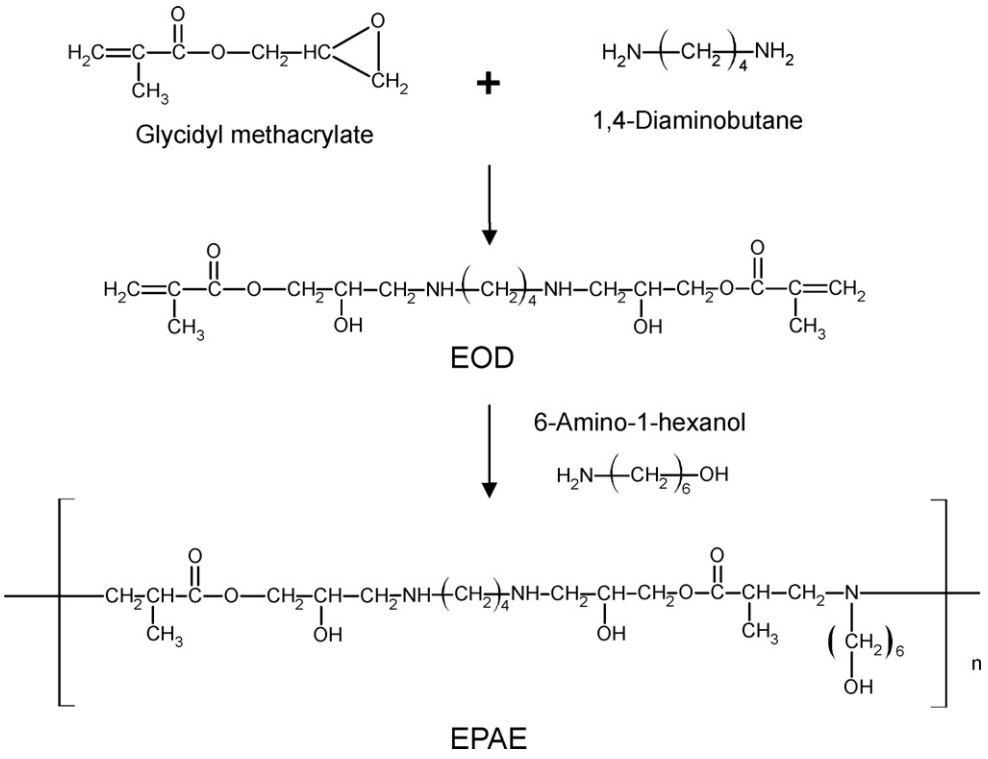
2.3. Synthesis of EOD

EOD was synthesized as shown in the first step of Scheme 1. 1.4-Diaminobutane and gylcidyl methacrylate with a molar ratio of 2/1 ($-NH_2$/epoxy) were mixed in anhydrous dichloromethane in a three-necked reaction flask under a dry nitrogen purge and then cooled to 18 °C to react for 24 h. The product was purified through column chromatography using elute solvent (acetone/ethyl acetate). The structure of EOD was characterized by FT-IR, ^{1}H NMR, and ^{13}C NMR.

EOD. ^{1}H NMR(400 MHz, d_6-DMSO, ppm) δ: 1.18(4H, $-NHCH_2C\underline{H}_2C\underline{H}_2CH_2NH-$), 2.08(2H, $-N\underline{H}CH_2CH_2CH_2CH_2N\underline{H}-$), 2.15(6H, $-C(C\underline{H}_3)=CH_2$), 2.60(4H, $-NHC\underline{H}_2CH_2CH_2C\underline{H}_2NH-$), 2.80(4H, $-NHC\underline{H}_2CH(OH)-$), 4.06(2H, $-NHCH_2C\underline{H}(OH)-$), 4.59(2H, $-C\underline{H}_2OCO-$), 5.66(2H, $-C(CH_3)=C\underline{H}_aH_b$), 6.07(2H, $-C(CH_3)=CH_a\underline{H}_b$). ^{13}C NMR(400 MHz, d_6-DMSO, ppm) δ: 18.53 ($-COO-C\underline{C}H_3=CH_2$), 30.71 ($-\underline{C}H_2CH_2NH-$), 49.12 ($-CH_2\underline{C}H_2NH-$), 49.23 ($-NH-\underline{C}H_2CHOH-$), 57.54 ($-CH_2-\underline{C}HOH-CH_2-$), 59.71 ($-CHOH-\underline{C}H_2-COO-$), 125.58 ($-CCH_3=\underline{C}H_2$), 136.15($-\underline{C}CH_3=CH_2$), 175.15 ($-\underline{C}OO-$)

2.4. Synthesis of EPAE

The monomers of EOD and 6-amino-1-hexanol with a $-C=C/-NH_2$ molar ratio of 1/1 were mixed in anhydrous N,N-



Scheme 1. Synthesis of EPAE.

dimethylformamide (DMF) in a three-necked reaction flask under a dry nitrogen purge and then heated to 85 °C to react for 8 h. The product was precipitated in anhydrous ethyl ether and vacuum-dried at 40 °C. The structure of EPAE was characterized by FT-IR, ^{1}H NMR, and ^{13}C NMR.

EPAE. ^{1}HNMR(400 MHz, d_6-DMSO, ppm) δ: 1.23∼1.35(16H, –NCH$_2$C<u>H$_2$</u>C<u>H$_2$</u>C<u>H$_2$</u>CH$_2$CH$_2$OH; –CH(C<u>H$_3$</u>)CH$_2$–; –NHCH$_2$C<u>H$_2$</u>C<u>H$_2$</u>CH$_2$NH–), 1.68(2H, –NCH$_2$CH$_2$CH$_2$CH$_2$C<u>H$_2$</u>CH$_2$OH), 2.12(2H, –N<u>H</u>CH$_2$CH$_2$CH$_2$CH$_2$N<u>H</u>–), 2.41(2H, –NC<u>H$_2$</u>CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$OH), 2.56(4H, –NHC<u>H$_2$</u>CH$_2$CH$_2$C<u>H$_2$</u>NH–), 2.73(2H, –OCOC<u>H</u>(CH$_3$)–), 2.77(4H, –NHC<u>H$_2$</u>CH(OH)–), 3.59(2H, –NCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$<u>H$_2$</u>OH), 4.07(2H, –NHCH$_2$C<u>H</u>(OH)–), 4.56(4H, –CH(OH)C<u>H$_2$</u>OCO–). ^{13}C NMR(400 MHz, d_6-DMSO, ppm) δ: 19.16(–CH<u>C</u>H$_3$–CH$_2$–), 25.27(–N–CH$_2$CH$_2$CH$_2$<u>C</u>H$_2$CH$_2$CH$_2$OH), 27.02(–N–CH$_2$CH$_2$<u>C</u>H$_2$CH$_2$CH$_2$CH$_2$OH), 27.11(–<u>C</u>H$_2$CH$_2$NH–), 32.51(–N–CH$_2$<u>C</u>H$_2$CH$_2$CH$_2$CH$_2$CH$_2$OH), 32.77(–COO–<u>C</u>HCH$_3$–CH$_2$–N–), 33.01(–N–CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$<u>C</u>H$_2$OH), 49.71(–CH$_2$<u>C</u>H$_2$NH–), 53.55(–NH<u>C</u>H$_2$CHOH–), 56.15(–N–<u>C</u>H$_2$CH$_2$–), 62.61(–CHCH$_3$<u>C</u>H$_2$N–), 63.87(–CH$_2$CH$_2$<u>C</u>H$_2$OH), 69.93(–NHCH$_2$CHOH<u>C</u>H$_2$COO–), 172.53(–<u>C</u>OO–).

2.5. Acid–base titration assay of polymers

Acid–base titration was used to evaluate the buffering capacity of synthesized cationic polymers. In this assay, 10 mg of EPAE was dissolved in 10 mL of 150 mM NaCl. 100 μL of 1N NaOH was then added to the solution to adjust the PH to 11.6 before it was titrated with acid. The solution was titrated with increasing volumes of 0.1 N HCl solutions, and the results were measured using a pH meter.

2.6. Hydrolytic degradation of polymers

EPAEs were 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).

2.7. 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 EPAEs/DNA for comparison with that of PEI25k/DNA were evaluated using the XTT assay (Funk et al., 2007; Scudiero et al., 1988). In a 96-well plate, COS-7(HeLa) 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.

2.8. Formation of polymer/DNA complexes

The plasmid pCMV-LacZ (pCMV-βgal) contained a CMV promoter to drive the β-galactosidase (LacZ) gene expression (Lim and Chae, 1989; Crystal, 1995). 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

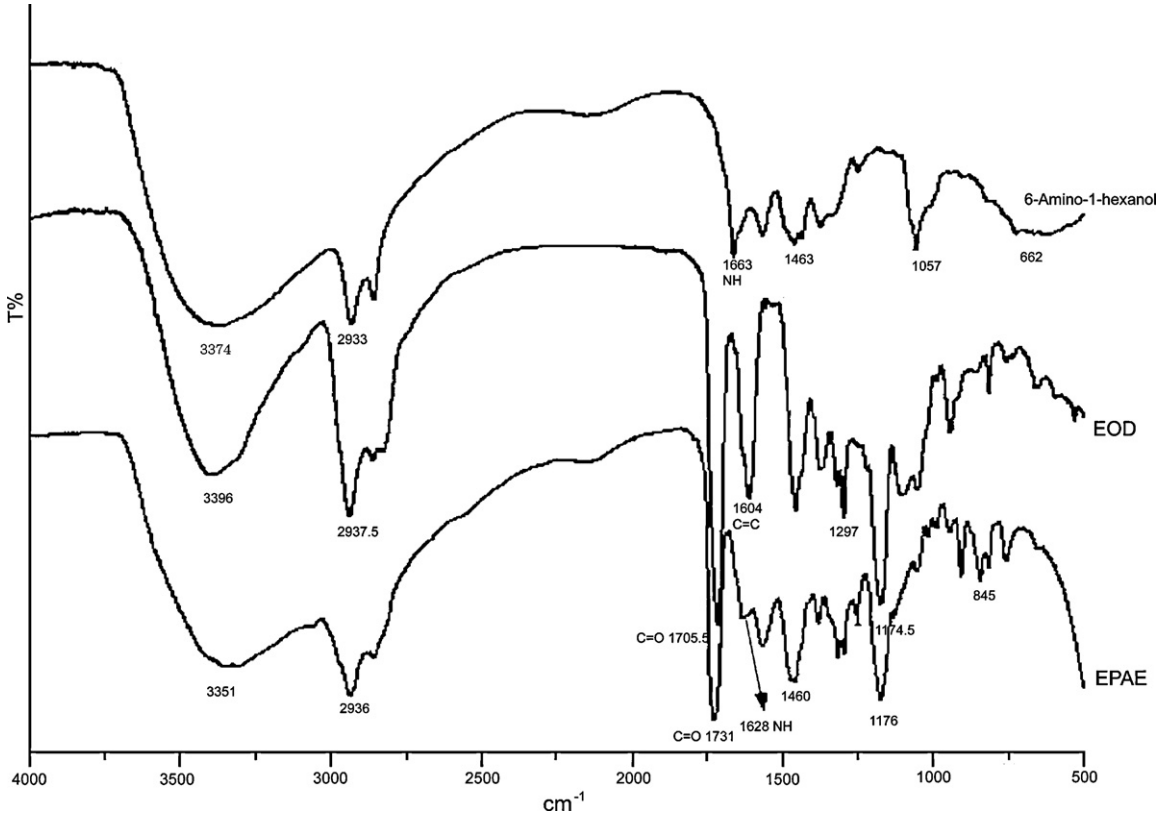


Fig. 1. FT-IR spectra of the synthesized EPAE.

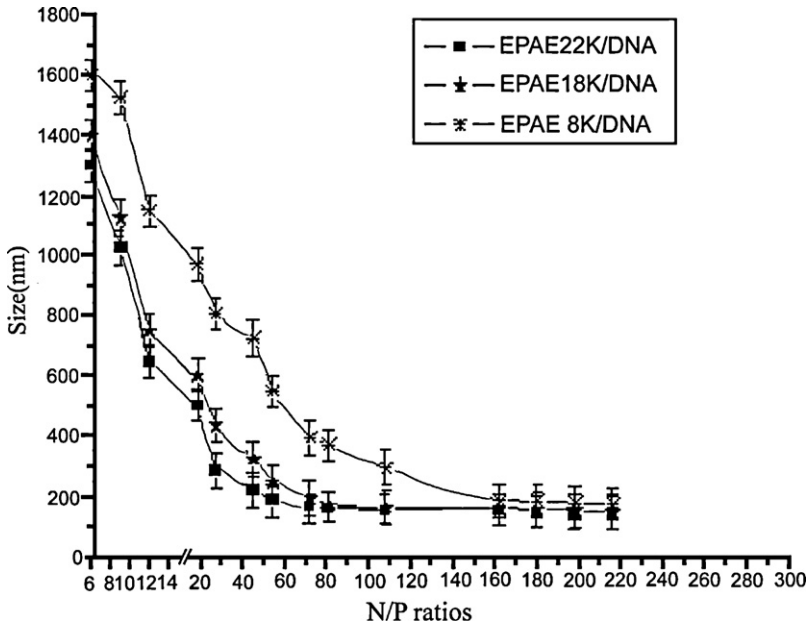


Fig. 2. Size of EPAEs/DNA complexes prepared at different N/P ratios. Results presented as the mean ± SD ($n = 3$).

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. Then, 10.0 mg/mL of the polymer was dissolved in 20 mM HEPES buffer (pH 7.4) and its serial dilutions were made with the various N/P ratios of polymers/DNA. The complexes were then allowed to self-assemble in the HEPES buffer. They incubated at room temperature for 30 min before measurements.

2.9. Characterizations of polymer/DNA complexes

The particle sizes and surface charges of the polymer/DNA complexes were determined by dynamic light scattering (Nicomp 380 system, USA) and electrophoretic mobility at 25 °C with a Zeta-potential system (Nicomp Instrument, USA).

2.10. DNA gel retardation assay of polymer/DNA complexes

EPAE/DNA complexes and PEI25k/DNA complexes were loaded into a 0.7% agarose gel containing ethidium bromide (0.3 g/mL) in a Tris–acetate–EDTA (TAE) buffer and performed at 100 V for 45 min. After electrophoresis, the DNA bands were visualized using UV-irradiation. EPAE/DNA complexes and PEI25k/DNA complexes with various N/P ratios were prepared.

2.11. 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.

3. Results and discussion

3.1. Structural characterizations of EPAE

EPAE was synthesized as shown in Scheme 1. The chemical structure of polymer was confirmed by FTIR and NMR spectroscopy. Fig. 1 shows the FT-IR spectra of the synthesized EPAE. The peaks at 1711 cm^{-1} (C=O, stretching, ester), 1606 cm^{-1} (N–H, bending, amine), and 3393 cm^{-1} (O–H, stretching, hydroxyl) represent the absorptions of ester and amine groups in EPAE, as shown at Fig. 1. The chemical shifts of characterized protons and carbons of EOD and EPAE are shown in the materials and methods section. In addition, the GPC data of EPAEs show that the weight-averaged molecular weights were 8350(EPAE8k), 18,038(EPAE18k), and 22,365(EPAE22k) with polydispersities of

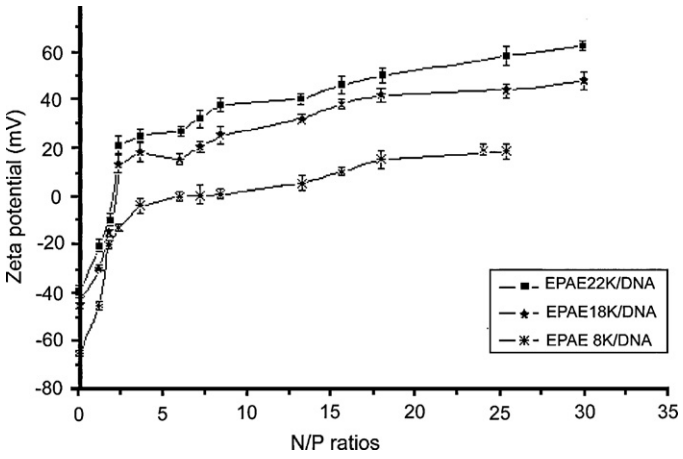


Fig. 3. Zeta-potential of EPAEs/DNA complexes prepared at different N/P ratios. Results are presented as the mean ± SD ($n = 3$).

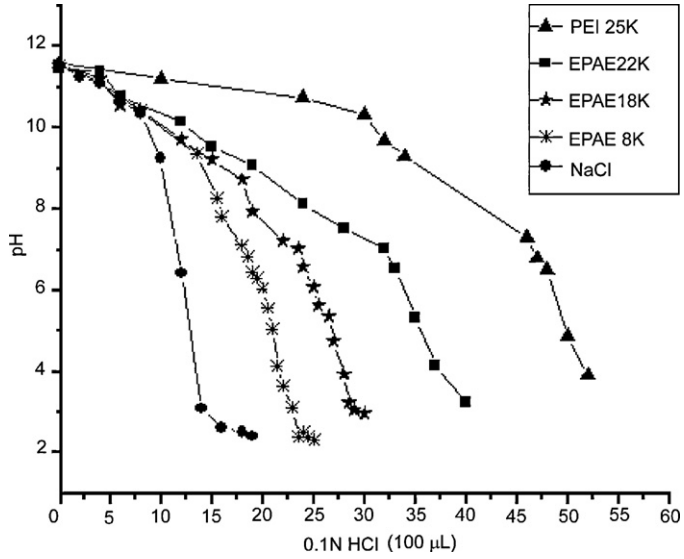


Fig. 4. Acid–base titration profile of various polymers with 0.1 N HCl.

1.386, 1.150, and 1.111, respectively, relative to polystyrene standards in THF.

3.2. Size and Zeta-potential analysis of polymer/DNA complexes

Figs. 2 and 3 show the size and Zeta-potential of EPAEs/DNA complexes at various mass ratios, determined using dynamic light scattering (DLS) and electrophoretic mobility at 25 °C with Zeta-potential analysis. The size of complexes decreased with increase of the mass content of the polymers until the N/P ratio of EPAE18k/DNA and EPAE22k/DNA reached 70/1 and that of EPAE8k/DNA reached 160/1. The results show that the average diameters (<200 nm at N/P 70/1) of EPAE18k/DNA and EPAE22k/DNA and the average diameter (<200 nm at N/P ratio 160/1) of EPAE8k/DNA fall within the optimal range (40–200 nm) required for cellular endocytosis. The above data demonstrates that EPAE22k and EPAE18k with higher molecular weight condense DNA better than EPAE8k with low molecular weight. The Zeta-potential of the resulting complex changed from negative charge to positive charge when the amounts of EPAEs increased. When the N/P ratio of EPAE22k/DNA and EPAE18k/DNA complexes was higher than 2/1, the surface of pCMV-β-gal was fully occupied with the EPAE molecules, forming positive charge complexes. When the N/P ratio of EPAE8k/DNA complexes was higher than 15/1, positive Zeta-potentials of the complexes formed. Complexes with extra positive charges on their surfaces had better interaction with the target cell membrane, resulting in an enhanced uptake.

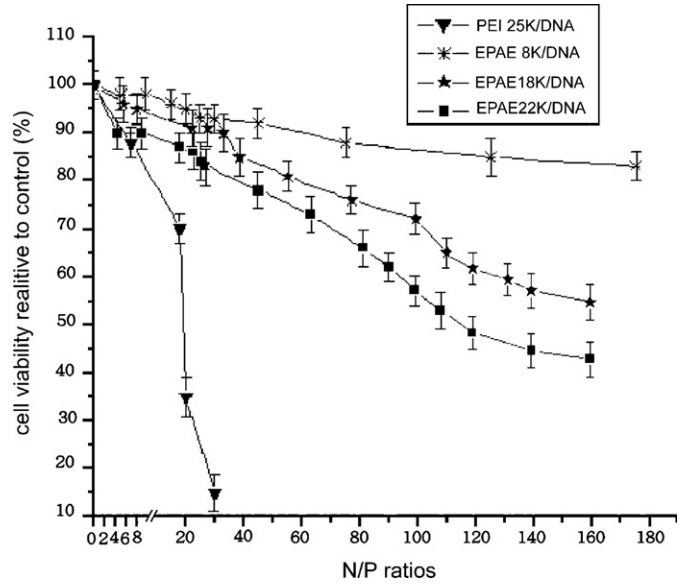


Fig. 5. Cytotoxicity of polymers/DNA complexes in COS-7 cells. Results are presented as the mean ± SD ($n = 3$).

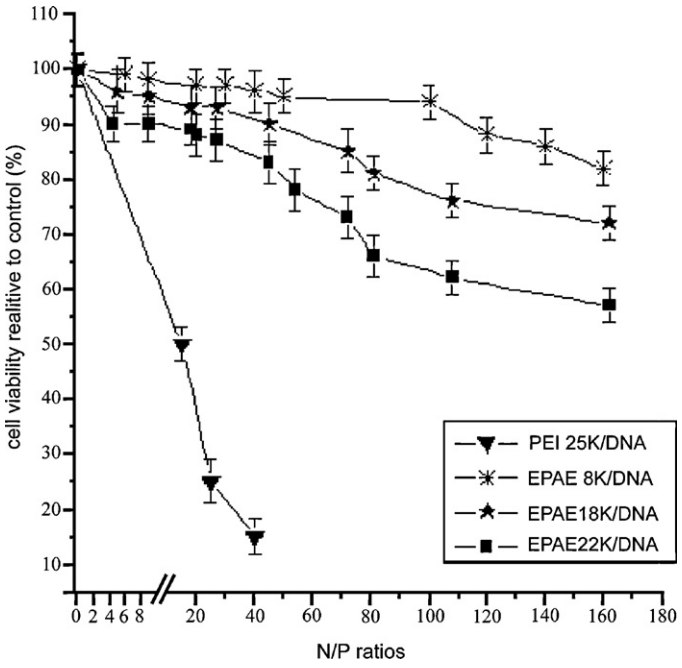


Fig. 6. Cytotoxicity of polymers/DNA complexes in HeLa cells. Results are presented as the mean ± SD ($n = 3$).

3.3. 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 PEI 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. Molecule weight of EPAE can change the buffering region of the polycation. The data show the pH range of EPAE22k to be between 11.8 and 5.7 and that of EPAE8k to be between 11.8 and

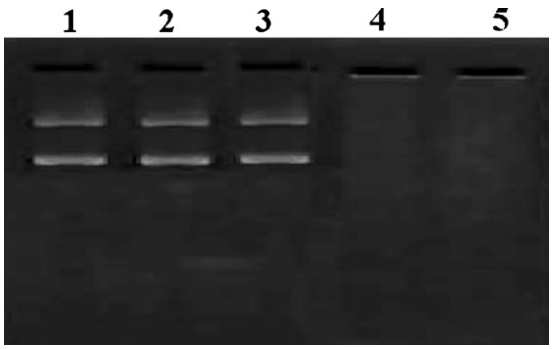


Fig. 7. DNA gel retardation assay of EPAE22k. Lanes: (1) free DNA. (2) EPAE22k/DNA (N/P): 1/1. (3) EPAE22k/DNA (N/P): 9/1. (4) EPAE22k/DNA (N/P): 18/1. (5) EPAE22k/DNA (N/P): 27/1.

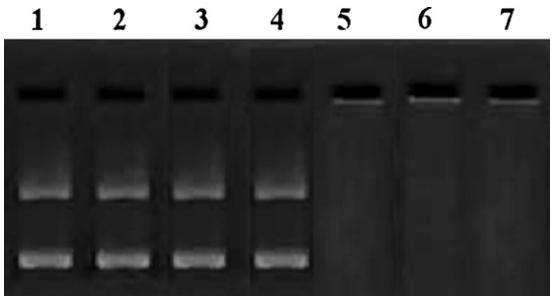


Fig. 8. DNA gel retardation assay of EPAE8k. Lanes: (1) free DNA. (2) EPAE8k/DNA (N/P): 1/1. (3) EPAE8k/DNA (N/P): 20/1. (4) EPAE8k/DNA (N/P): 60/1. (5) EPAE8k/DNA (N/P): 100/1. (6) EPAE8k/DNA (N/P): 200/1. (7) EPAE8k/DNA (N/P): 300/1.

6.6. It was found that the polymers with higher molecular weight have a high buffering capacity.

3.4. Cytotoxicity of polymers/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 EPAEs for comparison with that of PEI25k/DNA, we performed a XTT assay using the COS-7 and HeLa cell lines. Cells were incubated with increasing amounts of EPAEs/DNA and PEI25k/DNA. Relative cell viabilities of EPAEs/DNA and PEI25k/DNA were shown in Figs. 5 and 6. As can be seen, the cell viability of PEI25k/DNA complexes decreased rapidly with an increase in PEI25k concentration, whereas that of EPAEs/DNA did not change much with an increase in EPAEs/DNA. The results show that EPAEs/DNA exhibited substantially lower toxicity on COS-7 (HeLa) cells than did high molecule weight PEI25k/DNA. We infer that EPAEs/DNA can provide better cytotoxicity profiles than these presently used for PEI25k/DNA due to the differences in the chemical structure, which reduces the high charge density of the polycation.

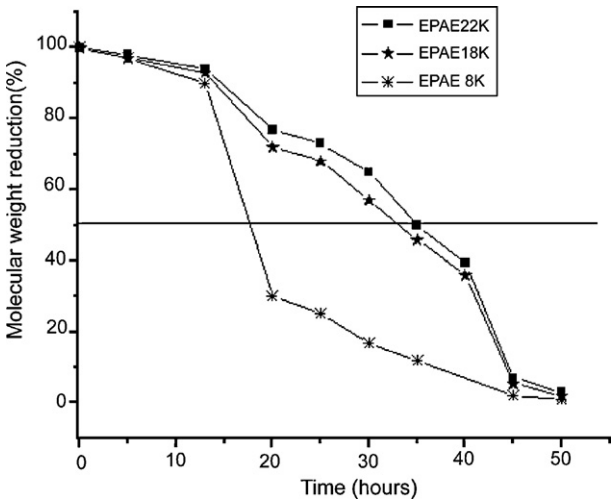


Fig. 9. Hydrolytic degradation of EPAEs.

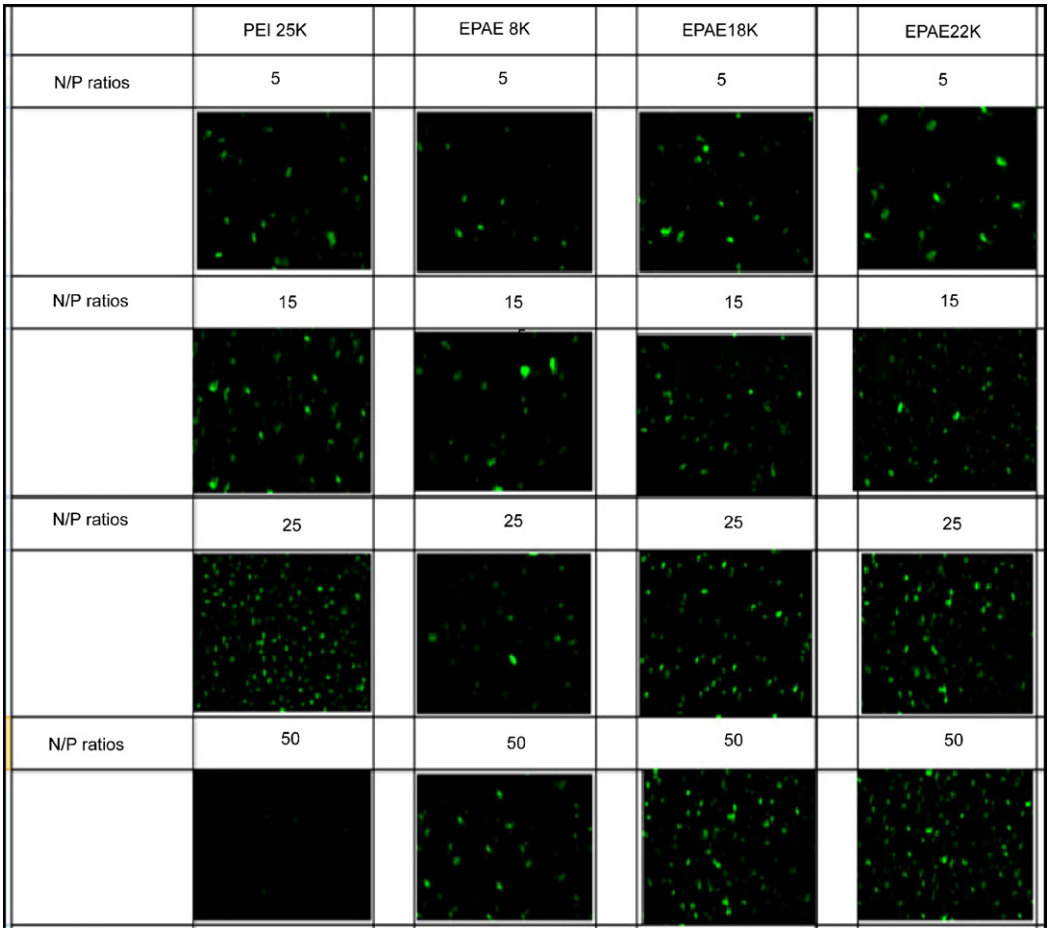


Fig. 10. Fluorescent microscopy of COS-7 cells transfected with plasmid encoding green fluorescence protein with different carriers.

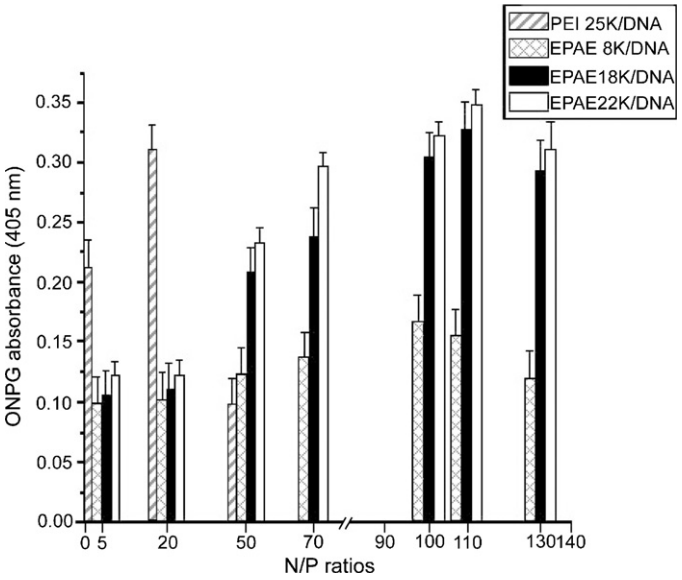


Fig. 11. Transfection efficiency of the polymers/DNA complexes into cultured COS-7 cells. Results are presented as the mean ± SD ($n = 3$).

3.5. DNA gel retardation assay

The electrophoretic mobility behavior of free DNA, EPAEs/DNA is shown in Figs. 7 and 8. Increasing amounts of EPAE 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 EPAE. With a low mass ratio of EPAE22k (N/P = 9/1), plasmid DNA was totally retained, as indicated in lane 4 of Fig. 7. The electrophoretic mobility behavior of EPAE18k is similar with EPAE22k (data not shown). Plasmid DNA was partially retained by the presence of EPAE8k at N/P ratio of 60/1 (lane 4 of Fig. 8) and totally retained at N/P ratio of 100/1 (lane 5 of Fig. 8). These results suggest that DNA was fully completed with EPAE22k and EPAE8k to form complexes, showing that amine groups of EPAEs with positive charges could interact with the charge of negative phosphate groups of DNA strands to form complexes.

3.6. In vitro hydrolysis of polymers

Fig. 9 shows the degradation profiles of polymers which were monitored at 37 °C at buffer pH values of 7.4 in order to approximate the environments within endosomal vesicles. The EPAE22k and EPAE18k generally degraded faster than that of EPAE8k at pH 7.4. The degradation studies indicate that the half-life of EPAE22k, EPAE18k, and EPAE8k in the HEPES buffer were 36, 33, and 18 h at pH 7.4, respectively. These results show that EPAE8k exhibited higher hydrolytic degradation rate than did EPAE22k and EPAE18k at pH 7.4.

3.7. Cellular delivery of plasmid DNA via EPAE vectors

The transfection efficiency is studied by fluorescence microscopy (Fig. 10) and the amount of β-galactosidase (equivalent to ONPG absorbance) measured in COS-7. The effect of N/P ratios of EPAEs/DNA and PEI25k/DNA complexes on transfection in COS-7 cells are shown in Fig. 11. As can be seen, EPAE22k and EPAE18k with higher molecular weight had the greater transfection efficiency than EPAE with low molecular weight. The transfection efficiencies of EPAE22k/DNA and EPAE18k/DNA complexes increased with an increase in N/P ratios, whereas that of PEI25k/DNA complexes did not observe. The best relative transfection efficiency of EPAE22k/DNA and EPAE18k/DNA complexes was reached at N/P ratio 110/1, which was higher than that of PEI25k/DNA and EPAE8k/DNA complexes. This discrepancy may be due to the difference in proton sponage effect and size distribution of relative complex. These results demonstrated that molecular weight and introduction of hydroxyl group into the backbone and side chain of poly(aminoester) have a significant effect on the size distribution and transfection ability of relative complex.

4. Conclusion

Poly(aminoester)s (EPAE22k and EPAE18k) bearing amino and hydroxyl groups with low cytotoxicity, high biodegradability, and high transfection efficiency were synthesized and then characterized using FT-IR, NMR, and GPC in this study. EPAE22k (EPAE18k) and DNA had a strong electrostatic interaction to self-assemble nano-particles. The positive charges in the backbone and side chain of EPAE22k (EPAE18k) condense DNA to form DNA-polycation complexes. The transfection efficiency of EPAE22k and EPAE18k into COS-7 cells was better than that of the EPAE8k, which resulted in numerous amine groups of EPAE22k (EPAE18k) in the backbone. Biodegradable EPAE22k and EPAE18k could potentially be used in a non-viral gene delivery system.

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