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Full Paper

Diglycidyl Esters Cross-Linked with Low Molecular Weight Polyethyleneimine for Magnetofection

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Magnetic polyethyleneimine (PEI) complexes have demonstrated to be simple and efficient vectors for enhancing gene transfection. However, the high cytotoxicity of PEI restricts its further application in vivo. In this study, we synthesized several low cytotoxicity biodegradable cationic polymers derived from PEI (M_w 600) linked with diglycidyl tartrate (DT-PEI) or its analogues (diglycidyl succinate (DS-PEI) and diglycidyl malate (DM-PEI); D-PEIs for all 3 polymers). Moreover, a type of biocompatible magnetic nanoparticles (MNPs) with negative charges was prepared to assemble with D-PEIs/DNA complexes via electrostatic interactions. The magnetic ternary complexes have appropriate sizes of 120–150 nm and zeta potential values of ~20–25 mV. The transfection ability and cell viability of D-PEIs increased as the amount of hydroxyl groups increased in the repeat unit, which indicated that increasing the hydroxyl number in the backbone of D-PEIs can enhance gene expression and decrease cytotoxicity in A549 cells. Magnetofection of DT-PEI showed similar transfection efficiency with 30 min incubation; in contrast, the standard incubation time was 4 h. All three magnetic complexes displayed lower cytotoxicity when compared with those of PEI complexes in COS-7 and A549. These results indicated that these series of magnetic PEI derivatives complexes could be potential nanocarriers for gene delivery.

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Introduction

Gene therapy is one of the promising therapeutic strategies in treating inherited and acquired diseases. Successful gene therapy depends on safe and efficient gene vectors. Although displaying high gene transfection efficiency, the application of viral vectors is limited by severe immunogenicity and inflammation problems.[1,2] Therefore, non-viral vectors as alternative gene carriers have been widely investigated to avoid the aforementioned problems.[3,4] In recent years, non-viral vectors have been chiefly divided into two types: organic component vectors and inorganic component nanoparticles. The first type includes polymers,[5,6] cationic lipids,[7] peptides,[8,9] and carbon nanotubes[10]; and the second type includes calcium phosphate particles,[11] quantum dots,[12] gold nanoparticles,[13] and magnetic nanoparticles.[14]

Cationic polymers are one of the most significant non-viral vectors for the delivery of negatively charged DNA. Among cationic polymers, polyethyleneimine (PEI) has been widely studied as the gold standard. However, it is difficult for PEI to be applied in the clinical field because of its cytotoxicity, non-degradability, and low-transfection efficiency.[15] High molecular weight (HMW) PEIs have a relatively high transfection efficiency (TE) due to their binding affinity with DNA and capacity of endosomal escape. However, the high molecular weight, charge density, and non-degradability lead to high cytotoxicity.[16,17] By contrast, low molecular weight (LMW) PEIs have been demonstrated to exhibit a relatively low cytotoxicity, associated with negligible transfection efficiency. To improve transfection activity and reduce cytotoxicity, many studies have been focussed on cross-linking LMW PEIs through biodegradable disulfide or ester bonds.[18–20]

The other critical factors affecting TE is the low amount of DNA that manages to reach the target cells. In this regard, the development of non-viral carriers that can be guided selectively into specific cells is very significant, and thus could improve the chance of gene expression.[21] Magnetic nanoparticles have been applied in targeted gene delivery using an external magnetic field. Furthermore, magnetofection can decrease the amount of DNA injected, shortening the time necessary to reach the desired target cells and improving significantly gene expression efficiency.[22] Considering these advantages, magnetofection has been widely researched and has achieved tremendous progress for gene delivery in the past few decades. However, substantial progress and successful clinical application depend on the efficient, specific delivery of genes without systemic toxicity. Furthermore, gene delivered and the therapeutic efficacy can be

accurately monitored noninvasively and spatiotemporally.[23] Because many magnetic nanoparticles (MNPs) have been used in clinical settings for many years, there is a high potential that these functional targeted MNPs will be applicable in clinical gene therapy in the future.[23] But there are also some possible limitations of magnetic gene delivery: (1) from a medical point of view, magnetic drug targeting makes sense mainly in localized stages of disease and less in metastatic stages where drug action may be required throughout the body;[24] (2) the other major limitation of magnetofection is the residual presence of a small amount of magnetic nanoparticles following transfection.[25] For magnetofection, gene carriers, such as PEI, are associated with magnetic nanoparticles. In order to absorb negatively charged DNA on their surface, PEI or other cationic polymers have been designed to tailor magnetic nanoparticles by physical adsorption method[26–28] or chemical conjugation method.[29–31] However, magnetic PEI complexes exhibit non-negligible cytotoxicity.[29] The cytotoxicity of a gene vector is significant to the clinical application, which powerfully depends on the biocompatibility of vector materials. In this paper, we synthesized biocompatible Fe_3O_4 magnetic nanoparticles and a series of low cytotoxicity cross-linked PEI 600 (D-PEIs) using diglycidyl esters with different numbers of hydroxyl groups. The ternary complexes, MNP@D-PEIs/DNA, were assembled by electrostatic interactions. The transfection efficiency and cytotoxicity of the ternary complexes were investigated in A549 and COS-7 cells.

Results and Discussion

Synthesis and Characterization of Polymer D-PEIs

The synthesis of D-PEIs is shown in Scheme 1. In a typical procedure, tartaric acid reacted with allyl alcohol in the presence of sulfuric acid to obtain the diester compound **1a**. Then, the product **1a** was oxidized by *m*-chloroperoxybenzoic acid (*m*-CPBA) to give the compound **2a**. The structure of **2** was confirmed by ^{1}H NMR (see Supplementary Material). DT-PEI (DT = diglycidyl tartrate), DM-PEI (DM = diglycidyl malate), and DS-PEI (DS = diglycidyl succinate) were prepared by cross-linking reaction between PEI 600 Da and **2a**, **2b**, and **2c**, respectively. In the polymerization reaction, the mole weight of PEI 600 was slightly higher than that of the diesters, otherwise the clear colourless reaction mixture would change to pale yellow gel-like materials. This could be due to the uncontrollable cross-linking between the diglycidyl esters and LMW PEI when excess diesters were used.[19] The structures of the D-PEIs were confirmed by ^{1}H NMR and the molecular weights of the D-PEIs were measured by gel permeation chromatography (GPC) (Fig. S1, Table S1).

Characterization of MNPs

The structure of the MNPs was confirmed by Fourier transform infrared (FT-IR) spectroscopy as shown in Fig. 1a. The FT-IR spectrum of typical MNPs shows absorption bands at 1618 and 1381 cm^{-1}, which are related to the stretching vibrations of carboxyl salt. And the peaks at 2857 and 2926 cm^{-1} are due to the vibrations of the symmetric and asymmetric stretching of the

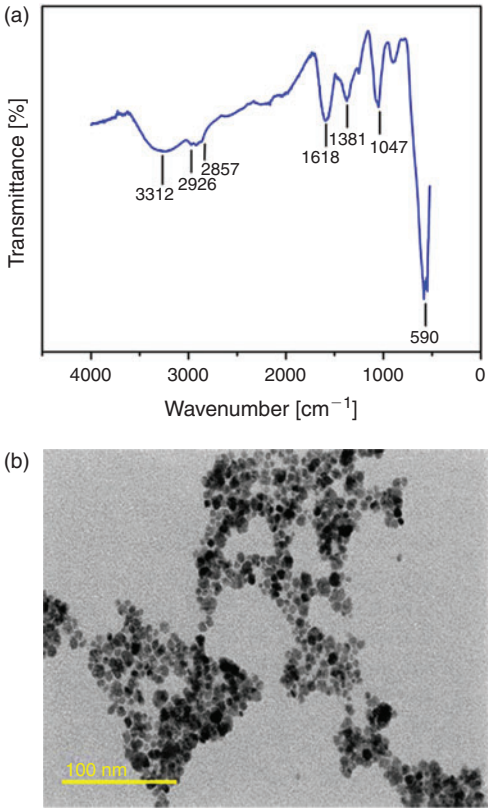
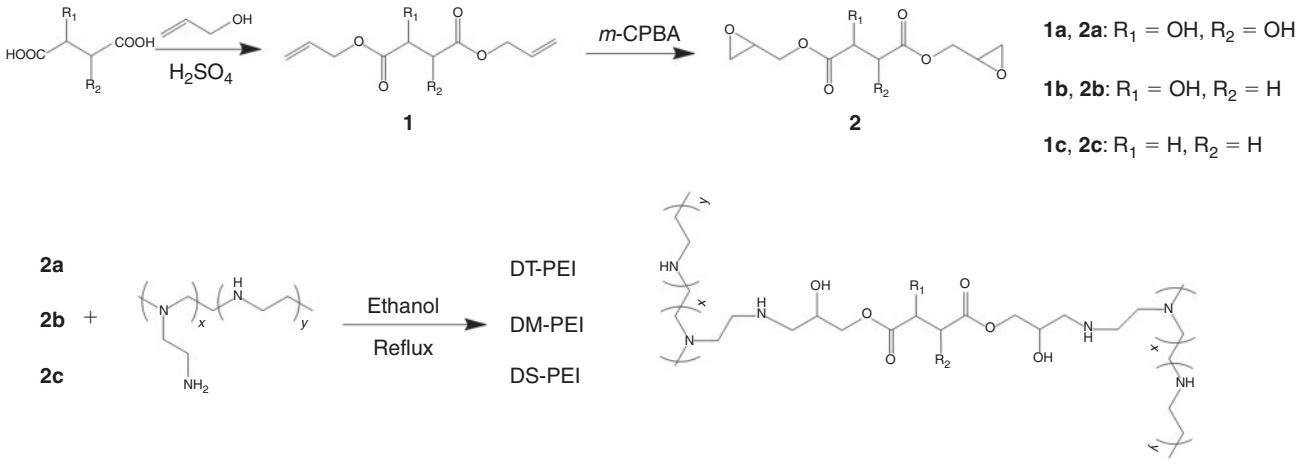


Fig. 1. (a) FT-IR spectrum of the Fe_3O_4 nanoparticles obtained with Na_3Cit. (b) TEM image of MNPs.



Scheme 1. Preparation route of D-PEIs.

methylene group, and the absorption band at 1047 cm^{-1} is probably related to the hydroxyl group from sodium citrate (Na_3Cit).[32] In addition, the peak at 590 cm^{-1} is attributed to (Fe–O) in the Fe_3O_4 nanoparticles.[33] The morphology and size were observed by transmission electron microscopy (TEM) as shown in Fig. 1b. The TEM image shows that the MNPs have an average diameter of ~10 nm. Photographs of the dispersions of the MNPs are given in Fig. 2. The MNPs were well dispersed in water under normal conditions (Fig. 2a). If the MNP solution is subjected to a strong magnetic field, the nanoparticles can rapidly gather on the side of the wall within 30 s (Fig. 2b). This result indicated that the MNPs can easily be manipulated by an external magnetic field.

Assembly and Characterization of Gene Complexes

The MNPs were functionalized with positively charged PEI due to its electrostatic interaction with negatively charged nucleic acid and the proton sponge effect, which enables release of MNP–PEI–nucleic acid complexes from endolysosomes into cytoplasm.[34] Therefore, ternary complexes of MNPs, D-PEIs, and DNA were assembled here by electrostatic interactions. The binary complexes of D-PEIs and DNA were prepared first and then complexed with MNPs.[27,35] The size and zeta potential of MNP@D-PEIs/DNA were determined at different weight ratios of MNP/DNA. For the application of magnetic gene delivery, the size of the nanoparticles prepared should be sufficiently small (<200 nm) to improve cellular uptake and blood circulation time within body.[36] As depicted in Fig. 3, the sizes of the complexes were between 130 and 150 nm and the zeta potentials were in the range of ~20.0–24.0 mV. The average sizes were increased by increasing the amount of MNP, this phenomenon is probably due to the increased aggregation by increasing the relative concentration of MNPs. The surface charge of the complexes were reduced by increasing the relative concentration of MNP; these results contribute to the formation of MNPs with a negative charge surface.[37] The morphology of MNP@DT-PEI/DNA was visualized by TEM (Fig. 3c), which showed that the MNPs were distributed in the magnetic complexes. To investigate the ability of the magnetic complexes to bind DNA, MNP@D-PEIs/DNA complexes were assessed using agarose gel retardation assays. As shown in Fig. 3d, the mobility of DNA was completely reduced, which suggests that the magnetic complexes can concentrate DNA under magnetofection conditions.

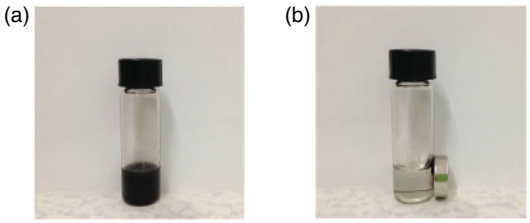


Fig. 2. Photographs of an aqueous MNP dispersion in a vial (a) in the absence of a magnetic field and (b) in the presence of a magnetic field for 30 s.

In Vitro Transfection

Optimization of Weight Ratio of D-PEIs/DNA on Transfection

To achieve optimized weight ratio of D-PEIs/DNA, the transfection efficiencies of these binary complexes (without

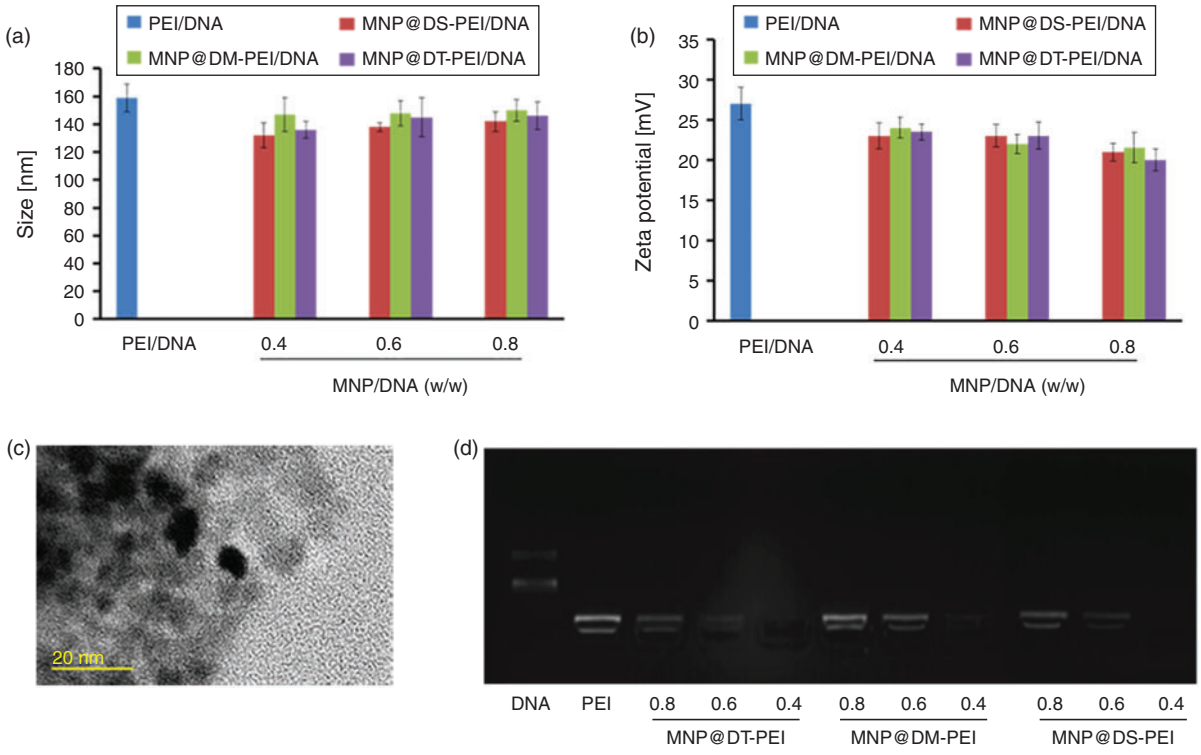


Fig. 3. Characterization of the magnetic complexes. (a) Size and (b) zeta potential of the ternary magnetic complexes at varying MNP-to-DNA weight ratios of 0.4, 0.6, and 0.8 (the weight ratio of D-PEI/DNA = 4) in water (mean ± s.d., $n = 3$). (c) TEM image of the magnetic complexes. (d) Agarose gel electrophoresis of PEI 25k and the ternary magnetic complexes at MNP-to-DNA weight ratios of 0.4, 0.6, and 0.8.

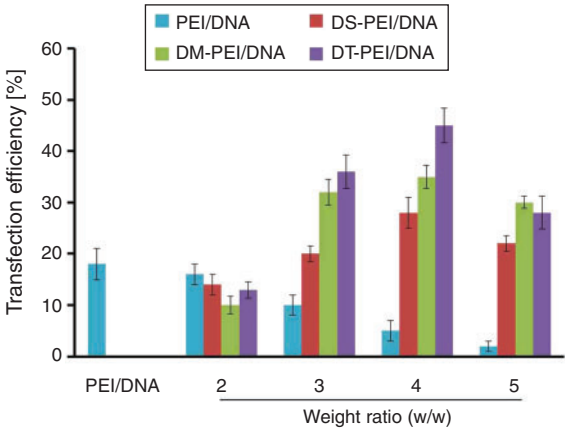


Fig. 4. Transfection of D-PEIs/DNA at weight ratios of 2, 3, 4 and 5 in A549 cells (mean ± s.d., $n = 3$).

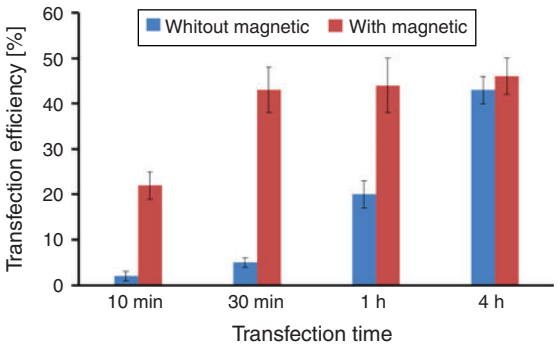


Fig. 5. Transfection of MNP@DT-PEI/DNA (at a weight ratio of 0.6 : 4 : 1) and DT-PEI/DNA (at a weight ratio of 4 : 1) was performed under magnetic field with different incubation times (10 min, 30 min, 1 h, 4 h) in A549 cells (mean ± s.d., $n = 3$).

MNP) were assessed in A549 cells. The plasmid of green fluorescent protein (GFP) was used as a reporter gene. PEI 25 kDa was prepared at a weight ratio of 1.39 (N/P = 10; N/P is the ratio of moles of the amine groups of polymers to those of the phosphate groups of DNA) as standard because of its high transfection efficiency.[19] D-PEIs/DNA complexes were prepared at different weight ratios including 2, 3, 4, and 5. The results are shown in Fig. 4. All three polymers showed better results when compared with PEI 25kDa under optimal weight ratio condition. Using a weight ratio of 4, DT-PEI exhibited the highest transfection efficiency, followed by DM-PEI and DS-PEI, which is consistent with the amount of hydroxyl groups in the polymer repeat unit. This result suggests that increasing the hydroxyl number in the backbone of D-PEIs enhances gene transfection. The introduction of hydroxyl groups in the backbone of D-PEIs may have an effect in improving the osmotic property, and the osmotic coefficient increases with increasing numbers of hydroxyls.[38] Furthermore, a hyperosmotic activity was reported to significantly improve the transfection capability by increasing the cellular uptake of the osmotically active gene carriers.[39]

Effect of Incubation Time on Magnetofection

The standard incubation time is approximately 4 h for PEI or other commercial transfection regents in order to achieve high transfection efficiency in vitro. However, a short period of gene delivery time might be significant for applications in vivo. Magnetofection is a way to efficiently shorten the time to 5 min to 2 h.[27,40,41] To optimize the incubation time, transfection of MNP@DT-PEI/DNA was performed under a magnetic field with different incubation times at a fixed MNP-to-DNA weight ratio of 0.6. The results are shown in Figs 5 and 6. The DT-PEI/DNA complexes showed optimal incubation times over 1 h, while the MNP@DT-PEI/DNA complexes achieved maximum transfection efficiency at 30 min of incubation. The reason could be attributed to the increasing sedimentation rates and internalization of the magnetic complexes. However, 30 min seems to be a little longer when compared with that displayed by commercial reagents (i.e. 15 min).[42] The phenomenon is partly attributed to the smaller diameters of the magnetic particles that we prepared (~10 nm), which required a longer time to reach the cell surface under a magnetic field. Furthermore, continuously increasing the incubation time did not obviously promote transfection, which is in accordance with the study by Xie et al.[40] Though magnetofection exhibited nearly 38 % higher gene expression with 30 min incubation, it showed a 3 % increase only in gene expression using a standard incubation time of 4 h. This result indicates that magnetofection of DT-PEI cannot significantly enhance transfection efficiency. The reason could be due to the fact that magnetic field itself cannot alter the uptake mechanism. DT-PEI/DNA, but not the magnetic complexes, cloud enter the nucleus and DT-PEI is crucial to magnetofection for magnetic PEI complexes.[41]

Optimization of the Amount of MNPs on Magnetofection

Sufficient amounts of MNPs are required in magnetic complexes for responding to the external magnetic field. However, an excess of MNPs would not enhance transfection.[40,43] The amount of MNPs optimization was performed under a fixed incubation time of 30 min with various amounts of MNPs. As shown in Fig. 7, the transfection efficiency improved by increasing the weight ratio (MNP/DNA) from 0.2 to 0.6 (fluorescent images of GFP expressed in A549 cells are shown in Fig. S2 in the Supplementary Material). However, further increases in the amount of MNPs led to a decrease in transfection efficiency, which indicated that an MNP/DNA ratio of 0.6 was optimal for the magnetofection of DT-PEI in A549 cells. This result indicates that low weight of MNPs is not enough for responding to the external magnetic field, which leads to the low efficiency. Moreover, an excess of MNPs would increase the aggregation of complexes, resulting in the reduction of transfection efficiency.[44]

Cytotoxicity

The cytotoxicity of the MNPs, D-PEIs/DNA, and MNP@D-PEIs/DNA were evaluated on A549 and COS-7 cells (Fig. 8). MNPs exhibited a negligible cytotoxicity after 48 h; more specifically, over 80 % cell viability was obtained when the MNPs concentration increased to 500 μg mL^{-1} (Fig. 8d). Three of the D-PEIs displayed lower cytotoxicity than 25 kDa PEI in both cell lines (Fig. 8 a, b). Until now, many groups have indicated that the introduction of hydroxyl groups into polycationic gene vectors aided in the reduction of cytotoxicity.[45,46] Thus, D-PEIs exhibited reduced cytotoxicity owing to an increased number of hydroxyl groups, which improved the biocompatibility of the carriers. In addition, the strong positive charge of PEI damages cell membranes, but the positive charge of the PEI backbone in D-PEI may be partially neutralized by electrostatically negative

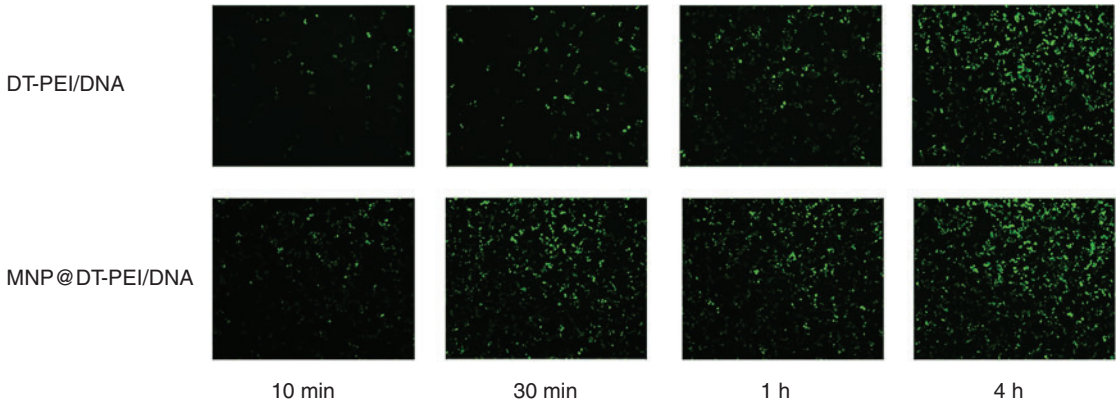


Fig. 6. GFP expression of MNP@DT-PEI/DNA and DT-PEI/DNA was performed under magnetic field with different incubation times (10 min, 30 min, 1h, 4h) in A549 cells (mean ± s.d., $n = 3$).

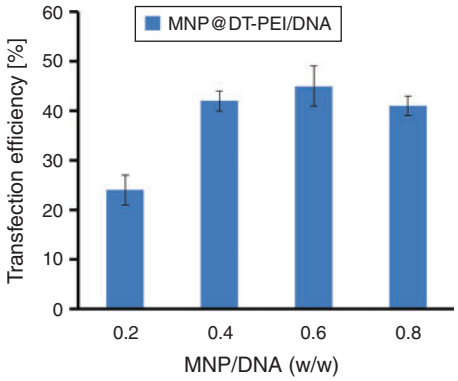


Fig. 7. Magnetofection efficiency of MNP@DT-PEI/DNA was performed under varying MNP/DNA ratios of 0.2, 0.4, 0.6, and 0.8 with 30 min incubation (DT-PEI/DNA = 4) in A549 cells (mean ± s.d., $n = 3$).

OH groups.[47] These results were attributed to the combination of the degradable ester linkage and the negative hydroxyl groups. As shown in Fig. 8c, all three MNP@D-PEIs/DNA showed more than 95 % cell viability in both cells under magnetofection conditions (MNP/D-PEIs/DNA = 0.6 : 4 : 1). These results showed that these magnetic complexes did not possess obvious toxicity because of the low cytotoxicity of MNPs and D-PEIs.

Conclusion

In this study, three polymers derived from PEI (M_w 600) linked with diglycidyl esters were prepared to assemble with DNA, then fabricated with biocompatible magnetic nanoparticles to form ternary complexes by electrostatic interactions. The magnetic ternary complexes showed good ability to condense DNA into nanoparticles under transfection conditions. The sizes and zeta potentials of the ternary complexes were suitable for cell endocytosis. The results of transfection of the binary complexes showed that the weight ratio of D-PEIs/DNA of 4 was the optimized ratio. DT-PEI showed the highest transfection and lowest cytotoxicity among the D-PEIs studied. This result indicated that increasing the hydroxyl density in the backbone of D-PEI can enhance gene expression and decrease cytotoxicity. The magnetofection experiments of MNP@DT-PEI/DNA showed that magnetofection can shorten incubation times when compared with standard protocols and the weight ratio of MNP/DNA of 0.6 was the optimized ratio. The MNPs exhibited negligible cytotoxicity. The magnetic ternary complexes also demonstrated low cytotoxicity under magnetofection conditions. These results indicate that this type of magnetic nanoparticle may be promising for gene delivery.

Due to the fact that MNPs have been used in clinical settings for more than a decade, we can foresee that these functional targeted MNPs will be applicable in clinical gene therapy in the future. To obtain an effective and low toxic transfection method for gene delivery in vitro, future work in will mainly focus on the development of multifunctional MNPs-vectors in vitro and in vivo, which will make a remarkable difference in tracking and disease diagnosis. In addition, the crude materials needed for the synthesis of MNPs-based gene vectors should have the properties of biocompatibility, safety, and degradability in the organism.

Experimental

Materials

All chemicals and reagents were obtained commercially and were used as received. Anhydrous ethanol was dried and purified under nitrogen by using standard methods and was distilled immediately before use. Polyethyleneimine (branched PEI 25 kDa) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich. LMW PEI (branched, average molecular weight 600 Da, 99 %) was purchased from Aladdin (Shanghai, China). Plasmid DNA encoding a red-shifted variant of wild-type GFP was from Aldevron, Inc. Dulbecco's Modified Eagle's Medium (DMEM), penicillin, streptomycin, and fetal bovine serum were obtained from GIBCO.

Preparation and Characterization of Diglycidyl Esters

Diglycidyl esters were prepared according to the reference.[48] In a typical procedure, tartaric acid (0.10 mol) and allyl alcohol (0.40 mol) were mixed and refluxed in the presence of 0.1 % (v/v) sulfuric acid for 24 h. The reaction was neutralized with sodium carbonate. Excess allyl alcohol was evaporated at 70°C under vacuum. The remaining solution was diluted with ethyl acetate and dried with anhydrous Na_2SO_4. After filtration, the crude product was collected by evaporation.

Diallyl tartaric acid (5 mmol) and *m*-chloroperoxybenzoic acid (15 mmol, 85 % purity) were mixed in methylene chloride, and refluxed at 55°C for 8 h. After that, the by-product *m*-chlorobenzoic acid was crystallized at −20°C overnight and filtered from the solution. The remaining co-products were removed by filtration and the filtrate was poured into column

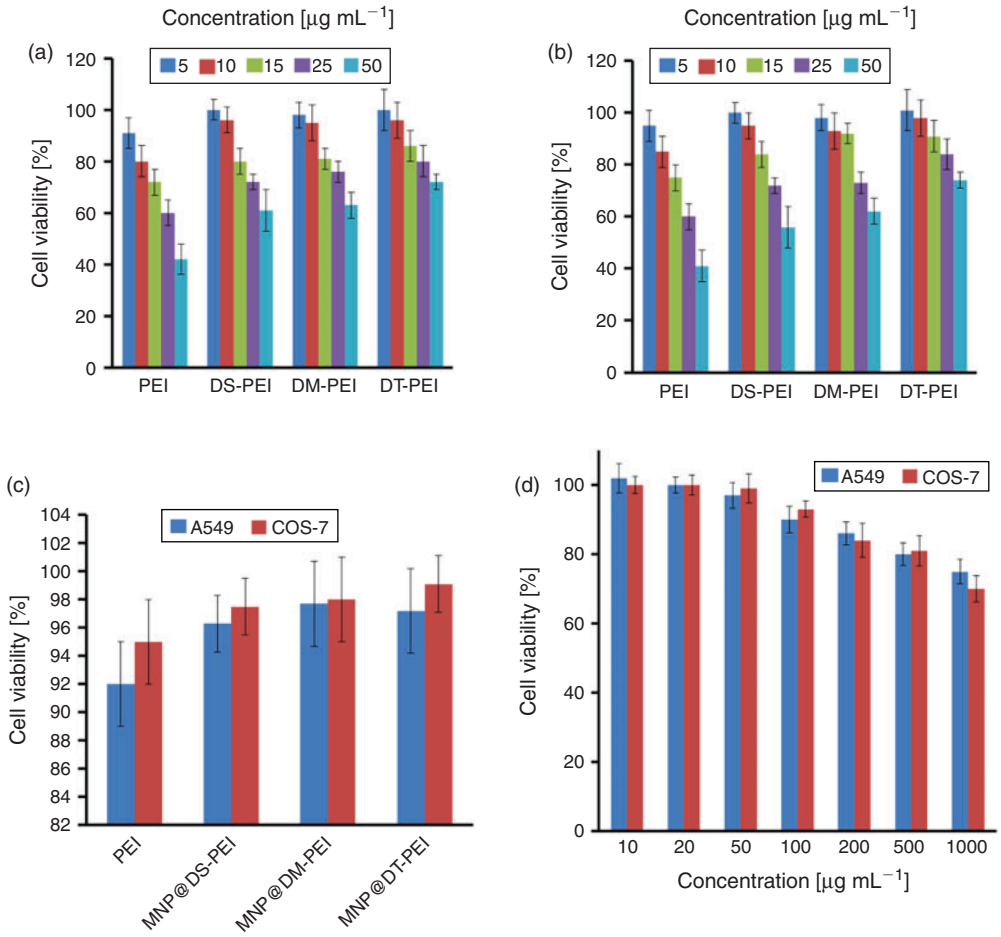


Fig. 8. Cell viability: D-PEIs under various concentration on (a) A549 cells and (b) COS-7 cells; (c) magnetic complexes under transfection conditions; and (d) MNPs with various concentrations (mean ± s.d., $n = 3$).

with ethyl acetate/petroleum ether (v/v 1 : 2) as eluent. These compounds were identified by ^{1}H NMR and ^{13}C NMR.

Synthesis of D-PEIs

In a typical example,[19] PEI 600 (1.50 mmol), diglycidyl tartaric or its analogues (1.33 mmol) and anhydrous ethanol (3 mL) were mixed in a 10-mL glass vial with a magnetic bar and sealed with a screw cap. It was heated in an oil bath at 79°C for 24 h with stirring. The product was purified by dialysis against water for 48 h (molecular weight cut-off 3500 kDa) and isolated by lyophilization. D-PEIs were characterized by ^{1}H NMR and GPC.

Preparation and Characterization of Magnetic Nanoparticles

The MNPs were prepared according to the reference.[37] In brief, anhydrous $FeCl_3$ (2.0 mmol) was added to 2-(2-hydroxyethoxy)-ethanol (DEG; 20 mL) with vigorous mechanical stirring to form a clear solution. Then, Na_3Cit (0.8 mmol) was added to the mixture and heated at 80°C with stirring to form a clear solution. After dissolving anhydrous sodium acetate (NaOAc; 6.0 mmol) in the above solution, the mixture was transferred into a 50-mL of Teflon-lined stainless steel autoclave and sealed in air. After this, the autoclave was kept at 240°C for 6 h. After the reaction, the black products were collected by centrifugation (8000 *g*, 30 min) and washed with ethanol and water thrice. The black precipitate was then dried at 60°C for further use.

FT-IR spectroscopy (Nicolet iS10) was performed to analyze the surface functionalization of the magnetic nanoparticles. The morphology and size were characterized using TEM (FEI TECNAI G2 20 LaB6).

Assembly and Characterization of MNP@D-PEIs/DNA

DNA, D-PEIs, and MNPs were diluted in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)-buffered glucose (HBG; 20 mM HEPES, 5 % w/v glucose, pH 7.4) for the preparation of gene complexes. D-PEIs and DNA were mixed at a w/w ratio of 4 and incubated for 15 min to form binary D-PEIs/DNA complexes. The MNPs were added gently to the pre-prepared complexes at different weight ratios (0.4, 0.6, or 0.8; MNP/DNA) for 30 min to form the ternary complexes of MNP@D-PEIs/DNA. PEI 25kDa/DNA polyplexes were also prepared at a w/w ratio of 1.39. The stability of DNA compaction in the gene complexes was evaluated by electrophoresis on 1 % (w/v) agarose gel in the presence of ethidium bromide (EB; 0.1 μg mL^{-1}) with a voltage of 120 V for 15 min. The gel was photographed using UV illuminator. The particle size and zeta potential of the MNP@D-PEIs/DNA complexes were evaluated on a Zetasizer 3000 HAS (Malvern Instrument, Inc., Worcestershire, UK). The size and morphology of the magnetic complexes were observed by TEM (FEI TECNAI G2 20 LaB6, USA).

Cell Culture

COS-7 or A549 cells were incubated, respectively, in DMEM and 1640 medium containing 10 % FBS, 100 μg mL^{-1} streptomycin, and 100 IU mL^{-1} penicillin at 37°C in an incubator with a humid atmosphere of 5 % CO_2.

In Vitro Transfection

A549 cells were seeded at a density of 6×10^4 cells per well in a 24-well plate with 0.5 mL medium containing 10 % FBS and incubated at 37°C for 24 h. Then, the medium was replaced with fresh serum-free medium, to which D-PEIs/DNA or MNP@D-PEIs/DNA complexes were added to achieve a final DNA concentration of 3 μg mL^{-1}. After various incubation times (10 min, 30 min, 1 h, or 4 h), the magnetic field was moved away and the medium was replaced by 900 μL fresh medium containing serum, and the cells were further incubated for 24 h at 37°C. The cells were directly observed and the microscopy images were recorded on a fluorescence microscope (80i, Nikon, Japan) at a magnification of 100×. The transfection efficiency was quantified using Fluorescence Activated Cell Shorter (FACS) (Becton Dickinson, San Jose, CA)

For magnetofection, an array of 24 neodymium-iron-boron (Nd-Fe-B) permanent magnets (diameter = 15 mm, height = 3 mm; Shanghai YuHang Magnet Co., China) in the format of a 24-well plate that was placed under the cell culture plate to offer the magnetic field.[32]

Cytotoxicity Assay

The cytotoxicity of each complex and MNPs was assessed by an MTT assay on A549 and COS-7 cells. Cells were seeded in 96-well plates at a density of 1×10^4 cells per well and cultured for 24 h. After that, the culture medium was replaced with 200 μL medium containing the complexes. After 48 h of incubation, the cells were washed with PBS once, and 50 μL of 1 × MTT buffer was added to each well. After 4 h, the medium was removed, and the formazan crystals were dissolved with 150 μL DMSO for 10 min on a shaker. The ultraviolet absorption of each well was measured by a PowerWave™ XS Microplate Reader (BioTek Instruments, Inc.) at a wavelength of 570 nm. Cell viability = $(OD_{treated}/OD_{control}) \times 100$ % where OD is the optical density. The results were expressed as mean values (± standard deviation s.d.) of six repeats.

Supplementary Material

The ^{1}H NMR and ^{13}C NMR data of diglycidyl esters, characterization of D-PEIs, and GFP expression of MNP@DT-PEI/DNA are available on the Journal's website.

Acknowledgements

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