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Synthesis of biodegradable polycationic methoxy poly(ethylene glycol)–polyethylenimine–chitosan and its potential as gene carrier

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ABSTRACT

Methoxy poly(ethylene glycol)–polyethylenimine–chitosan (mPEG–PEI–CS) was synthesized via chitosan conjugated with polyethylenimine and methoxy poly(ethylene glycol). The intermediates and final copolymer were characterized and confirmed by ^{1}H NMR and FT-IR spectra. mPEG–PEI–CS was water soluble and its intrinsic viscosity was 0.446 dL/g. The contents of mPEG and PEI conjugated in the copolymer were 51.3% (*w*/*w*) and 28.9% (*w*/*w*), and the degree of substitution of PEI by mPEG was 176%. Gel electrophoresis confirmed that DNA was retained completely by the copolymer nanoparticles. The average diameter and zeta potential of mPEG–PEI–CS/DNA were 155 nm and 17.5 mV. The transfection of human embryonic kidney 293 (HEK293) cells proved that mPEG–PEI–CS/VRfat-1 plasmid had little toxicity on the growth and gene expression of cells, and the ratio of ω-3/ω-6 fatty acids was obviously increased after 72 h transfection compared to CS/VRfat-1 ($P < 0.05$). These indicated that mPEG–PEI–CS was a promising effective gene delivery and package molecule.

1. Introduction

Polymers can play an important role in the control of drug release. As a non-toxic, biocompatible and biodegradable polysaccharide, chitosan (CS) has attracted much interest, and a number of applications in drug delivery have been found due to its favorable biological properties (Liu, Li, Li, & Fang, 2004; Mao et al., 2004a). Further more, CS has been shown to condense DNA effectively and protect DNA from nuclease degradation (Liu & Yao, 2002; Mao et al., 2004b). However, CS is insoluble at neutral or basic pHs and it can only be dissolved in some specific acids (Chung, Kuo, & Chen, 2005). To solve this problem, many derivatives have been studied such as the modification of CS by quaternization of the amino groups (Le Dung, Milas, Rinando, & Desbrieres, 1994; Sieval et al., 1998), *N*-carboxymethylation (Muzzarelli, Tanfani, Emanuelli, & Mariotti, 1982) and PEGylation (Ohya, Cai, Nishizawa, Hara, & Ouchi, 2000; Saito, Wu, Harris, & Hoffman, 1997).

Poly(ethylene glycol) (PEG) is amphiphilic and non-toxic, and shows reduced reticuloendothelial system (RES) clearance. Extensive research has been carried out on PEGylation of biomolecules to prolong blood circulation time (Sugimoto, Morimoto, Sashiwa, Saimoto, & Shigemasa, 1998). On the other hand, PEG is used as a main additive in film coating materials, and it acts as a pore-forming agent to create interconnected channels for drug release (Lin & Lee, 2003). Polyethylenimine (PEI), a highly branched polyamine, is one of the most frequently used polycations for gene delivery. Escape of PEI/DNA complexes from the lysosomal compartment due to its 'proton sponge' mechanism was thought to be an attractive feature (Boussif et al., 1995). PEI has a strong influence on the efficiency of gene transfer with regard to transfection activity and cytotoxicity. The cytotoxicity of PEI is depends on its molecular weight with higher molecular weights giving cytotoxicity (Bieber & Elsasser, 2001; Godbey, Wu, & Mikos, 1999; Remy et al., 1998). A rational approach was suggested to combine low molecular weight PEIs together with suitable high molecular compounds such as CS, PEG and so on. It was expected that the toxicity would be reduced, while the long-term safety and transfection efficiency would be improved.

Omega-3 fatty acids can reduce cardiac events and decrease progression of atherosclerosis in coronary patients (Kris-Etherton, Harris, & Appel, 2002), while the omega-3 and omega-6 fatty acids are normally not interconvertible in the human body, therefore, how to increase the content of ω-3 fatty acids attracted much interest in recent years (Kang, Wang, Wu, & Kang, 2004).

Taking this information into account, mPEG–PEI–CS was synthesized, where mPEG was used to increase the slow-releasing property and water solubility while PEI was used to improve the

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transfection efficiency. The synthesized mPEG–PEI–CS was characterized by ^{1}H NMR, FT-IR, as well as transmission electron microscopy (TEM). The nanoparticle of mPEG–PEI–CS was prepared and its potentials as a gene delivery vector were investigated, the results showed that mPEG–PEI–CS/VR fat-1 could successfully convert omega-6 to omega-3 fatty acids.

2. Experimental

2.1. Materials

CS (Mn = 4.8×10^4 Da, degree of deacetylation >90%) was provided by Nanjing Weikang Biotechnology Co. Ltd (China), mPEG (Mn = 2×10^3 Da) was obtained from Han-nong Chemicals Inc. (Korea). PEI (Mn = 2×10^3 Da) was purchased from Wuhan Qianglong New Chemical Materials Co. Ltd. Dialysis tubing (MWCO 8×10^3–1.4×10^4 Da) was purchased from Beijing Solarbio Technology Co. Ltd (China). VRfat-1 plasmid: recombinant eukaryotic VR1012 plasmid containing fat-1 gene constructed in our laboratory, human embryonic kidney 293 (HEK293) cells were purchased from American Type Culture Collection (Rockvile, MD, USA). All the other commercially available chemicals were used as received without further purification.

2.2. Preparation of mPEG iodide (**1**) and mPEG–PEI (**2**)

mPEG iodide was prepared based on the literature (Hu, Jiang, Xu, Wang, & Zhu, 2005). Synthesis of mPEG–PEI (**2**) was via the mPEG iodide attacking the amino groups of PEI. mPEG iodide (12.0 g, 6.0 mmol) was dissolved in DMF (50 mL) with stirring under argon atmosphere, then the solution of PEI (6.0 g, 3.0 mmol) in DMF (50 mL) was added into the reaction mixture and a clear solution obtained, which was heated at 70 °C and stirred for 6 h under an argon atmosphere. The solution was concentrated by the removal of DMF, the residue was poured into ether (300 mL) with vigorous stirring and the precipitate was washed with ether thoroughly. The product was received as a white fibrous powder after dried under vacuum.

2.3. Preparation of N-phthaloyl CS (**3**) and Iodination of N-phthaloyl CS (**4**)

N-phthaloyl CS (**3**) was prepared based on the literature (Nishimura, Kohgo, Kurita, & Kuzuhara, 1991). The Iodination of *N*-phthaloyl CS was prepared as follows (Ashton, Koeniger, Stoddart, Alker, & Harding, 1996). PPh_3 (21.5 g, 82.2 mmol) was dissolved in anhydrous DMF (100 mL). To this solution I_2 (18.3 g, 72 mmol) was carefully added over 20 min with the evolution of heat. Sometimes the reaction mixture reaches approximately 55 °C. When cooled down to room temperature, imidazole (9.8 g, 144 mmol) was added partially, then *N*-phthaloyl CS (1.7 g, 6.0 mmol) in 30 mL DMF was added and the temperature was raised to 70 °C. At this temperature, the solution was stirred under argon atmosphere for 24 h. Then the reaction mixture was concentrated under reduced pressure, NaOMe solution was poured into the reaction vessel with effective cooling and the mixture was stirred for 30 min at 5 °C. Subsequently poured into MeOH (600 mL) to form a precipitate, which was collected by filtration and Soxhlet extracted with MeOH for 24 h. The product was obtained as a yellow powder.

2.4. Synthesis of mPEG–PEI–CS (**6**)

N-phthaloyl CS iodide (2.5 g, 6.0 mmol) was dissolved in DMF (200 mL) under argon atmosphere, the mixture was stirred for 1 h, then the solution of mPEG–PEI in DMF (150 mL) was added and a clear brown solution was obtained, which was heated to 70 °C and stirred for 20 h. The solution was concentrated by the removal of DMF, a viscous residue was obtained, which was dissolved in distilled water (100 mL) and purified by dialysis against distilled water for 72 h with several water changes times. Hydrazine monohydrate (150 mL) was added to the above solution with stirring under argon atmosphere, the mixture was heated to 100 °C and stirring for 15 h. Subsequently, the brown solution was diluted with distilled water and then evaporated, this procedure was repeated 3 times by the removal of hydrazine monohydrate. The crude product was dialyzed first against brine (5%) for 48 h then against distilled water for 72 h.

2.5. ^{1}H NMR and FT-IR spectrometry measurements

The ^{1}H NMR spectra of the intermediates and copolymer were measured with a 400-MHz NMR spectrometer (Avance 400 FT-NMR, Bruker, Germany), and the FT-IR spectra were measured with a Perkin Elmer spectrometer by the KBr pellets method.

2.6. Measurement of viscosity and determination of mPEG and PEI contents

Intrinsic viscosity of the copolymer solutions in 0.5 mol/L CH_3COOH/CH_3COONa was measured using an Ubbelode viscometer at 25 ± 0.01 °C. The content of mPEG units in mPEG–PEI and copolymer were determined by a modified colorimetric method based on the partitioning of a chromophore present in ammonium ferrothiocyanate regent from the aqueous to a chloroform phase in the presence of mPEG (Nag, Mitra, & Ghosh, 1996). Calibration curves were obtained by measuring the adsorbance at the various mPEG concentrations. The concentration of mPEG was calculated from the calibration curve according to its measured absorbance. The calculation for the content of PEI in copolymer was based on the content of mPEG.

2.7. Preparation of copolymer/DNA nanoparticles

The nanoparticles of CS and mPEG–PEI–CS complexes were prepared following the method of ionic cross linkage (Bodmeier, Chen, & Paeratakul, 1989). Briefly, CS and mPEG–PEI–CS were diluted separately in CH_3COOH/CH_3COONa (pH 5.5) containing appropriate amount of triphosphate and heated at 65 °C for 10 min with mild magnetic stirring. After that, an appropriate amount of polymer solution was added to the solution of plasmid DNA and the solution was mixed then left for 5 min.

2.8. Nanoparticle sizes and zeta potential

The average diameter and zeta potential of the polymeric micelles were measured by Zetasizer 3000 HS/IHPL instrument (Malvern Instruments Ltd., Malvern, UK).

2.9. Transmission electron microscopy (TEM)

The morphology of the nanoparticles was examined via TEM. A drop of polyioncomplex micelle was placed on a copper grid covered with nitrocellulose, which was air-dried and negatively stained with tungstophosphoric acid at room temperature then the observation was performed with the electron microscopy.

2.10. Agarose gel electrophoresis

The DNA binding ability of CS and mPEG–PEI–CS were evaluated by agarose gel electrophoresis. The nanoparticle solutions

of plasmid DNA with CS and mPEG–PEI–CS copolymer were loaded into individual wells of 0.7% agarose gel, electrophoresed at 100 V for 45 min and stained with 0.5 mg/mL ethidium bromide. The plasmid migration pattern was revealed under UV irradiation.

2.11. The transfection of HEK293 cells and cell viability

HEK293 cells were seeded in 12-well plates (1.0×10^5 cells/well), respectively. The cells were incubated in Dulbecco's modified Eagle medium (DMEM, Invitorgen Corparration.) supplemented with 10% fetal bovine serum (FBS), streptomycin at 100 μg/mL, penicillin at 100 μg/mL, and were maintained at 37 °C in a 5% environment of CO_2 until the plates were covered with 80% excess of cells. The complexes of nanoparticles enwraped with CS and mPEG–PEI–CS each containing 3 μg of DNA were added into the wells, respectively, then the cells were incubated with the complexes at 37 °C in a 5% carbon dioxide humidified atmosphere.

2.12. Analysis of polyunsaturated fatty acids

The cells mentioned above were collected and centrifuged for 5 min then washed with 0.9% NaCl, and the precipitate was extracted with chloroform/methanol (2:1, v/v) containing 0.2% acetic acid. Subsequently, the chloroform phase containing fatty acids of cells was collected, and the fatty acids were esterified (Kang & Wang, 2005). In brief, lipid sample was mixed with 1 mL hexane and 1 mL of BF_3/MeOH, the mixture was heated at 100 °C for 1 h, then methyl esters were extracted in hexane phase and analyzed via gas chromatography–mass spectrometry (GCMS QP2010, Japan) as described (Kang et al., 2001).

3. Results and discussion

3.1. Analysis of mPEG iodide and mPEG–PEI

mPEG iodide and mPEG–PEI were synthesized rapidly as shown in Scheme 1. The structure of mPEG iodide was determined by ^{1}H NMR (Fig. 1). mPEG iodide had its specific peaks at 3.4 ($-OCH_3$) ppm and 3.2 ($-CH_2I$) ppm, the degree of the substitution (DS) from –OH to –I groups, estimated based on the data mentioned above, was 0.89. Compared with the literature (Tian et al., 2005), mPEG–PEI was prepared via a simple synthesis route (Scheme 1). The structure of mPEG–PEI was determined by FT-IR spectra (Fig. 2). The appearance of characteristic peak at 1112 (C–O stretch) cm^{-1} and the growing of the peak at 2867 (C–H stretch) cm^{-1} indicated the successful attachment of the mPEG onto PEI. On the other hand, the critical peak at 1665 cm^{-1} assigned to the amino I which was another demonstration of the successful linking. The grafting mPEG onto PEI also can be confirmed by ^{1}H NMR (Fig. 1). The chemical shift at 2.6–2.8 ppm referred to the methylene of PEI and the shift at 3.4 ppm assigned to methoxy group of mPEG. All the information mentioned above indicated that mPEG grafted onto PEI successfully. The aim of introducing mPEG onto PEI was to enlarge the molecular weight and reduce the cytotoxicity of PEI. The amount of mPEG in mPEG–PEI could be calculated following formula (1) and the DS_{mPEG} of mPEG–PEI was calculated as shown in formula (2).

$$\begin{aligned} \mathrm{mPEG}(\%w/w) &= \frac{m_{\mathrm{mPEG}}}{m_S} \times 100\% \\ \mathrm{PEI}(\%w/w) &= \frac{m_S - m_{\mathrm{mPEG}}}{m_S} \times 100\% \\ \omega &= \frac{m_S - m_{\mathrm{mPEG}}}{m_{\mathrm{mPEG}}} \times 100\% \end{aligned} \tag{1}$$

$$\mathrm{DS}_{\mathrm{mPEG}}(\%) = \frac{\frac{m_{\mathrm{mPEG}}}{M_{\mathrm{mPEG}}}}{\frac{\omega \times m_{\mathrm{mPEG}}}{M_{\mathrm{PEI}}}} \times 100\% \tag{2}$$

Among the two formulas, m_S is the weight of the sample and m_{mPEG} is the measured mPEG weight in the sample, while M_{mPEG} and M_{PEI} are the molecular weight of mPEG and PEI, respectively.

From the formulae, the amount of mPEG in mPEG–PEI was 63.8% (*w*/*w*), as a result, the content of PEI was 36.2% (*w*/*w*), and the DS_{mPEG} was 176%. As is known, PEI was high branched, and had many primary amino groups. As a mater of fact, there were excess of mPEG grafted onto PEI, therefore, the DS_{mPEG} exceeded 100%. The grafting mPEG onto PEI can not only improve the solubility but also decrease the toxicity of the copolymer.

3.2. Analysis of mPEG–PEI–CS

mPEG–PEI–CS was successfully synthesized as shown in Scheme 1. Briefly, the *N*-phthaloyl CS was activated by iodination at the C_6–OH position according to the literature (Ashton et al., 1996), and mPEG–PEI was conjugated onto CS under mild reaction conditions. The structure of mPEG–PEI–CS was determined by FT-IR (Fig. 2). Compared with CS, The mPEG–PEI–CS showed new absorptions or intensified ones at 2867, 1112 and 843 cm^{-1} due to the introduction of mPEG and the classic peak at 1655 (amide I) and 1576 (amide II) cm^{-1} indicated that PEI was grafted onto CS successfully. More information about the graft copolymer was obtained by ^{1}H NMR spectra (Fig. 1). The characteristic peaks at 7.5–7.9 (phthaloyl moiety) ppm due to the protecting amino groups of CS, disappeared in the copolymer of mPEG–PEI–CS indicated the successful removal of the phthaloyl groups, while the chemical shift at 2.6–2.8 ($-NH-CH_2-$) ppm assigned to $-CH_2$ of PEI, and the critical signals at 3.3 ($-OCH_3$) ppm and 3.6 ($-OCH_2-$) ppm due to the existence of mPEG. All the information indicated that mPEG and PEI were grafted onto CS successfully. The amounts of mPEG and PEI in mPEG–PEI–CS could be calculated as formula (3) and the DS_{PEI} of the copolymer by PEI was calculated following formula (4).

$$\begin{aligned} \mathrm{mPEG}'(\%w/w) &= \frac{m'_{\mathrm{mPEG}}}{m'_S} \times 100\% \\ \mathrm{PEI}'(\%w/w) &= \frac{m'_{\mathrm{mPEG}} \times \omega}{m'_S} \times 100\% \end{aligned} \tag{3}$$

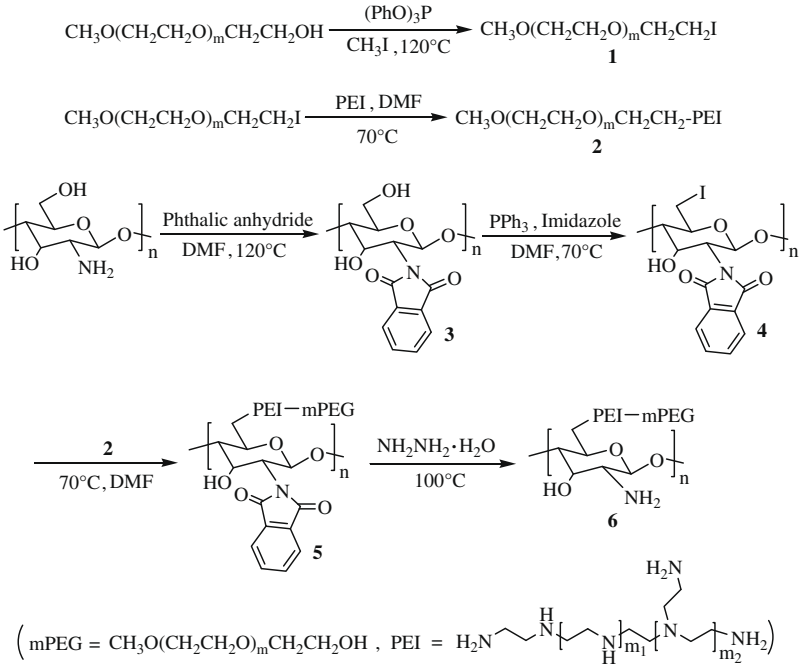
$$\mathrm{DS}_{\mathrm{PEI}}(\%) = \frac{\frac{m'_{\mathrm{mPEG}} \times \omega}{M'_{\mathrm{PEI}}}}{\frac{m'_S - (1+\omega)m'_{\mathrm{mPEG}}}{M'_{\mathrm{chitosan}}}} \tag{4}$$

Among the formula, m'_S and m'_{mPEG} represent the weight of the mPEG–PEI–CS sample and the measured mPEG weight in the sample, while M'_{PEI} and $M'_{chitosan}$ are the molecular weight of PEI and CS, respectively.

The contents of mPEG and PEI in the copolymer were 50.9% (*w*/*w*) and 28.9% (*w*/*w*), therefore the amount of CS in mPEG–PEI–CS was 20.2% (*w*/*w*) based on the data displayed above. As was shown in the results, the DS_{PEI} was 11.8%, which was quite suitable for the copolymer as our expected. If the DS_{PEI} were too high, the solubility of mPEG–PEI–CS either in water or organic solvents would be reduced. The intrinsic viscosity (η) of PEI, mPEG, CS and mPEG–PEI–CS in NaOAc aqueous solution were 0.135, 0.089, 3.761 and 0.464 dL/g, respectively, which were calculated as following.

$$\eta_\gamma = t/t_0 \quad \eta_{SP} = \eta_\gamma - 1 \quad \eta = [2(\eta_{SP} - \ln \eta_\gamma)]^{\frac{1}{2}}/C$$

where t represents the outflow time of polymer solution, t_0 represents the outflow time of the solvent, while C is the concentration of the polymer.



Scheme 1. Synthesis of the mPEG–PEI–CS copolymer.

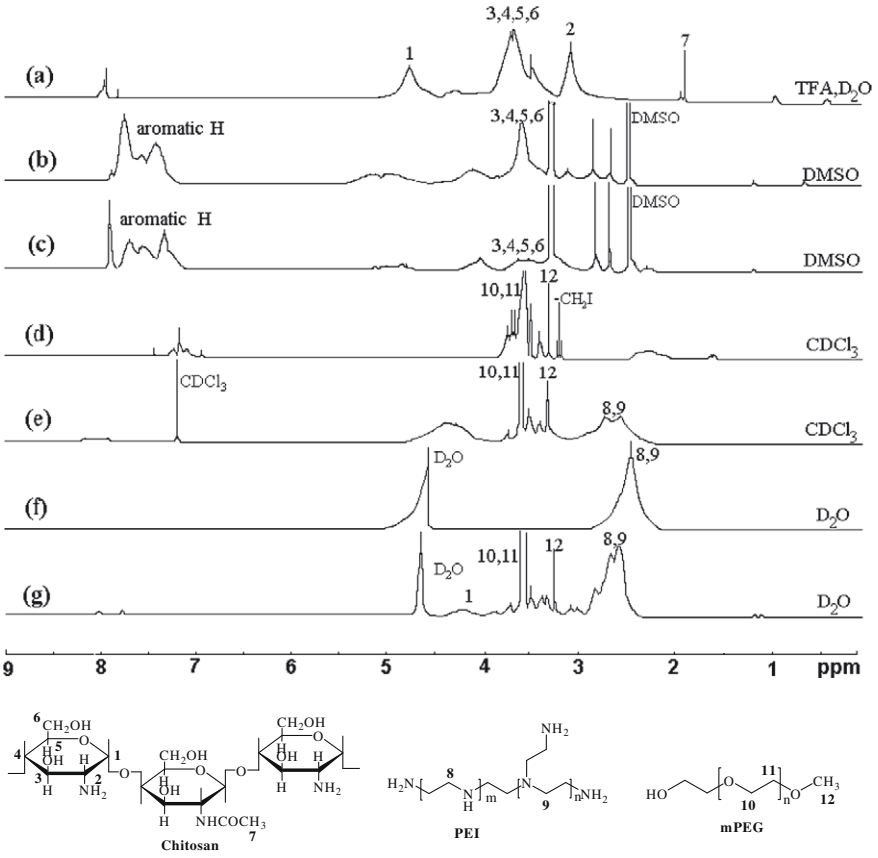


Fig. 1. ^{1}H NMR spectra of CS and its derivates: (a) CS dissolved in Trifluoroacetic acid and D_2O, (b) *N*-phthaloyl CS iodide dissolved in DMSO, (c) *N*-phthaloyl CS dissolved in DMSO, (d) mPEG iodide dissolved in $CDCl_3$, (e) mPEG-PEI dissolved in $CDCl_3$, (f) PEI dissolved in D_2O, (g) mPEG–PEI–CS dissolved in D_2O.

The viscosity of mPEG–PEI–CS was higher than that of PEI and mPEG, while lower than that of CS, which could be explained that the destruction of the crystal structure of CS, while the introduction of liner chain of mPEG could be another factor.

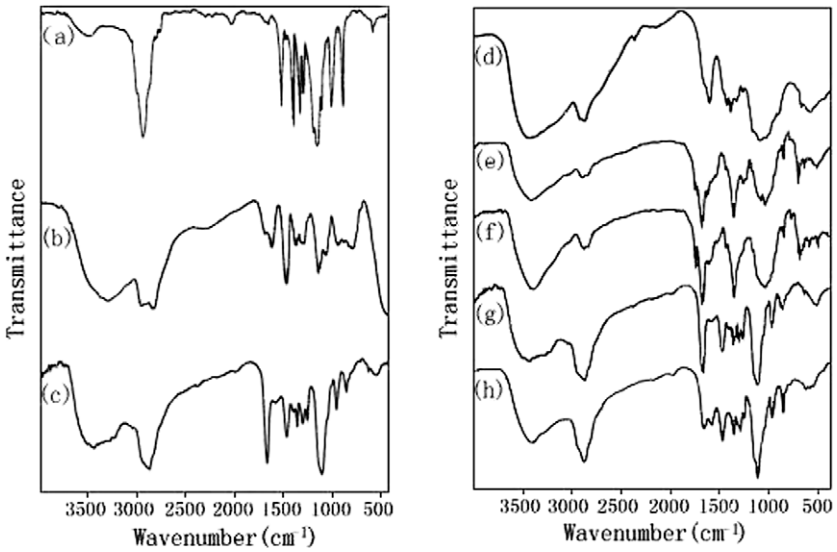


Fig. 2. FT-IR spectra of (a) mPEG, (b) PEI, (c) mPEG-PEI, (d) CS, (e) *N*-phthaloyl CS, (f) *N*-phthaloyl CS iodide, (g) mPEG-PEI, (h) mPEG–PEI–CS.

3.3. Characterization of nanoparticles

Small particles are necessary for endocytosis into cells, while a positive surface charge of untargeted polyplexes is necessary for banding to anionic cell surface (Klaus et al., 2003; Mansouri et al., 2006). It is well known that CS and PEI as the cationic polymers have been considered a good candidates for the preparation of nanoparticles or polymeric micelles. While researches showed that many of drugs, proteins and DNA complexes were sensitive to acidic solution and could be easily inactivated in a highly acidic environment (Kim, Jeong, & Nah, 2007), therefore, polycationic PEI was introduced onto CS for the purpose of enhancing the positive charge of the copolymer. In this work, the average diameter and its surface charges were determined as revealed in Table 1 and Fig. 3. As was shown, the average diameter of mPEG–PEI–CS was smaller than that of CS probably due to the increased DNA condensation capability, further more, the zeta potential of mPEG–PEI–CS was in the positive range and it was higher than that of CS due to the introduction of polymeric PEI. As expected, with the increasing of the mass ratio, the zeta potential became more positive and the average diameter became smaller. When the mass ratio reached 30:1, the perfect particle size of 155 nm and higher zeta potential of 17.5 mV were obtained.

The condensation capability of mPEG–PEI–CS with DNA was evaluated by measuring the fluorescence emitted when adding the ethidium bromide into the polyplexes (Fig. 4). As was shown,

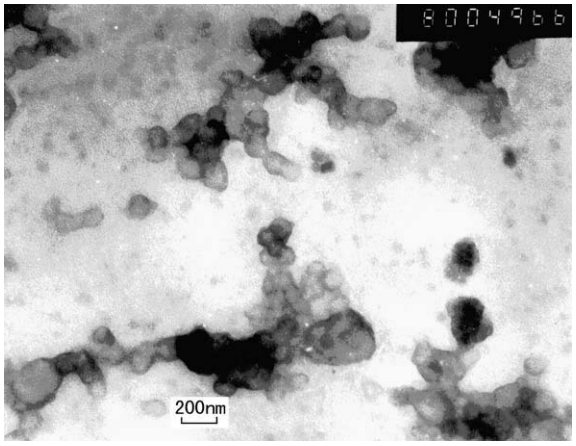


Fig. 3. TEM photograph of mPEG–PEI–CS nanoparticles (100,000×).

Table 1
The average diameter and zeta potential of CS and mPEG–PEI–CS.

Sample	*T* (°C)	*w*/*w*	Z-Ave (d.nm)	Zeta Potential (mV)
CS1[a]/DNA	25	10:1	490	9.60
	25	20:1	349	15.6
	25	30:1	230	15.8
mPEG–PEI–CS	25	10:1	406	−1.54
	25	20:1	374	0.44
	25	30:1	155	17.5
CS2[b]/DNA	25	10:1	380	−1.59
	25	20:1	398	0.60
	25	30:1	381	11.1

[a] Mn = 2.93 × 10^5 Da.
[b] Mn = 4.8 × 10^4 Da.

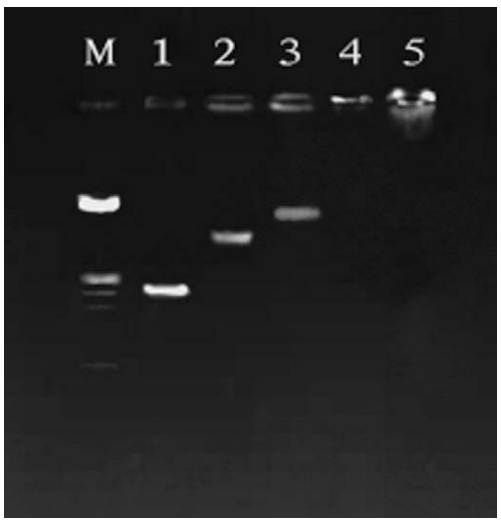


Fig. 4. Gel retardation assay (0.7% agarose gel) of mPEG–PEI–CS/DNA complex. M: marker; Lane 1: VRfat-1; Lane 2–5: mPEG–PEI–CS/VRfat-1, mass ratios of 5:1, 10:1, 20:1, 30:1, respectively.

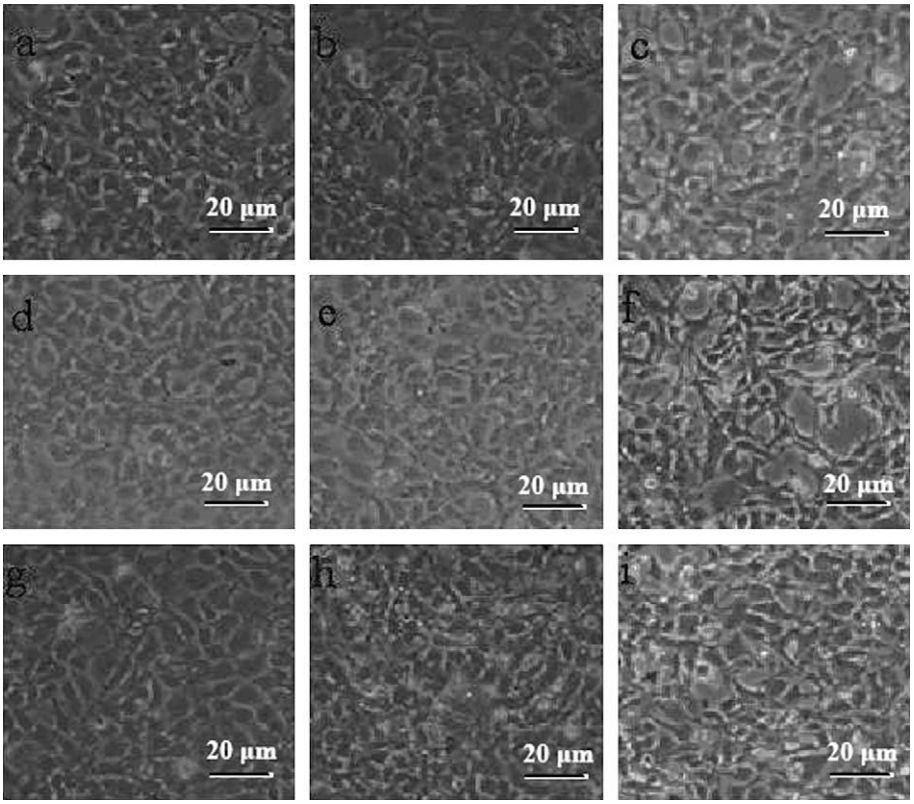


Fig. 5. The cell viability after incubation for 24, 48 and 72 h. (a) CS1/DNA (24 h); (b) CS1/DNA (48 h); (c) CS1/DNA (72 h); (d) mPEG–PEI–CS/DNA (24 h); (e) mPEG–PEI–CS/DNA (48 h); (f) mPEG–PEI–CS/DNA (72 h); (g) CS2/DNA (24 h); (h) CS2/DNA (48 h); (i) CS2/DNA (72 h). All figures are of the same magnification (200×).

the complexes were negatively charged when the mass ratios were 5:1 or 10:1, while the migration of DNA was suppressed completely when mass ratio of mPEG–PEI–CS/DNA was 20:1. It showed that DNA plasmids were entrapped into mPEG–PEI–CS successfully.

3.4. Cell viability

Cationic polymers are able to condense more DNA than lipids. They form complexes with DNA and protect it against nuclease degradation (Gao & Huang, 1996). As well known, the polycationic PEI is a good candidate for gene delivery, but of which the application is limited due to its cytotoxicity, because the non-cytotoxicity is a precondition for gene delivery systems as eventual applicability in *vitro* and *in vivo*. In this work, the cytotoxicity of PEI was greatly reduced due to the introduction of mPEG and CS. As shown in Fig. 5, the cell viability of CS and mPEG–PEI–CS/DNA complexes was over 95% after 24 h and 48 h incubation. Even after 72 h, the HEK293 cells still grew well. The results revealed that mPEG–PEI–CS/DNA didn't decrease the cells viability in comparison with the CS/DNA nanoparticles.

3.5. Analysis of polyunsaturated fatty acids

Studies on nonhuman primates and human newborns indicated that eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were essential for normal functional development of the retina and brain, particularly in premature infants (Simopoulos, 2000). Further more, EPA and DHA are the chief ω-3 fatty acids used as dietary supplements. The importance of ω-3 polyunsaturated fatty acids (PUFAs) has been recognized due to their therapeutic value on human health. In contrast to ω-6 fatty acids, ω-3 fatty acids can influence cardiovascular disease pathology by beneficially modulating inflammation (Wang, Reiterer, Toborek, & Hennig, 2008). Mammals normally can not convert ω-6 to ω-3 fatty acids because they lack the ω-3 fatty acid desaturase gene. In this article, mPEG–PEI–CS–VRfat-1 was used to transfer HEK293 cells and the content of the fatty-acid was investigated. As shown in the Fig. 6, statistical analysis indicated that the content of EPA increased from 1.32% to 5.89% ($P < 0.05$) and DHA increased from 1.39% to 3.98% ($P < 0.05$) after 72 h of transfection. Further more, as expected, the quantity of ω-6 PUFAs significantly reduced from 56.12% to 28.52% ($P < 0.05$), while ω-3 PUFAs had a distinct increase from 5.10% to 18.45% ($P < 0.05$). As a result, the ratio of ω-6/ω-3 was decreased from 11.0 to 1.55. These results demonstrated that VRfat-1 was successfully transfected into HEK293 cells by mPEG–PEI–CS, and the transfection efficiency of the copolymer merits further investigation.

4. Conclusions

A novel fully water-soluble and biodegradable cationic mPEG–PEI–CS was synthesized, the characterization results of ^{1}H MNR and FT-IR for the intermediates and final product conformed that the mPEG-PEI was successfully grafted onto CS at the C-6 position via CS iodide precursor. The complex of mPEG–PEI–CS/DNA micelle had an appropriate particle size of 155 nm and positive surface charge of 17.5 mV. The cationic copolymer showed a good DNA condensation capability and exhibited negligible toxicity. Using mPEG–PEI–CS as transfection agent, the converting of ω-6 to ω-3 fatty acids was successfully realized. Based on these superior properties, mPEG–PEI–CS should be a promising non-viral gene carrier

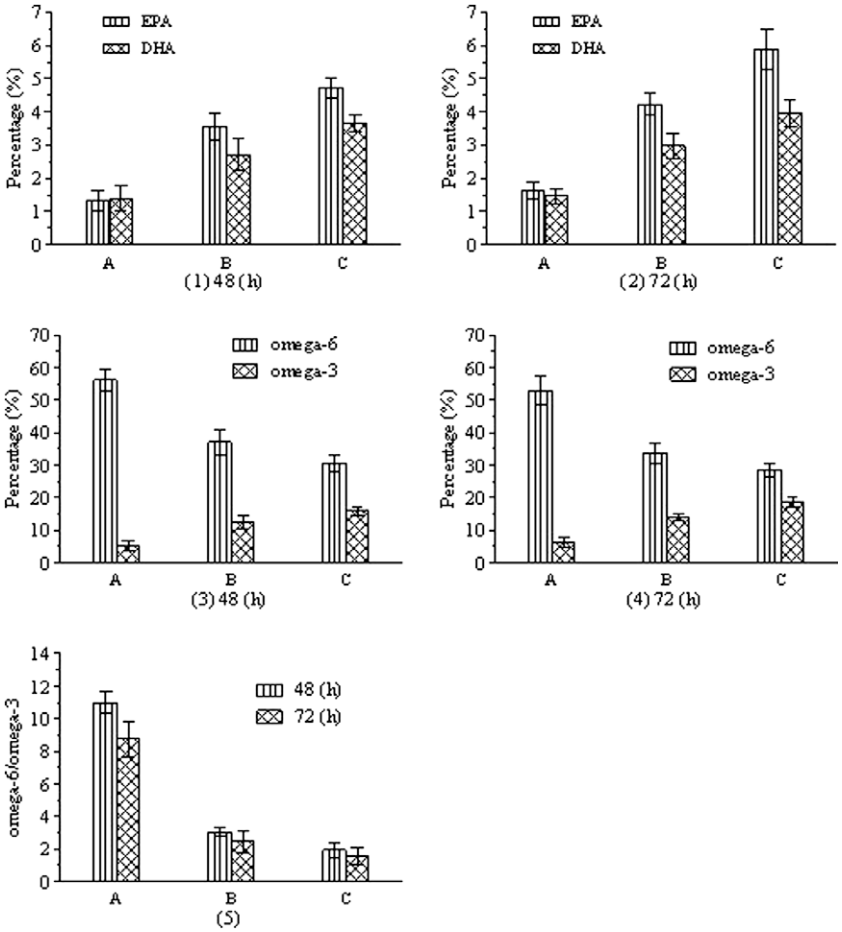


Fig. 6. Changes of the fatty acids in HEK293 cells after transfection. (A) contranst; (B) CS–VRfat-1; (C) mPEG–PEI–CS–VRfat-1; (1) and (2): changes of EPA and DHA in cells after 48 and 72 h transfection, respectively; (3) and (4): changes of ω-6 and ω-3 fatty acids in cells after 48 and 72 h transfection, respectively; (5): change tendency of ω-6/ω-3 fatty acids after 48 h and 72 h transfection. Values are means of three measurements.

for which applications will be extensively investigated later in biological fields.

Acknowledgements

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