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Synthesis of diamine maleyl chitosans, and in vitro transfection studies

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ABSTRACT

Three novel diamine-modified chitosan derivatives were synthesized from *N*-maleyl chitosan via Michael addition reaction with 1,2-diaminoethane, 1,4-diaminobutane, and 1,6-diaminohexane, respectively. These chitosan derivatives exhibited well binding ability of condensing plasmid DNA to form complexes with size ranging from 150 to 500 nm when the chitosan derivative/DNA weight ratios were above 10. The complexes observed by scanning electron microscopy (SEM) exhibited a compact and spherical morphology. The cytotoxicity of the chitosan derivatives presented a dependence on their side-chain structures. The gene transfection experiments were evaluated in 293 T and HeLa cells. The data obtained demonstrated that the gene transfection efficiencies of these chitosan derivatives were better than that of chitosan, suggesting these chitosan derivatives as potential gene vectors in vitro.

1. Introduction

It is an important subject to develop an efficient gene delivery system into cells for the success of gene therapy (Lehrman, 1999; Verma & Somia, 1998). Recent years, several non-viral gene vectors have been developed to enhance gene transfection efficiency. Among non-viral systems, cationic polymers have gained increasing attention because they can form stable polycationic complexes between plasmid DNA and cationic polymers by electrostatic attraction interaction. Up to now, a number of cationic polymers have been employed for gene delivery. For example, polyethylenimine (PEI) (Huh et al., 2007; Kim et al., 2005), poly-L-lysine (PLL) (Collins & Fabre, 2004; Park & Healy, 2004), polyamidoamine dendrimers (Kim et al., 2007; Zhou et al., 2006), and poly(propylenimine) dendrimers (PPI) (Lee, Ko, Park, Yamaguchi, & Kim, 2001). These cationic polymers mediate transfection via condensing genetic material into compact nanoparticles, protecting DNA from enzymatic degradation, and thus facilitating the cell uptake and endolysosomal escape.

Chitosan is a linear, natural cationic polysaccharide comprising β (1→4) linked glucosamine partly containing *N*-acetyl-D-glucosamine (Köping-Höggård et al., 2004; Leong et al., 1998). Since the polysaccharides are derived from natural sources, chitosan is one of the most intensively studied cationic polymers among the non-viral systems due to its inherent properties such as biocompatibility, biodegradability and low toxicity (Köping-Höggård et al., 2004; Li et al., 2010; Muzzarelli, 2010; Park et al., 2010). In spite of the presence of the unique properties described above, the transfection efficiency of chitosan is poor, which is attributed to the poor solubility of chitosan in aqueous solution and the strong interactions between chitosan and DNA (Köping-Höggård et al., 2004). Because the poor solubility of chitosan can significantly affect the formation of DNA/chitosan complex. Simultaneously, the strong interaction between the chitosan and DNA will result in the formation of highly stable DNA/chitosan complex, thereby preventing dissociation of the complex within the cell and ultimately precluding translation of the DNA. In order to improve the transfection efficiency of chitosan, great attention has been paid to modify the molecular structure of chitosan. For example, Wong et al. (2006) synthesized PEI-graft-chitosan by cationic polymerization of aziridine to water-soluble chitosan. Kim, Ihm, Choi, Nah, and Cho (2003) reported that chitosan coupled with urocanic acid can improve the corresponding transfection efficiency. And also, chemical medications of chitosan using hydrophilic (Germershaus, Mao, Sitterberg, Bakowsky, & Kissel, 2008), hydrophobic (Chae, Son, Lee, Jang, & Nah, 2005), thermosensitive (Sun et al., 2005) entities have been also reported, and the results indicated that the transfection efficiencies of the modified chitosan vectors were superior to that of pure chitosan vector.

In this work, in order to overcome the disadvantages of its poor solubility in water and improve the transfection efficiency, chitosan was modified with maleic anhydride to obtained *N*-maleyl chitosan (Lacerda et al., 2009; Sun, Zhu, Xie, & Yin, 2011; Vanichvattanadecha, Supaphol, & Rujiravanit, 2008) and then

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coupled by three diamines with different length using Michael addition reaction. The in vitro gene transfection of these modified chitosans containing primary amines in the side chains was evaluated.

2. Experimental

2.1. Materials

Chitosan (degree of deacetylation: 85%, Mw: ~10 kDa) was purchased from Haidebei Co. Ltd., Jinan, China. The dimethyl sulphoxide (DMSO) was obtained from Shanghai Chemical Reagent Co., China and dried before use. 1,2-Diaminoethane and 1,6-diaminohexane were obtained from domestic suppliers and distilled before use. 1,4-Diaminobutane was provided by Sigma-Aldrich and used directly. Dulbecco's Modified Eagle's Medium (DMEM), penicillin–streptomycin, trypsin, fetal bovine serum (FBS), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), and Dubelcco's phosphate buffered saline (PBS) were purchased from Invitrogen Corp. The reporter plasmids, pEGFP-C1 and pGL-3, were purchased from Invitrogen and Promega, respectively. Plasmids were amplified in *Escherichia coli* and extracted and purified by E.Z.N.A. fast filter endo-free plasmid maxi kit (Omega). The plasmid DNA was stored at −20 °C until the transfection experiments. All other reagents were analytical grade and used as received.

2.2. Synthesis of N-maleyl chitosan

N-maleyl chitosan was synthesized according to our previous study (Lu, Xu, Zhang, Cheng, & Zhuo, 2008). In brief, 2 g of chitosan was dissolved in 50 mL of acetic acid aqueous solution (0.1 M), precipitated with 40 mL of 0.2 M NaOH, collected by filtration, and then washed with water to pH 7. The treated chitosan was dispersed in 150 mL DMSO. Then, DMSO solution containing 3.5 g maleic anhydride was added. After reacting at 60 °C for 8 h, the product was collected by precipitation in 500 mL acetone.

2.3. Synthesis of chitosan-based derivates with pendant primary amine (chitosan derivatives 1a–1c)

Chitosan derivative 1*a*: To graft diamine onto *N*-maleyl chitosan, 0.2 g *N*-maleyl chitosan was dissolved in 20 mL 0.25% (w/v) sodium hydroxide solution. Then an aqueous solution containing 3 g of 1,2-diaminoethane was added. After stirring at 60 °C for 24 h, hydrochloric acid was added to adjust the pH of the solution to 7.0. Thereafter, the solution was dialysized (MWCO: 3.5 kDa) against distilled water for 3 days and then lyophilized for 3 days to obtain polymer 1a.

Chitosan derivative 1*b*: Chitosan derivative 1b was synthesized from *N*-maleyl chitosan and 1,4-diaminobutane according to the procedure described above.

Chitosan derivative 1*c*: Chitosan derivative 1c was synthesized from *N*-maleyl chitosan and 1,6-diaminohexane according to the procedure described above.

2.4. Chitosan derivatives characterization

^{1}H NMR spectra of chitosan derivatives 1a, 1b, 1c were recorded on a Mercury VX-300 spectrometer at 300 MHz (Varian, USA) by using D_2O as a solvent and TMS as an internal. The molecular weights of the polymers were measured by a gel permeation chromatography (GPC) equipped with a Waters 2690 separation module and a Waters 2410 refractive index detector. Acetic acid/ammonium acetate (pH 5.3) buffer solution was used as elute at a flow rate of 1.0 mL/min. The column temperature was maintained at 35 °C.

2.5. Cell culture

293 T and HeLa cells were incubated in DMEM supplemented with 10% (v/v) FBS and 1% antibiotics (penicillin–streptomycin, 10,000 U/mL) at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were sub-cultured prior to confluence using trypsin–EDTA.

2.6. Preparation of chitosan derivative/DNA complexes

Chitosan derivatives 1a, 1b, 1c were respectively dissolved in NaCl solution (150 mM, which is physiological saline) with a concentration of 2 mg/mL and then filtered using a 0.22 μm filter. A plasmid DNA stock solution (120 ng/μL) was also prepared in 40 mM Tris–HCl buffer solution. Nanoparticles were prepared by adding copolymer solution to equal volumes of DNA solution (containing 1 μg DNA) at various weight ratios with gentle vortexing and incubated at 37 °C for 30 min before use.

2.7. Cytotoxicity assay

Cells were seeded in the 96-well plate at a density of 6000 cells/well and cultured 24 h in 200 μL DMEM containing 10% FBS. After the polymer was added for 48 h, the medium was replaced with 200 μL of fresh medium. Then 20 μL MTT solutions (5 mg/mL) were added for 4 h. Thereafter, the medium was removed and 150 μL DMSO was added. Plates were incubated for 5 min at 37 °C, and the absorbance was measured at 570 nm using a microplate reader (BIO-RAD, Model 550, USA). The relative cell viability was calculated as: cell viability (%) = $(OD_{sample}/OD_{control}) \times 100$, where $OD_{control}$ was obtained in the absence of chitosan derivatives and OD_{sample} was obtained in the presence of chitosan derivatives. Each value was averaged from 4 independent experiments.

2.8. Agarose gel electrophoresis

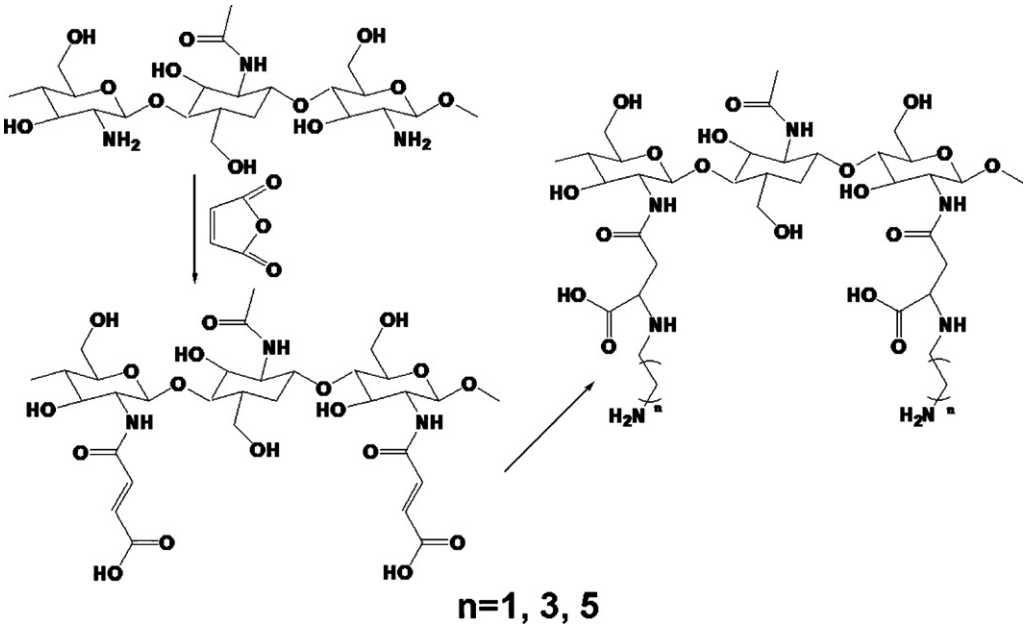
The chitosan derivative/DNA complexes with different weight ratios ranging from 5 to 11 were formed according to the conditions described above. Then 6 μL complexes suspension containing 0.1 μg DNA was electrophoresed on the 0.7% (w/v) agarose gel containing GelRed™ and with Tris-acetate (TAE) running buffer at 80 V for 80 min. DNA retardation was observed by irradiation with UV light and assayed with Cam2com software.

2.9. Measurement of particle size and zeta-potential

The particle size and zeta-potential measurements were performed at pH 7.0 in triplicates by Nano-ZS ZEN3600 (MALVERN Instrument) at 37 °C. The chitosan derivative/DNA complexes were prepared by adding appropriate volume of copolymer solution (150 mM NaCl solution) to 1 μg of DNA (in 40 mM Tris–HCl buffer solution) at various weight ratios. The solution containing complexes was diluted by 150 mM NaCl solution for particle size measurement or diluted by distilled water for zeta-potential measurement to 1 mL, and then incubated at 37 °C for 30 min.

2.10. Scanning electron microscopy (SEM)

The chitosan derivative/DNA complexes were prepared as described above. 100 μL of complex suspension was deposited onto a glass slide. After drying at room temperature, the morphology of



Scheme 1. Synthesis of diamine grafted *N*-maleyl chitosan (chitosan derivatives 1a–1c).

the sample was observed by a scanning electron microscope (SEM, FEI-QUANTA 200, Holland). Before the SEM observation, the samples were fixed on an aluminum stub and coated with gold for 7 min.

2.11. In vitro transfection

Cells were seeded in 24-well plates at an initial density of 6×10^4 cells/well with 1 mL DMEM containing 10% FBS and incubated at 37 °C for 24 h in 5% CO_2 (to reach 70% confluence at the time of transfection). The chitosan derivative/DNA (pEGFP-C1 or pGL3-Luc) complexes were formed at different weight ratios ranging from 10 to 60 according to the conditions described above (containing 1 μg DNA in each weight ratio). Before transfection, the cells were washed by phosphate buffered saline (PBS, 0.1 M, pH 7.4), and the cells were incubated with the complexes in serum-free or 10% serum-containing culture medium for 4 h at 37 °C. Then the medium was replaced with fresh medium containing serum and incubated for 48 h. And the cells were analyzed for green fluorescence protein (pEGFP-C1) expression with a fluorescence microscope (OLYMPUS IX70, Japan).

To assay the expression of luciferase, the medium was removed and the cells were rinsed gently by PBS. After thorough lysis of the cells with reporter lysis buffer (Promega) (200 μL/well), the luciferase activity was determined by detecting the light emission from an aliquot of cell lysate incubated with 100 μL of luciferin substrate (Promega) in a luminometer (Lumat LB9507, Berthold). The protein content of the cell lysate was determined by BCA protein assay kit (Pierce) (Smith et al., 1985). All the experiments were carried out in triplicate to ascertain the reproducibility.

3. Results and discussion

3.1. Polymer synthesis and characterization

The synthesis routes of chitosan derivatives 1a–1c are shown in Scheme 1. The products were obtained via Michael addition reaction between amino groups of diamine to the double bonds of *N*-maleyl chitosan.

The ^{1}H NMR spectra of chitosan, *N*-maleyl chitosan, and chitosan derivatives 1a-1c are shown in Fig. 1. After the maleyl reaction, new peak appears at 6.2 ppm and 5.7 ppm in the spectrum of *N*-maleyl chitosan (Fig. 1), which is assigned to proton of –CH=CH–. By comparing the intensity of peaks at 3.4–3.6 ppm (multiplet, assigned to glucosamine unit, H-3, H-4, H-5, H-6, H-6′), the maleyl chitosan degree is calculated as 81.2%. In this study, chitosan derivatives 1a, 1b, 1c were synthesized via Michael addition reaction between *N*-maleyl chitosan and excess diamine. The reaction was carried out in basic environment with two advantages. First, *N*-maleyl chitosan can be completely dissolved in basic water. The second, the basic environment is in favor of Michael addition reaction. The molecular weights of chitosan derivative 1a, 1b and 1c are 15,500 Da, 16,600 Da and 17,300 Da, respectively.

Chitosan derivative 1*a*: ^{1}H NMR (D_2O, ppm) d: δ = 3.321-3.65 (multiplet, D-glucosamine unit, H-3, H-4, H-5, H-6, H-6′); δ = 2.4–2.78 (–$NHCH_2CH_2$–); δ = 1.903 ($COCH_3$).

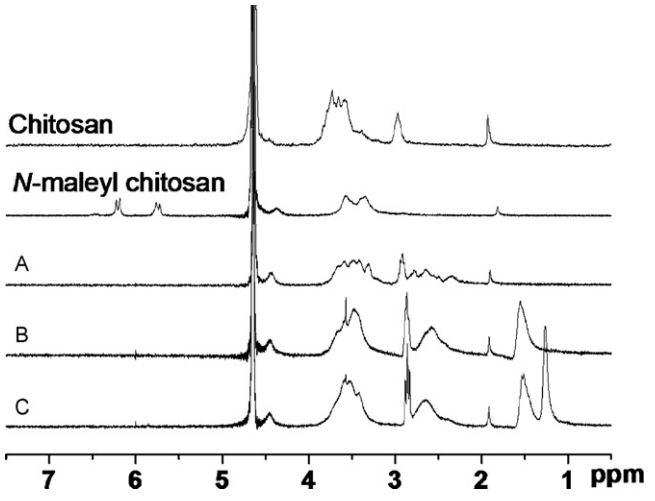


Fig. 1. ^{1}H NMR spectra of (A) chitosan derivative 1a, (B) chitosan derivative 1b, and (C) chitosan derivative 1c in D_2O.

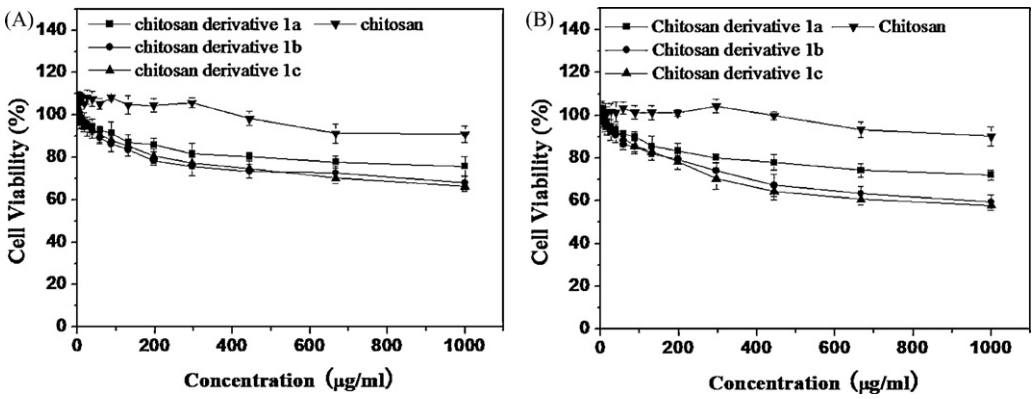


Fig. 2. Cytotoxicity of polymers at various concentrations for (A) 293 T, and (B) HeLa cells.

Chitosan derivative 1*b*: ^{1}H NMR (D_2O, ppm) d: δ = 3.41–3.60 (multiplet, D-glucosamine unit, H-3, H-4, H-5, H-6, H-6′); δ = 2.582 (4H, $-NHCH_2-$); δ = 1.915 ($COCH_3$); δ = 1.556 (4H, $-CH_2-$).
Chitosan derivative 1c: ^{1}H NMR (D_2O, ppm) d: δ = 3.4–3.65 (multiplet, D-glucosamine unit, H-3, H-4, H-5, H-6, H-6′); δ = 2.68 (4H, $-NHCH_2-$); δ = 1.921 ($COCH_3$); δ = 1.537 (4H, $-CH_2-$); δ = 1.270 (4H, $-CH_2-$).

3.2. Cell viability

The sample without treatment of chitosan derivative was considered as a positive control with a cell viability of 100%. As illustrated in Fig. 2, chitosan exhibited non-toxic in both cell lines, and the cytotoxicity of all synthesized chitosan derivatives was found to be concentration dependent. Namely, the cell viability decreased slightly with increasing chitosan derivative concentration, which meant all of chitosan derivatives had very low cytotoxicity. Meanwhile, the cytotoxicity of chitosan derivative 1b and 1c was lower than that of chitosan derivative 1a. The results indicated that the cytotoxicity of chitosan derivatives depended on the structure of the side chains. The cytotoxicity increased with the increasing length of the side-chain, which was similar to the results reported by Liu et al. (2010) for the cytotoxicity of poly(amidoamine)s with pendant primary amines.

3.3. Aagrose gel electrophpresis

The complexes of polycation/DNA at weight ratios ranging from 5 to 11 were electrophoresed separately in agarose gel. Naked DNA was used as a control. The migration of naked DNA and polymer/DNA complexes in agarose gel is shown in Fig. 3. All chitosan derivatives showed good DNA binding ability, and the migration of DNA in agarose gel was completely retarded when the weight ratio was above 5.

3.4. Particle size and zeta potential of polycation/DNA complexes

Fig. 4 gives the particles sizes and zeta potentials of 1a–1c at various weight ratios. As shown in Fig. 4a, it was found that the particle sizes of the complexes tended to decrease with the increase of weight ratio, and the particle sizes were also dependent on the structures of the chitosan derivatives. When the weight ratios were above 20, the average diameters of chitosan derivative 1b, 1c/DNA complexes were between 150 and 300 nm, while the average diameters of chitosan derivative 1a/DNA were between 400 and 500 nm at the same weight ratio. It suggested that the binding ability of chitosan derivative 1b and 1c was higher than that of chitosan derivative 1a, which was consistent with the results measured by agarose gel electrophoresis. The morphology of the polycation/DNA complexes was observed by SEM. As shown in Fig. 5, the typical image showed that the complexes had a spherical shape and a compacted structure, and the diameter of the formed complexes was about 200 nm. Therefore, the results confirmed that the chitosan derivatives could form nanoparticles with DNA and the average diameters of the complexes were within the size requirements for efficient cellular endocytosis (Köping-Höggård et al., 2001; Liu & Reineke, 2005).

The zeta potential of chitosan derivative/DNA complexes in Fig. 4b showed that the complexes had negative zeta potentials at low weight ratios, suggesting that the amount of chitosan derivatives was not enough to condense the DNA completely. When the weight ratio was above 6, the surface charge of the complexes became positive. The surface charge of the complexes increased

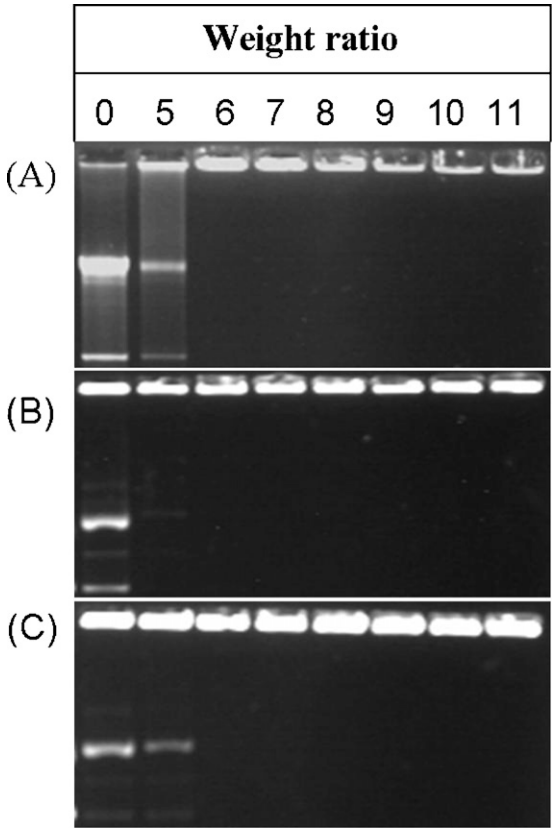


Fig. 3. Agarose gel electrophoresis retardation assay of the chitosan derivatives at various weight ratios of chitosan derivatives/DNA complexes. (A) chitosan derivative 1a, (B) chitosan derivative 1b, and (C) chitosan derivative 1c.

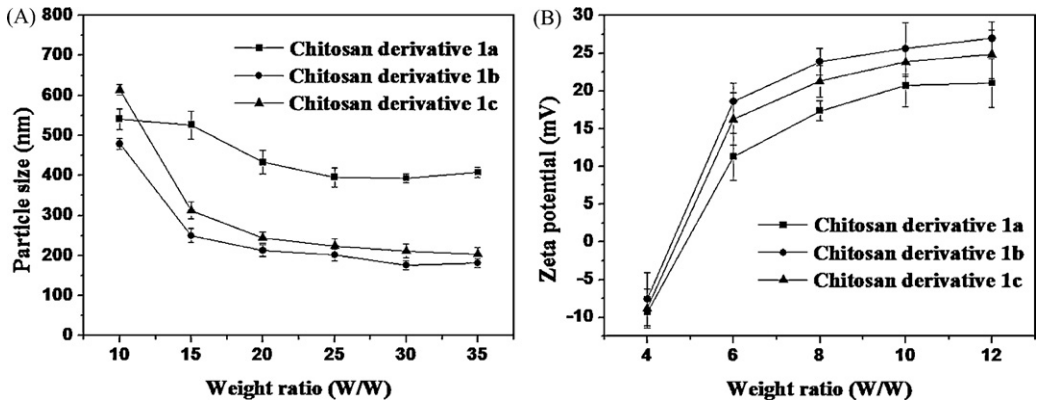


Fig. 4. (A) The particle size of chitosan derivatives/DNA complexes at weight ratios ranging from 10 to 35 in 150 mmol/L NaCl. (B) Surface charge of chitosan derivatives/DNA complexes in pure water at various weight ratios.

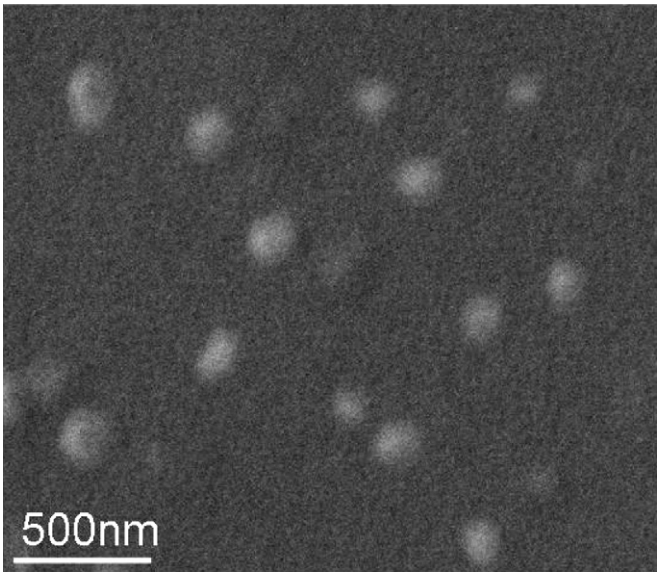


Fig. 5. The typical morphology of chitosan derivatives/DNA complexes determined by scanning electron microscopy (SEM, FEI Quanta 200), chitosan derivative 1b/DNA (W/W = 15).

with the increasing weight ratio, and gradually approached a plateau due to the saturation of polycations complexed with DNA.

3.5. In vitro gene transfection

The transfected cells that expressed green fluorescent proteins were directly observed by a reverse fluorescent microscope. Fig. 6 displays the fluorescence images of the transfected 293 T cells. It can be seen that the transfection efficiencies of chitosan derivative 1b/DNA and chitosan derivative 1c/DNA complexes were higher than that of chitosan derivative 1a/DNA complexes. The gene transfection activity of the polymers was also evaluated in terms of luciferase assay, which is a more sensitive method than fluorescence intensity determination. The transfection efficiencies of chitosan derivative/DNA complexes were evaluated at various weight ratios from 10/1 to 60/1. From Table 1, we can find that the transfection efficiencies of synthesized gene vectors were greatly improved than that of chitosan. Among all the polymers, chitosan derivative 1b showed that the highest luciferase expression at the weight ratio of 50 in 293 T cells and 40 in HeLa cells. We also found that the transfection efficiencies of the chitosan derivative/DNA complexes were greatly dependent on the structures of pendant side chains of chitosan derivatives. The transfection efficiencies

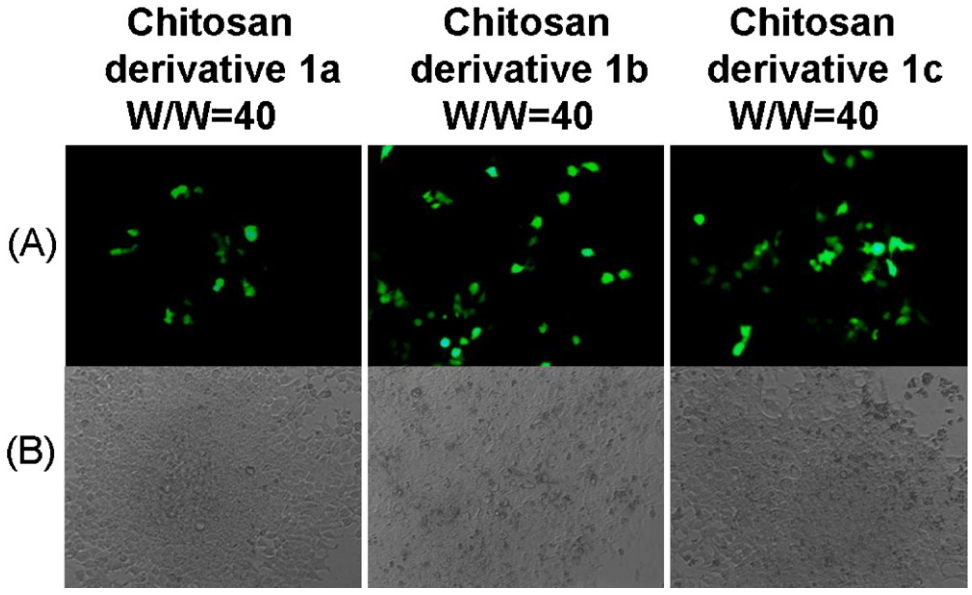


Fig. 6. Typical fluorescence images of 293 T cells transfected by chitosan derivatives/DNA complexes at the weight ratio 40. (A) fluorescence field images, and (B) bright field images.

Table 1
In vitro transfection efficiencies of chitosan derivatives 1a–1c/pGL-3 complexes in 293 T cells (a) and HeLa cells (b). The cells were incubated in the absence or presence of 10% serum with chitosan derivatives/pGL-3 complexes ($n = 3$).

(a)				
Samples (polymer:DNA weight ratios)	RLU/mg protein + sd			
	Chitosan	Chitosan derivative la	Chitosan derivative lb	Chitosan derivative lc
			Without serum	
10	265,257 ± 86,845	946,841 ± 602,173	4,362,891 ± 2,134,640	1,372,137±333,829
20		1,184,135 ± 339,130	18,392,463 ± 3,452,791	2,346,231 ± 583,423
30		2,669,324 ± 1,252,621	30,048,322 ± 5,453,657	9,947,324 ± 4,552,930
40		4,643,927 ± 1,880,342	45,382,472 ± 8,563,345	18,933,765 ± 5,883,942
50		4,334,289 ± 1,578,231	54,323,688 ± 2,113,431(5	4,793,434 ± 2,348,921
60		2,683,922 ± 1,132,423	18,936,542 ± 4,943,820	5,557,489 ± 2,143,224
			In the presence of serum	
10	431,267 ± 188,935	1,429,653 ± 667,497	10,605,755 ± 8,074,923	2,133,463 ± 433,151
20		2,286,637 ± 1,857,448	43,499,671 ± 5,817,725	4,637,754 ± 671,672
30		4,544,336 ± 935,478	50,681,573 ± 9,493,981	22,886,657 ± 8,042,432
40		7,703,671 ± 3,880,345	106,294,564 ± 46,272,312	31,899,674 ± 8,222,415
50		8,278,126 ± 3,398,324	86,563,347 ± 35,638,944	12,835,594 ± 7,895,243
60		4,937,523 ± 2,872,453	43,325,859 ± 13,458,331	9,341,748 ± 5,378,244
(b)				
			Without serum	
10	146,743 ± 40,314	545,820 ± 43,829	2,242,536 ± 800,123	1,785,479 ± 703,229
20		717,513 ± 68,943	3,564,324 ± 611,254	2,674,751 ± 528,913
30		846,840 ± 130,245	6,544,122 ± 423,147	4,143,266 ± 639,420
40		764,786 ± 140,346	5,432,673 ± 634,785	4,341,875 ± 784,732
50		678,453 ± 34,259	4,547,844 ± 834,345	3,675,253 ± 599,372
60		559,432 ± 45,492	2,193,568 ± 682,531	1,857,437 ± 153,492
			In the presence of serum	
10	203,580 ± 33,356	732,415 ± 212,943	1,983,025 ± 583,210	1,075,394 ± 403,229
20		1,123,438 ± 399,390	5,345,649 ± 541,391	2,356,735 ± 307,650
30		1,294,126 ± 245,326	6,998,346 ± 674,831	5,674,556 ± 884,292
40		1,833,023 ± 388,302	8,175,842 ± 1,684,957	6,657,533 ± 1,227,853
50		937,821 ± 250,332	5,536,241 ± 1,488,420	4,001,347 ± 576,403
60		893,372 ± 292,021	3,892,319 ± 443,795	2,873,955 ± 348,644

of chitosan derivative 1a/DNA and chitosan derivative 1c/DNA at various weight ratios were lower than that of chitosan derivative 1b/DNA. Liu et al. (2010) also investigated the effect of the length of diamine in poly(amindoamine)s on transfection efficiency, and found the transfection efficiency of poly(amindoamine)s with pendant diaminobutane was higher than that of poly(amindoamine)s with pendant diaminoethane and poly(amindoamine)s with pendant diaminohexane. The reason may be ascribe to the effect of the length of methylene on the charge density of the synthesized polymers. The particle size and zeta potential in Fig. 4 exhibited that chitosan derivative 1b/DNA complex with the smallest particle size and the highest zeta potential, which is helpful for cell uptake and efficient transfection. The transfection efficiencies of chitosan derivatives 1a–1c were further evaluated in HeLa cells. From Table 1b, the results displayed that the lower transfection ability was observed in HeLa cell lines, which meant that the transfection ability of the polymers was dependent on cell lines.

The stability of gene vectors in serum is very important for the practical application of gene therapy in vivo. In this study, the effect of serum on the transfection ability of chitosan derivatives 1a–1c was investigated. As show in Table 1, the gene expression of those polymers was not inhibited, and the presence of serum even led to the enhancement of gene transfection efficiencies in both cell lines. Similar findings were also reported in the literature. For instance, Yoo, Lee, Chung, Kwon, and Jeong, 2005 reported that a hydrophobically modified glycol chitosan showed increasing in vitro transfection efficiency in the presence of serum. Hu et al. (2006) reported that stearic acid grafted chitosan oligosaccharide displayed increasing transfection ability in the presence of 10% serum-containing medium. Conventionally, large steric barriers of carbohydrate polymer are often grafted on the periphery of nanoparticles to prohibit flocculation in biological media. Srinivasachari, Liu, Zhang, Prevette, and Reineke (2006) used trehalose click polymers to inhibit nanoparticle aggregation and promote DNA delivery in presence of serum. Tseng & Jong (2003) used dextran grafting branched polyethylenimine to improve the stability of polycationic vectors and promote gene delivery in serum. These polymers successfully prevented aggregation through steric stabilization (Maruyama, Ishihara, Kim, Kim, & Araike, 1997), and increased the gene transfection efficiencies in serum. Therefore, the improved gene transfection in the presence of serum may be speculated that the saccharide ring chains of chitosan in the chitosan-based polymers played an important role in these complexes to resist the inhibitation of serum.

4. Conclusions

Three novel diamine-modified chitosan derivatives were synthesized by Michael addition reaction. All chitosan derivatives exhibited improved water solubility. These polymers were evaluated as new gene vectors and they showed good DNA binding capability. The particle sizes were ranging from 150 to 500 nm and the majority of these particles had spherical morphology. Along with increase of the weight ratio, the zeta potentials of the particles were monotonously increased. In comparison with chitosan (10 kDa), these chitosan derivatives presented relatively lower cytotoxicity and much higher transfection efficiencies. The results suggest that the kind of novel modified chitosan in this study may be used as a new non-viral gene delivery vector.

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