J Mater Sci: Mater Med (2009) 20:673–680
DOI 10.1007/s10856-008-3620-1

Synthesis and properties of a novel methoxy poly(ethylene glycol)-modified galactosylated chitosan derivative

Tao Zhang · Yuan Yuan Yu · Dong Li · Rong Peng · Ying Li · Qing Jiang · Ping Dai · Rong Gao

Received: 17 July 2008 / Accepted: 6 October 2008 / Published online: 24 October 2008

Abstract Chitosan and its derivatives are attractive non-viral vectors. To produce target-cell specificity and improve the solubility of chitosan, a novel chitosan derivative, modified with galactose and methoxy poly(ethylene glycol) (mPEG) was synthesized, and structure changes of chitosan and its derivatives were characterized. Compared to chitosan, the solution viscosity of the novel chitosan derivative drastically decreased. And, the degree of substitution (DS) of chitosan by galactose and mPEG were calculated as 0.09 and 0.30. The average diameter and zeta potential of mPEGylated galactosylated chitosan (GaC) nanoparticle containing VRMFat plasmid were 178 nm and +2.93 mV, suggesting suitable properties for gene delivery system. The gel electrophoresis confirmed that the plasmid DNA was remained completely by the mPEGylated GaC nanoparticle. And, the cytotoxic effect of mPEGylated GaC nanoparticles on human embryonic kidney (HEK 293) cells was negligible in comparison with that of control chitosans. Therefore, it is expected that the mPEGylated GaC will have the potential as a targeting gene delivery system for a further application.

Tao Zhang and Dong Li equally contributed to this research.

T. Zhang · Y. Y. Yu · Y. Li (✉) · Q. Jiang · P. Dai
College of Chemistry, Sichuan University,
Chengdu 610064, People's Republic of China
e-mail: profliying@sina.com

D. Li · R. Peng · R. Gao (✉)
College of Life Science, Sichuan University,
Chengdu 610064, People's Republic of China

1 Introduction

Gene therapy, as a transfer of genetic materials to specific cells to have a therapeutic effect, is a promising approach to treat genetic or acquired diseases by producing bioactive agents or stopping abnormal functions of the cells. Therefore, efficient and safe gene delivery vectors are essential for the success of gene therapy [1, 2]. Basically, gene delivery vectors are classified into two categories: viral vectors and non-viral vectors. Although viral vectors are more effective transfection agents, the use of viral vectors is limited by the size of the gene they can deliver, immunogenic, oncogenic potential and target-cell specificity problems. In contrast, non-viral vectors have been increasingly proposed as a promising alternative because of their excellent safety profile, unrestricted gene size, low immune response, targetability, stability in storage and easy of synthesis [3, 4]. Non-viral vectors can be divided into two categories: cationic polymers and cationic lipid carriers. Cationic polymer/DNA complexes tend to be more stable than cationic lipid/DNA complexes [5]. So that, cationic polymers have been attracted great attention recently because they can easily form self-assembling polyelectrolyte complexes with plasmid DNA, and can protect DNA from enzymatic degradation [6].

As a natural cationic polymer, chitosan is considered to be a good candidate for drug carrier and delivery system, since it is known as a biocompatible, biodegradable, non-toxic, cationic polysaccharide obtained by deacetylation of a naturally occurring polymer chitin [7]. It consists of β-(1-4)-2-amino-2-deoxy-D-glucopyranose residues and has small or moderate amount of β-(1-4)-2-acetamino-2-deoxy-D-glucopyranose residues. Being positively charged and its biodegradability and biocompatibility, chitosan has also been attractive in some other fields including

agriculture, biotechnology, pharmaceutics, cosmetics, food, water-treatment materials and personal care [8]. However, applications of chitosan are limited by poor solubility due to strong intermolecular hydrogen bonding of amino and hydroxyl groups. To improve the hydrophilic property of chitosan as well as produce derivatives with new properties, many different hydrophilic substitutes to chemical modification of chitosan have been used.

Among those hydrophilic systems, poly(ethylene glycol) (PEG) is often used to modify chitosan for the hydrophilic gene delivery system. PEG is one of the most suitable graft-forming polymers because of its unique physicochemical and biological properties, including hydrophilicity, solubility in both water and organic solvents, lack of toxicity, easy of chemical modification and absence of antigenicity and immunogenicity [9]. Enormous researches have been carried out on PEG grafted chitosan. PEG acts as a pore-forming agent to create interconnected channels for drug release in the film coating material of PEG grafted chitosan. Except for hydrophilic property purpose, chitosan has also been used to have target-cell specificity. As mentioned before, galactose modified oligosaccharides show a high affinity for asialoglycoprotein receptor (ASGR) of hepatocyte [10].

The purpose of this study was to develop a novel functional cationic polymer via modification of chitosan with galactose and mPEG. mPEG was used to improve the solubility of chitosan and prolong blood circulation time and galactose was acted as a liver-targeting moiety. The galactosylated chitosan (GaC) and mPEGylated GaC were characterized by FT-IR and ^{1}H NMR spectra, and the amounts of galactose and mPEG with chitosan were measured by colorimetric assay using anthrone sulfuric acid and ammonium ferrothiocyanate, respectively. We had prepared nanoparticle of mPEGylated GaC, and investigated its potentials as a gene delivery vector. The results showed that the mPEGylated GaC copolymer had high binding ability to plasmid DNA and negligible toxicity.

2 Experimental

2.1 Materials

Chitosan (Mn = 48 kDa, deacetylation degree 90%) was purchased by Nanjing Weikang Biotechnology Co. Ltd. (China). mPEG (Mn = 2 kDa) was purchased from Hannong Chemicals Inc. (Korea). Galactose was purchased from Shanghai Boao Biotechnology Co. Ltd. (China). Dialysis membrane (8–12 kDa molecular weight cut-off) was purchased from Beijing Solarbio Technology Co. Ltd. (China). All other chemicals used were of analytical grade and were used without further purification.

2.2 Methods

2.2.1 *Synthesis of mPEG-aldehyde*

mPEG-aldehyde (mPEG-CHO) was prepared by oxidation of mPEG with anhydrous DMSO/Ac_2O according to Harris and Dong's method [11, 12]. Ac_2O (5.0 ml, 50 mmol) was added to the solution of mPEG (10 g, 5 mmol) in 33 ml anhydrous DMSO containing 3 ml $CHCl_3$ under argon atmosphere. The mixture was stirred for 9 h at room temperature. The reaction mixture was then poured into 400 ml anhydrous diethylether. The precipitate was filtered and reprecipitated twice from $CHCl_3$ with anhydrous diethylether. The precipitate was dried in a vacuum at room temperature for 24 h. After drying, 8.1 g of white powder was obtained.

2.2.2 *Synthesis of galactosylated chitosan [13]*

Chitosan (0.70 g, 4.2 mmol), D (+)-galactose (1.51 g, 8.4 mmol), and $BF_3 \cdot OEt_2$ (10.5 ml, 84 mmol) were dissolved in 150 ml dry THF. The reaction was stirred at 60°C under argon atmosphere for 20 h. The solution was concentrated with rotary evaporator. The condensed viscous liquid was poured into anhydrous methanol. The precipitate was filtered, washed several times with anhydrous methanol and dried in a vacuum oven to give 1.20 g of galactosylated chitosan (GaC).

2.2.3 *Synthesis of mPEGylated GaC [14–16]*

GaC (1.1 g, 6.0 mmol) was dissolved in a mixture of water (40 ml) and methanol (10 ml). Then, mPEG-CHO (6.0 g, 3.0 mmol) was added and stirred at room temperature for 24 h. Reduction was carried out by adding dropwise a solution of potassium borohydride (1.0 g, 18 mmol) dissolved in 10 ml of water to the reaction mixture for 30 min. The solution was stirred for 72 h at room temperature and then neutralized with aqueous 1 mol/l HCl solution. The solution was filtered and then concentrated with rotary evaporator to remove methanol of the mixture solution. The concentrated viscous liquid was poured into saturated aqueous solution of ammonium sulfate for removing the excess of mPEG-CHO. The above layer solution was dialyzed against demineralized water. The dialyzed solution was concentrated until solid residue using rotating evaporator and the obtained product was dried in a vacuum oven.

2.2.4 *Characterization*

^{1}H NMR spectra were recorded on a Bruker Avance 400 spectrometer. Chitosan was dissolved in the mixed solvent CF_3COOD and D_2O. GaC and mPEGylated GaC were

dissolved in D_2O according to their solubility. FT-IR spectra were recorded with a Perkin Elmer spectrometer by the KBr pellets method. The content of galactose and mPEG of the final product were determined by a UV/Vis spectrometer.

2.2.5 Determination of galactose content

The content of galactose in the copolymer was determined by a modified anthrone sulfuric acid method [17]. Several concentrations of galactose solutions were prepared and placed in wells. The fresh anthrone sulfuric acid was prepared in an ice bath, added into the wells, and heated at boiling water for 5 min. The absorbance was determined by spectrophotometer at 628 nm. The calibration curve was constructed based on the various galactose concentrations and the corresponded absorbances. The concentration of galactose was calculated from the calibration curve according to its measured absorbance.

2.2.6 Determination of mPEG content

The content of mPEG units in the copolymer was 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 [18]. Several concentrations of mPEG aqueous solutions were prepared. mPEG solution, ammonium ferrothiocyanate, and chloroform in the volume ratio of 1:10:10 were mixed vigorously for 30 min at room temperature. The solution was centrifuged, and the lower chloroform phase was collected and determined by spectrophotometer at 509 nm. The calibration curve was constructed based on the various mPEG concentrations and the corresponded absorbances. The concentration of mPEG was calculated from the calibration curve according to its measured absorbance.

2.2.7 Viscosity measurement

Intrinsic viscosity and reduced viscosity of the copolymer solutions in aqueous 0.5 mol/l CH_3COOH/CH_3COONa at 25°C were measured using Ubbelode viscometer.

2.2.8 Preparation of polymer/DNA nanoparticles

The nanoparticles of chitosan and mPEGylated GaC complexes were prepared using the method of ionic cross linking [19]. First, chitosan and mPEGylated GaC were diluted separately in CH_3COOH/CH_3COONa (pH 5.5), and heated at 65–70°C for 10 min. Then, an appropriate amount of polymer solution, the mass ratio of chitosan and mPEGylated GaC/DNA, VRMFat, a 5.6 Kb eukoryotic expression plasmid, containing appropriate amount of triple phosphate, were 10:1, 20:1, and 30:1, respectively, was added to the solution of plasmid DNA and the resulting solution was mixed and left for 5 min.

2.2.9 Transmission electron microscopy (TEM)

The morphology of the nanoparticles was examined using a TEM and measured as granule diameter. Appropriate sample was placed on a copper grill covered with nitrocellulose. It was dried at room temperature negatively stained with tungsten phosphate and was then examined with the electron microscopy.

2.2.10 Measurement of nanoparticle sizes and zeta potential

The average granule diameter and zeta electricity potential of mPEGylated GaC nanoparticle containing VRMFat plasmid were measured by Zetasizer 3000 HS/IHPL instrument (Malvern Instruments Ltd., Malvern, UK).

2.2.11 Agarose gel electrophoresis

The DNA binding ability of chitosan and the mPEGylated GaC were evaluated by agarose gel electrophoresis. The nanoparticle solutions of plasmid DNA with chitosan and the mPEGylated GaC copolymer were loaded into individual wells of 0.8% agarose gel, electrophoresed at 80 V for 45 min, and stained with 0.5 g/ml ethidium bromide. The resulting plasmid migration pattern was revealed under UV irradiation.

2.2.12 Cell viability

The cell viability assay of the copolymer used human embryonic kidney (HEK 293) cells, which were incubated in Dulbecco's modified Eagle medium (DMEM, Invitrogen Corporation) supplemented with 10% fetal bovine serum (FBS), streptomycin at 100 μg/ml, penicillin at 100 μg/ml. The cells were maintained at 37°C in a 5% carbon dioxide humidified atmosphere. Cells were seeded at 1×10^5 cells per well in 12-well plates and incubated to obtain over 80% confluence prior to the addition of the complexes containing about 3 μg DNA per well. Cells were incubated with the complexes at 37°C in a 5% carbon dioxide humidified atmosphere.

3 Results and discussion

3.1 Synthesis of mPEGylated GaC

The advancement of gene therapy largely depends on the development of delivery systems that can introduce

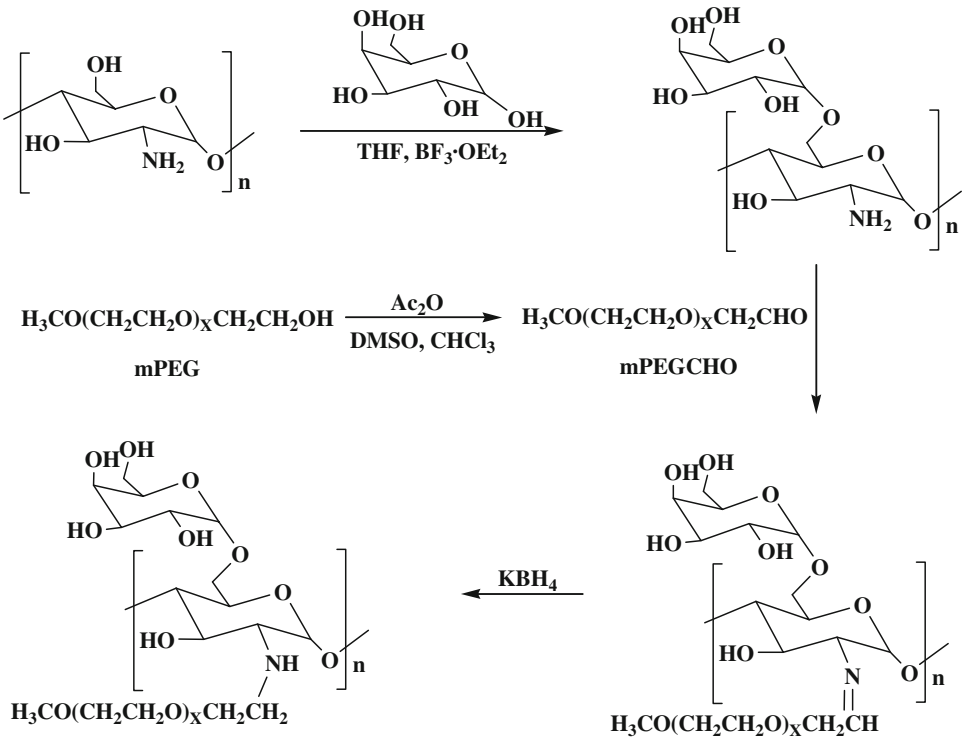
therapeutic genes into the target cells efficiently and safely. It was found that chitosan with a high degree of deacetylation induced greater effect on the package efficiency [20]. To increase the transfection efficiency and the permeability of the cell membranes, highly deacetylated (90%) chitosan used in this study. PEG has been employed extensively in biomedical and pharmaceutical applications with high hydrophilicity, biocompatibility and biodegradability. And, a long circulation time in blood is an important and fundamental factor for reaching the target cells in the efficient and safe gene delivery vectors. The mPEG was used to prolong circulation time in blood and improve the solubility of the chitosan. Galactose was used as a specific adhesive ligand to ASGR of hepatocyte.

In this paper, mPEG was used instead of PEG to avoid crosslinking of copolymer. The mPEGylated GaC, modified with galactose and mPEG was prepared as depicted in Scheme 1. According to Harris and Dong's method [11, 12], mPEG was oxidized to mPEG-CHO with acetic anhydride and DMSO. It was noted from the FT-IR and ^{1}H NMR spectra that the final product was the mixture of mPEG and mPEG-CHO. It could not be separate from reaction mixture and was used without further purification. The degree of conversion from –OH to –CHO group, estimated by the relative intensities of ^{1}H NMR spectra between –CHO (δ 9.73 ppm) and –OCH_3 (δ 3.38 ppm), was 0.45. An efficient method was used for the direct stereoselective synthesis of glycofuranosides with the C-6 hydroxy of chitosan [13]. The synthesis of GaC was carried in anhydrous THF in the presence of $BF_3 \cdot OEt_2$. The synthesis of mPEGylated GaC was performed according to a procedure reported elsewhere [14–16]. The amino groups of GaC were combined with an aldehyde group of mPEG-CHO, and the Schiff bases were reduced by KBH_4 into secondary amino groups. It was known that neutral pH suppresses the degradation of Schiff bases. The reaction condition was in water at room temperature because the GaC was soluble in water. The dropwise addition of aqueous KBH_4 solution was to prevent excessive KBH_4 changing the pH of the solution to basic rapidly. The unreacted mPEG was hardly separated from the reaction mixture by dialysis against water. But it was well removed by washing extensively several times with acetone.

3.2 Characterization of mPEGylated GaC

Structure changes of chitosan and its derivatives were characterized with FT-IR and ^{1}H NMR spectra. A comparative IR spectra study of chitosan, GaC, and mPEGylated GaC, were shown in Fig. 1. From the chitosan IR spectra, it was found that distinctive absorption bands appear at 1,645 cm^{-1} (amide I), 1,606 cm^{-1} (amide II, $-NH_2$ bending) and 1,383 cm^{-1} (amide III). The absorption band at 3,439 cm^{-1} that overlapped with N–H stretch was O–H stretching vibrations. And the characteristic absorption bands of chitosan saccharine structure were 1,154 cm^{-1} (asymmetric stretching of the C–O–C bridge), 1,075 cm^{-1} and 1,033 cm^{-1} (skeletal vibrations involving the C–O

Scheme 1 Synthesis of mPEGylated GaC



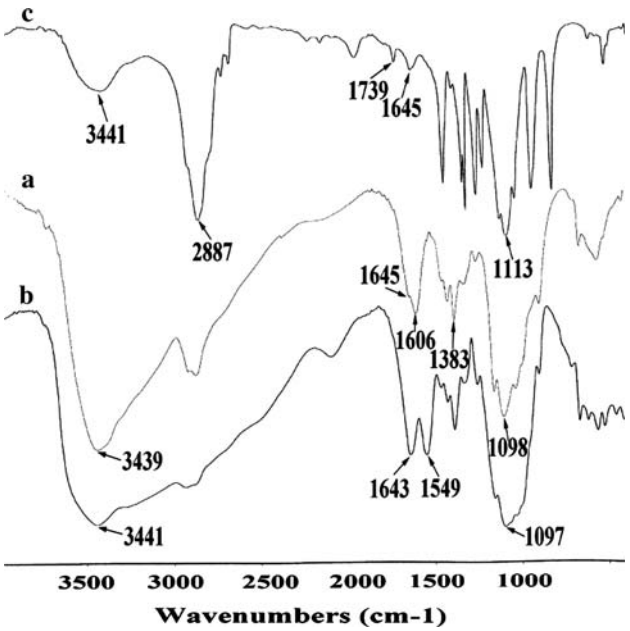


Fig. 1 The IR spectra of chitosan (a), GaC (b), and mPEGylated GaC (c)

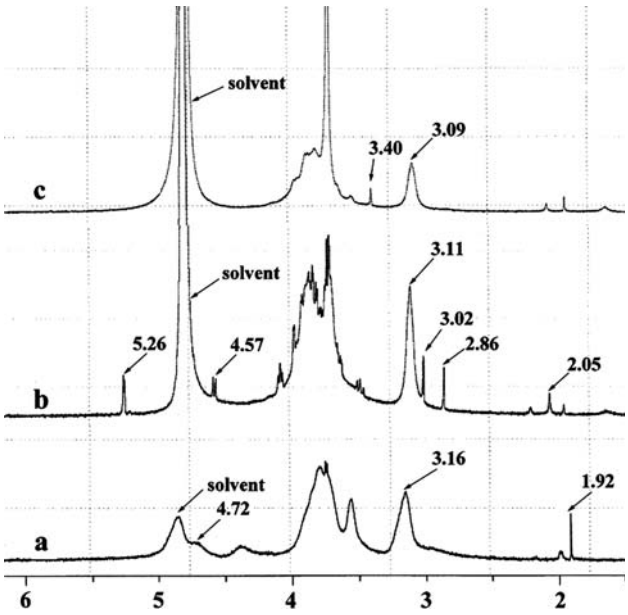


Fig. 2 The ^{1}H NMR spectra of chitosan (a), GaC (b), and mPEGylated GaC (c)

stretching) [21, 22]. Compared with chitosan, the IR spectra of GaC exhibited a broad O–H absorption around 3,441 cm^{-1} which indicated that the intermolecular hydrogen bonding between GaC chains increased due to the introduction of galactose into the chitosan chains [23]. And, the peaks of amide I and amide II of GaC slightly shifted to 1,643 cm^{-1} and 1,549 cm^{-1}, respectively, which indicates the conformational change of chitosan. For the mPEGylated GaC sample, the peaks intensities corresponding to the hydroxyl group, amino group and amide group of GaC were significantly reduced as a result of mPEG-CHO grafting. Compared to the amide I peak at 1,643 cm^{-1}, the peak intensity of amide II significantly decreased, which shows that the $–NH_2$ groups of GaC were partially grafted with mPEG-CHO. Also, distinctive absorption bands of mPEG at 1,113 cm^{-1} (C–O stretching) and 2,887 cm^{-1} (C–H stretching) appeared and at 3,441 cm^{-1} (O–H stretching), 1,643 cm^{-1} (amide I) and 1,549 cm^{-1} (amide II) declined in the IR spectra of mPEGylated GaC. However, the unreacted mPEG-CHO was not fully separated from the mPEGylated GaC by dialysis against water because of the absorption band at 1,739 cm^{-1}, which can be assigned to the absorption peak of the C=O stretching of mPEG-CHO.

Successful synthesis of GaC and mPEGylated GaC were also confirmed by ^{1}H NMR spectra. Typical ^{1}H NMR spectra information of the molecular structure of chitosan, GaC, and mPEGylated GaC was shown in Fig. 2. The assignments and chemical shifts of chitosan [24, 25] were: ^{1}H NMR δ 1.92 ppm ($–COCH_3$, acetyl group); δ 3.16 ppm (H-2 of glucosamine ring); δ 3.56–3.79 ppm (H-3, H-4, H-5, and H-6 of glucosamine ring); δ 4.72 ppm (H-1 of glucosamine ring). Compared with chitosan, the ^{1}H NMR spectra of GaC showed the signals at δ 2.05 ppm ($–COCH_3$, acetyl group); δ 2.86 ppm (H-2 of *N*-acetyl glucosamine ring); δ 3.02 ppm (H-2 of galactose ring); δ 3.11 ppm (H-2 of glucosamine ring); δ 3.49–4.10 ppm (H-3, H-4, H-5, and H-6 of glucosamine ring and galactose ring); δ 4.57 ppm (H-1 of glucosamine ring); δ 5.26 ppm (H-1 of *N*-acetyl glucosamine ring). The ^{1}H NMR spectra of mPEGylated GaC provided little information on its structure. After washing extensively several times with acetone, the ^{1}H NMR spectra of mPEGylated GaC showed the strong broad signal of oxymethyl groups of mPEG at 3.55–4.07 ppm and partially covered over the signals of the glucosamine ring of chitosan. The signals at around 3.09 and 3.40 ppm are assigned to the H-2 signal of glucosamine ring and the proton signal of methoxy of mPEGylated GaC.

mPEG was used as PEG source in order to avoid the crosslinking reaction by bifunctional PEG-CHO. The amount of galactose and mPEG of the copolymer was calculated as follows:

$$\omega(w/w) = m_0/m \times 100\%$$

where m_0 represents the measured galactose or mPEG weight in the sample (g) and m represents the sample weight (g).

The degree of substitution (DS) of chitosan by galactose (DS_{gal}) and chitosan by mPEG (DS_{mPEG}) was calculated as follow:

$$\mathrm{DS} = \frac{m_0/\mathrm{M}_0}{(m - m_1 - m_2)/\mathrm{M}} \times 100\%$$

where m represents the sample weight (g); m_1 and m_2 represents the measured galactose and mPEG weight in the sample (g); M represents the molecular weight of chitosan monomer (165 g/mol); M_0 represents the molecular weight of galactose (180 g/mol) or mPEG (2,000 g/mol).

The amount of galactose and mPEG of the copolymer were 9% and 90% and the DS_{gal} and DS_{mPEG} were 0.09 and 0.30.

The intrinsic viscosity (η) and reduced viscosity (η_{sp}/C) of mPEG, chitosan, and mPEGylated GaC in an aqueous solution were calculated by Formula 1 and Formula 2.

$$\eta_{sp}/C = \frac{t - t_0}{t_0 C} \tag{1}$$

$$\eta = \frac{\sqrt{2\left(\frac{t-t_0}{t_0} - \ln\frac{t}{t_0}\right)}}{C} \tag{2}$$

where t_0 is the outflow time of the solvent, t is the outflow time of copolymer solution, and C is the concentration of copolymer solution.

It is known that the intrinsic viscosity and reduced viscosity of aqueous solution of the copolymer strongly depend on the copolymer composition and it's DS [26]. And solution of chitosan derivatives with low DS was significantly more viscous than the solution of the copolymer with high DS. As shown in the Table 1, the η and η_{sp}/C of the copolymer were higher than that of mPEG (2000) and very lower than that of chitosan because of the partically crystalline structure and very tight hydrogen bond between amino and hydroxyl groups of chitosan. It means that grafting of mPEG-CHO onto chitosan separates chitosan backbonds, decreases hydrogen bonding, so that drastically decreases solution viscosity of the chitosan. And also, the low solution viscosity of the mPEGylated GaC is probably determined by mPEG properties. It is known [26] that in an aqueous medium a long chain of PEG molecules is heavily hydrated and brush structure of the copolymer, and makes macromolecules stiffer and distort preventing the approach of other molecules or hydrogen bonds.

3.3 Characterization of nanoparticles

Chitosan and its derivatives are attractive non-viral vectors as they can form complexes with DNA based on electrostatic interaction between the positive amino groups of chitosan and negative phosphate groups of DNA. In the ionic cross linking method, chitosan is dissolved in aqueous acidic solution (pH 5.5), the majority amino groups of chitosan protonated, to obtain the polycation of chitosan. This solution is then added under constant stirring to the anionic plasmids solution containing appropriate amount of triple phosphate. The solution of plasmids and triple phosphate is negative, which can interact with the polycation of chitosan by electrostatic forces to form the nanoparticles with plasmids [27]. The formation of copolymer/DNA complexes is an important prerequisite for gene delivery using cationic polymers. Typically prepared mPEGylated GaC nanoparticles showed spherical and polydisperse nature as observed with transmission electron microscope (Fig. 3).

The average granule diameter, and zeta electronic potential, analysis by Zetasizer 3000 HS/IHPL with different mass ratio were depicted in Table 2. It was shown that the average granule diameter was 178 nm (diameter less than 400 nm) and the particle size of the complexes was smaller

Table 1 The η and η_{sp} of compounds

	η (dl/g)	η_{sp}/C (dl/g)
mPEG (2000)	0.083	0.086
Chitosan	3.884	3.985
mPEGylated GaC	0.514	0.536

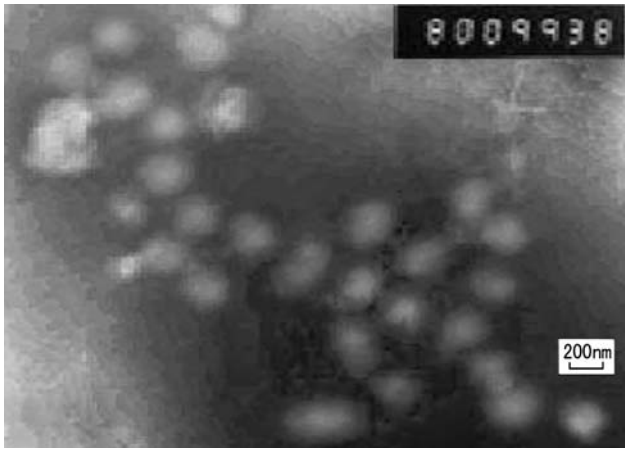


Fig. 3 TEM photograph of mPEGylated GaC nanoparticles (80,000×)

Table 2 The average diameter and zeta electronic potential of chitosan and mPEGylated GaC

Sample	T (°C)	w/w	Z-Ave (d.nm)	Zeta potential (mV)
Chitosan 1/DNA	25	10:1	490	9.60
	25	20:1	349	15.6
	25	30:1	230	15.8
mPEGylated GaC/DNA	25	10:1	256	−2.93
	25	20:1	226	−0.32
	25	30:1	178	2.93
Chitosan 2/DNA	25	10:1	380	−1.59
	25	20:1	398	0.60
	25	30:1	381	11.1

Chitosan 1 (Mn = 293 kDa) and chitosan 2 (Mn = 48 kDa)

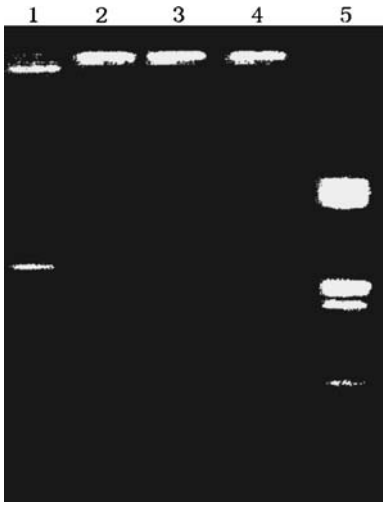


Fig. 4 Gel retardation assay (0.8% agarose gel) of chitosan and mPEGylated GaC/DNA complexes (mass ratio of 30:1). Line 1: VRL; Line 2: mPEGylated GaC/VRL; Line 3: chitosan/VRL; Line 4: chitosan/VR1020; Line 5: λ DNA *Eco*RI/*Hin*dIII marker

than the results of chitosan at the mass ratio of 30:1. And, it was known that the sizes of the complexes depend on the molecular weight of chitosan [28] and the N/P ratio of chitosan to DNA [29]. As shown in the Table 2, the particle size of mPEGylated GaC/DNA decreased with increasing mass ratio. The results indicated that the mass ratio of the complex had an important effect on the compaction of DNA and had average granule diameter at 178 nm, which was appropriate for hepatocyte targeting delivery vector. The surface charge of the DNA delivery systems is known as a major factor influencing their biodistribution [30]. The zeta electricity potential was +2.93 mV, suggesting that the complex was positively charged at the mass ratio of 30:1. The mPEGylated GaC/DNA complex showed negative zeta electricity potential at mass ratio of 10 and 20, indicating that the complex could not be formed completely. Also, the zeta electricity potential slightly increased with increasing mass ratio and molecular weight of chitosan. As mentioned before, the slightly positive zeta electricity potential resulted in the best transfection efficiency [31], so the mPEGylated GaC at the mass ratio of 30 is profitable for the gene delivery vector into cells.

The package efficiency of chitosan, and mPEGylated GaC was analyzed by 0.8% agarose gel electrophoresis versus control naked DNA plasmid (Fig. 4). It was found that the DNA plasmids packed with chitosan and mPEGylated GaC did not move out of the sample wells and were entirely bound, demonstrating that the DNA plasmids were successfully entrapped into the chitosan and

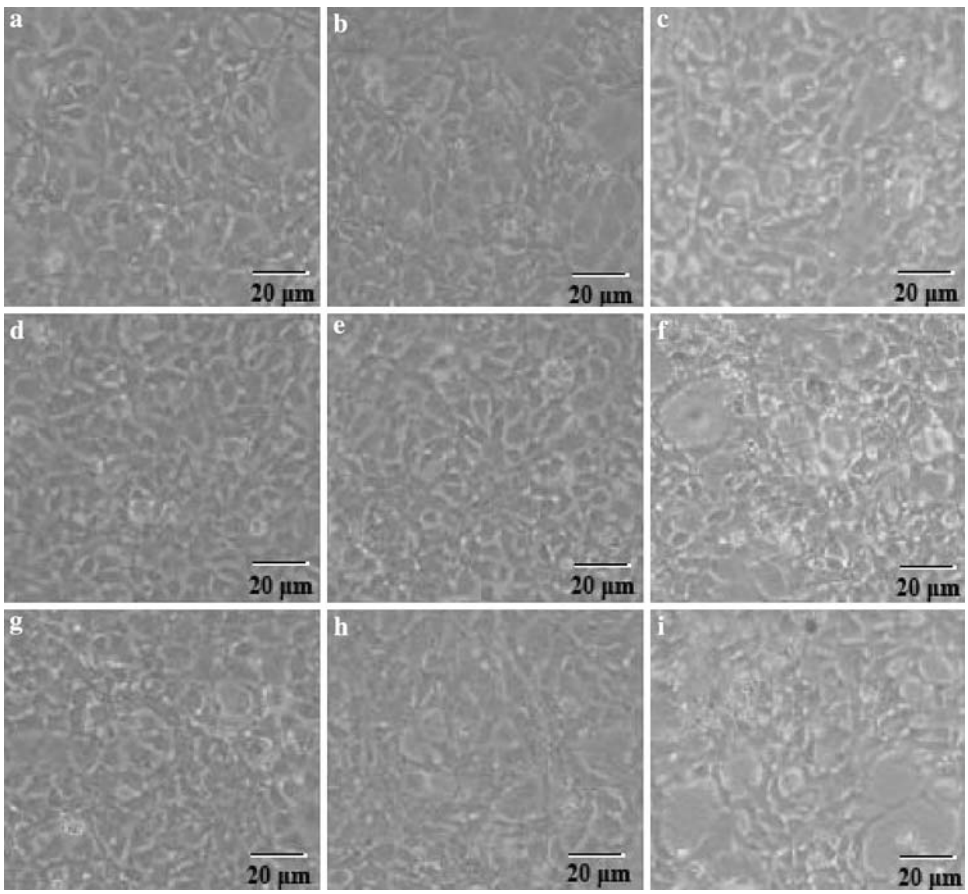


Fig. 5 The cell viability after incubated for 24, 48, and 72 h. **a** chitosan 1/DNA (24 h); **b** chitosan 1/DNA (48 h); **c** chitosan 1/DNA (72 h); **d** mPEGylated GaC/DNA (24 h); **e** mPEGylated GaC/DNA (48 h); **f** mPEGylated GaC/DNA (72 h); **g** chitosan 2/DNA (24 h); **h** chitosan 2/DNA (48 h); **i** chitosan 2/DNA (72 h). All figures are of the same magnification (200×)

mPEGylated GaC nanoparticles, and the introduction of galactose and mPEG did not affect its DNA binding ability.

3.4 Cell viability

The application of cationic polymer vectors and treatment with plasmid DNA often suffered from serious cytotoxic side effects [20]. To investigate a potential cytotoxic effect of mPEGylated GaC nanoparticles, the cell viability of HEK 293 was determined by the light microscopy photograph. As shown in Fig. 5, both chitosan and mPEGylated GaC nanocomplexes had negligible cytotoxic effect on HEK 293 cells, and the cells grew well after incubated for 24 h, 48 h, and 72 h. The results further demonstrate that mPEGylated GaC is biocompatible, and is a safe vector.

4 Conclusions

In this paper, the mPEGylated GaC, which was successfully grafted galactose and mPEG onto chitosan to produce target-cell specificity and improve the solubility, was demonstrated and the chemical structures of GaC and mPEGylated GaC were characterized by FT-IR, and ^{1}H NMR spectra. Also, the potential of mPEGylated GaC as a gene delivery system was evaluated. The mPEGylated GaC nanoparticle was showed suitable physicochemical properties for gene delivery system and great ability to form complexes with DNA. The copolymer was showed negligible toxicity against HEK 293 cells. Therefore, it is expected that the mPEGylated GaC will have much potentials for safe hepatocyte targeting gene carrier.

Acknowledgments This research was supported by the foundation of education ministry of China (Project No. 20070610053).

References

1. T.H. Kim, S.I. Kim, T. Akaike, C.S. Cho, J. Control Release **105**, 354 (2005). doi:10.1016/j.jconrel.2005.03.024
2. S. Selvam, P.B. Thomas, S.F. Hamm-Alvarez, Adv. Drug Deliv. Rev. **58**, 1243 (2006). doi:10.1016/j.addr.2006.07.021
3. U. Guliyeva, F. Oner, S. Ozsoy, R. Haziroglu, Eur. J. Pharm. Biopharm. **62**, 17 (2006). doi:10.1016/j.ejpb.2005.08.006
4. T. Schmitz, I. Bravo-Osuna, C. Vauthier, G. Ponchel, B. Loretz, B.S. Andreas, Biomaterials **28**, 524 (2007). doi:10.1016/j.biomaterials.2006.08.017
5. D.W. Park, A.S. Hoffman, S. Pun, P.S. Stayton, Nat. Rev. Drug Discov. **4**, 581 (2005). doi:10.1038/nrd1775
6. S. Mao, X. Shuai, F. Unger, M. Simon, D. Bi, T. Kissel, Int. J. Pharm. **281**, 45 (2004). doi:10.1016/j.ijpharm.2004.05.019
7. M.L. Lorenzo-Lamosa, C. Remunan-Lopez, J.L. Vila-Jato, M.J. Alonso, J. Control Release **52**, 109 (1998). doi:10.1016/S0168-3659(97)00203-4
8. M. Rinaudo, Prog. Polym. Sci. **31**, 603 (2006). doi:10.1016/j.progpolymsci.2006.06.001
9. J.G. Ryu, Y.I. Jeong, I.S. Kim, J.H. Lee, J.W. Nah, S.H. Kim, Int. J. Pharm. **200**, 231 (2000). doi:10.1016/S0378-5173(00)00392-6
10. R. Gref, J. Rodrigues, P. Couvreur, Macromolecules **35**, 9861 (2002). doi:10.1021/ma021132a
11. J.M. Harris, E.C. Struck, M.G. Case, M.S. Paley, M. Yalpani, J.M. Van Alstine, D.E. Brooks, J. Polym. Sci. Polym. Chem. Ed. **22**, 341 (1984). doi:10.1002/pol.1984.170220207
12. A.J. Dong, M.H. Feng, H.Y. Qi, S.S. Li, L.D. Deng, J. Mater. Sci.: Mater. Med. **19**, 869 (2008). doi:10.1007/s10856-007-3183-6
13. V. Ferrieres, J.N. Bertho, D. Piusquellec, Tetrahedron Lett. **36**, 2749 (1995). doi:10.1016/0040-4039(95)00356-H
14. X. Zhang, H. Zhang, Z. Wu, Z. Wang, H. Niu, C. Li, Eur. J. Pharm. Biopharm. **68**, 526 (2008). doi:10.1016/j.ejpb.2007.08.009
15. N. Bhattarai, H.R. Ramay, J. Gunn, F.A. Matsen, M. Zhang, J. Control Release **103**, 609 (2005). doi:10.1016/j.jconrel.2004.12.019
16. V.M. Ramos, N.M. Rodriguez, I. Henning, M.F. Diza, M.P. Monachesi, M.S. Rodriguez, A. Abarrategi, V.C. Magana, J.L. Lopez-Lacomba, E. Agullo, Carbohydr. Polym. **64**, 328 (2006). doi:10.1016/j.carbpol.2005.12.025
17. A. Laurentin, C.A. Edwards, Anal. Biochem. **315**, 143 (2003). doi:10.1016/S0003-2697(02)00704-2
18. A. Nag, G. Mitra, C. Ghosh, Anal. Biochem. **237**, 224 (1996). doi:10.1006/abio.1996.0233
19. R. Bodmeier, H.G. Chen, O. Paeratakul, Pharm. Res. **6**, 413 (1989). doi:10.1023/A:1015987516796
20. M.K. Lee, S.K. Chun, W.J. Choi, J.K. Kim, S.H. Choi, A. Kim, K. Oungbho, J.S. Park, W.S. Ahn, C.K. Kim, Biomaterials **26**, 2147 (2005). doi:10.1016/j.biomaterials.2004.07.008
21. K. Van De Velde, P. Kiehens, Carbohydr. Polym. **58**, 409 (2004). doi:10.1016/j.carbpol.2004.08.004
22. M.R. Kasaai, Carbohydr. Polym. **71**, 497 (2008). doi:10.1016/j.carbpol.2007.07.009
23. T.W. Chung, J. Yang, T. Akaike, K.Y. Cho, J.W. Nah, S.I. Kim, C.S. Cho, Biomaterials **23**, 2827 (2002). doi:10.1016/S0142-9612(01)00399-4
24. M. Sugimoto, M. Morimoto, H. Sashiwa, H. Saimoto, Y. Shigemasa, Carbohydr. Polym. **36**, 49 (1998). doi:10.1016/S0144-8617(97)00235-X
25. Y.I. Jeong, D.G. Kim, M.K. Jang, J.W. Nah, Carbohydr. Res. **343**, 282 (2008). doi:10.1016/j.carres.2007.10.025
26. N. Gorochovceva, R. Makuska, Eur. Polym. J. **40**, 685 (2004). doi:10.1016/j.eurpolymj.2003.12.005
27. S.A. Agnihotri, N.N. Mallikarjuna, T.M. Aminabhavi, J. Control Release **100**, 5 (2004). doi:10.1016/j.jconrel.2004.08.010
28. G. Borchard, Adv. Drug Deliv. Rev. **52**, 145 (2001). doi:10.1016/S0169-409X(01)00198-3
29. M. Thanou, B. Florea, M. Geldof, H.E. Junginger, G. Borehard, Biomaterials **23**, 153 (2002). doi:10.1016/S0142-9612(01)00090-4
30. I.K. Park, T.H. Kim, Y.H. Park, B.A. Shin, E.S. Choi, E.H. Chowdhury, T. Akaike, C.S. Cho, J. Control Release **76**, 349 (2001). doi:10.1016/S0168-3659(01)00448-5
31. J.S. Kim, B.I. Kim, A. Maruyama, T. Akaike, S.W. Kim, J. Control Release **53**, 175 (1998). doi:10.1016/S0168-3659(97)00251-4