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Preparation of gene drug delivery systems of cationic peptide lipid with 0G-PAMAM as hydrophilic end and its biological properties evaluation

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ABSTRACT

As an efficient gene delivery, non-viral vectors should have high transfection efficiency, excellent endosomal escape, low cytotoxicity, and the ability to rapidly release the gene into the cytoplasm.Cationic liposome have been widely used as efficient gene carriers, but the cytotoxicity, rapid degradation and low cellular uptake are major drawback impeding its further appolication. Herein, with double lauric acid as hydrophobic chains, tartaric acid as skeleton, 0 generation PAMAM modified with lysine as hydrophilic head, a new type cationic peptide lipid was synthetised. The alkyl chain promote lipid across cell membranes and with membrane fusion, 0 generation PAMAM modified with lysine hydrophilic end amino can contain a large number of protons which can change into ammonium and combine with the DNA negatively charge phosphate groups. It is expected that this carrier has low toxicity, high transfection efficiency and targeting property. By adjusting the cationic liposome/gene weight ratio, the transfection system was optimized to improved gene transfection efficiency, reduce cytotoxicity, and increase property and stability, etc.

1. Introduction

Gene therapy has potential to be one of the most important fields in medicine, offering the prospect for the treatment of various inherited and acquired disease. with it, disease like cancer, cardiovascular disorders, or primary immune deficiencies can be possibly cured (Islam et al., 2014; Junquera and Aicart, 2014). Therefore, the need for effective and safe systems for gene delivery is quite obvious. Gene delivery carriers are generally categorized into viral and non-viral (Nayerossadat and Maedeh, 2012), which used in vitro and in vivo.

viral system such as retroviruses, adenoviruses, adeno-associcted viruses are the potent tools for efficient gene delivery, however, they have potential rick of immunogenicity, inflammation, toxicity, which restrict their further application (Lin et al., 2015; Liu et al., 2011; Lentz et al., 2012; FDA, 2005; Yin et al., 2014; Kaneti et al., 2016; Liu and Berkhout, 2011; Men et al., 2010; Toita et al., 2011). Non-viral gene delivery system have been widely explored and applicated in clinical because of its inexpensive synthesis, scalability, high transfection efficiency and low cytotoxicity (Zhao et al., 2015a; Xiong and Mi, 2011). So non-viral system hold great potential for systemic gene delivery but favorable clinical outcomes cure still a long way off.

Most non-viral gene vectors investigated are positive charged material, such as cationic liposome, polyethylenimine, polyaminoamine dendrimers (Stefanutti et al., 2014). Among these various catonic carriers, cationic liposome based gene delivery has attracted considerable attention and become a routine technique in basic research (Naicker et al., 2014; Mahmoudi et al., 2014; Junquera and Aicart, 2014; Caracciolo and Amenitsch, 2012). Since the development of the earliest examples of synthetic cationic liposome as gene transfer reagents three decades ago, many others have since been reported. By enhanced intracellular penetration or improve tumor targeting, cationic liposome has the ability to deliver chemotherapeutic agents to the targeted tissues or inside the cancerous cell. Cationic liposome has obvious advantages of low immunogenicity, targeting feasible and ease of handling, but it is impeded by some hurdles such as colloidal instability, low transfection and too much positive charges (Zhao et al., 2015b; Masotti et al., 2009). Therefore, it is necessary to modify cationic liposome to minimize its disadvantage for further clinical applications.

Polyamidoamine (PAMAM), one of the most effective cationic polymers carriers, have been used to deliver plasmid DNA (pDNA) (Eichman et al., 2000; Braun et al., 2005) due to their potential for modification and flexible chemistry. PAMAM carriers can interactions with the pDNA electrostatically and condense it into positively charged nanoparticles which are more easily taken up by cells. It is reported that PAMAM dendrimers complex effectively with negatively charged nucleic acids (Svenson, 2009), and enhance the transfection efficiency

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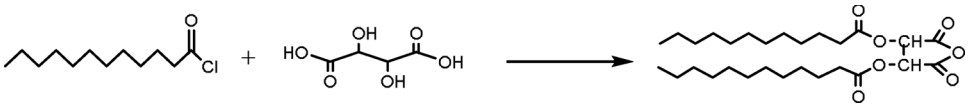

Fig. 1. The synthesis of Di lauroyl tartaric anhydride.

(Sun and Zhang, 2010). PAMAM is a prototypical "proton-sponge" polymers that are hypothesized to facilitate endosome escape via their strong buffering capacity, which leads to osmotic swelling and rupture of acidified endocytic vesicles (Sonawane et al., 2003; Behr, 1997). Thus, PAMAM provides a safer and more effective vector design for the study of gene delivery systems (Reyes et al., 2013; Durán et al., 2013).

Natural tartaric acid is inexpensive and readily available and widely used in drinks and other food (Sinkó et al., 2010). Tartaric acid, multifunctional molecule, has two hydroxyl and two carboxyl groups as reactive sites, which is suitable as the backbone in the design of cationic lipids through easily modified with different head group and alkyl hydrophobic tail. Natural tartaric acid was used as a backbone to design and synthesize the cationic lipids for gene delivery.

In this paper, natural tartaric acid was used as a backbone, double lauric acid as hydrophobic chains, and lysine modified 0 generation PAMAM as hydrophilic head to design and synthesize the cationic lipids for gene delivery. The alkyl chain is promote lipid across cell membranes and with membrane fusion, lysine modified 0 generation PAMAM hydrophilic end amino can contain a large number of protons which can be changed into ammonium and combined with the DNA negatively charged phosphate groups. The particle size, Zeta potential, transfection efficiency and cytotoxicity of the cationic liposome was also evaluated.

2. Materials and methods

2.1. Materials

laurel chloride ($C_{12}H_{23}ClO$), L-tartaric acid ($C_4H_6O_6$), lysine ($C_6H_{14}N_2O_2$), Di-tert-butyl dicarbonate ($C_{10}H_{18}O_5$), *N,N'*-isopropylcarbonimine (DIC), *N*-Hydroxy succinamide (NHS), hydrochloric acid (HCL)was purchased from Aladdin Co. Ltd. (Guangzhou, China). Dioleoyl Phosphoethanolamine were purchased from Beijing biotech Co. Ltd. (Beijing, China). Tetrahydrofuran (THF) from Guangzhou Chemical Reagent Factory(Guangzhou, China), and dichloromethane and *N,N*-Dimethylformamide (DMF) were purchased from Tianjin Fuyu Fine Chemical Co.Ltd.(Tianjin,China). Sodium bicarbonate and methanol from Tianjin great chemical reagent factory.(Tianjin,China). Plasmid pEGFP-C1 was extracted by our lab. Plasmid Extraction Kit were purchased from Bio teke Co.Ltd.(Beijing,China). Agarose and Powdered agar from Guangzhou whiga Technology Co.Ltd. (Guangzhou,China). Tryptone and yeast powder were purchased from Oxoid Co.Ltd. (Beijing,China). RPMI1640 medium was purchased from GIBCO Co.Ltd.(Shangha,China). L-glutamic acid from Shanghai poly Biotechnology Co.Ltd.(Shanghai,China). Fetal bovine serum was purchased Thermo Fisher biochemical products Co.Ltd. (Beijing,China).MTT from Guangzhou whiga Technology Co.Ltd. (Guangzhou,China). Dimethyl sulfoxide (DMSO) from Tianjin Bai shi Chemical Co.Ltd.(Tianjin,China). Human hepatocellular carcinoma cell lines (HepG2) and Human cervix carcinoma cells (HeLa) were cultured by our lab.

2.2. Cell culture, materials and reagents

Hela and HepG2 cells were cultured in our lab containing 10% heat-inactivated fetal bovine serum (FBS). All cells were maintained under standard culture conditions of 37°Cand 5% CO_2.

2.3. The synthesis of Di lauroyl tartaric anhydride

Lauroylchloride (13.1256 g, 0.06 mol) was added to tartaric acid (3.0018 g, 0.02 mol) in a dry round-bottom flask under stirring and the resulting mixture was heated to 70 ℃ for 24 h, and then cooled to room temperature. A total of 20 ml n-hexane was added and the precipitate was filtered, and washed thoroughly with 200 ml n-hexane, and dried under a vacuum (Altenbach et al., 2010). The product was obtained as a white powder (90% Yield). The Fig. 1 show the synthesis of Di lauroyl tartaric anhydride.

2.4. The synthesis of bis(N,N'-tert-butoxycarbonyl) Lysine acid

lysine (5.84 g, 0.04 mol) was dissolved in tetrahydrofuran(40 ml), spotting with hydroxide solution (120 ml) under ice bath (a). Then Di-tert-butyl dicarbonate (18.33 g,0.084 mol) was dissolved in tetrahydrofuran (40 ml), and drops into (a) under the ice bath. The ice bath was removed and the mixture react at room temperature overnight. The solution was evaporated by evaporation to remove tetrahydrofuran, adjusted pH to 2–3, extracted with dichloromethane, and the organic phase was dried with sodium sulfate, evaporated to remove dichloromethane. The product was obtain by vacuum drying for 6 h. The Fig. 2 show the synthesis of Bis(*N,N'*-tert-butoxycarbonyl) Lysine Acid.

2.5. The synthesis of H2N-PAMAM -Lys-Boc

Boc-Lys-Boc (5.50 g, 0.03 mol) was dissolved in anhydrous DMF (100 ml) (b),and then *N*-Hydroxy succinimide (NHS) (1.59 g,12.8 mmol) and *N,N*-disopropylcarbodiimide (DIC) (6.36 g, 50.53 mol) were added to (b), the resulting mixture(c) was reacted at room temperature and avoid light for 4 h. After 4 h, PAMAM (2.74 g, 0.01 mol) was dissolved in DMF (50 ml), and then added to (c), the resulting mixture (d) stirred at room temperature for 24 h. After the end of the reaction, a large number of deionized water was added in (d), and a lot of white precipitate was separated out. A lot of white power solid were obtain after centrifugal separation and vacuum drying for 6 h. The Fig. 3 show the synthesis of H2N-PAMAM-Lys-Boc.

2.6. The synthesis of Di lauroyl tartaric anhydride-PAMAM-Lys

Laurie acid acyl tartaric acid lipid (0.996 g, 0.002mpl) was dissolved in 10 ml of anhydrous DMF (20 ml) (e), and N-Hydroxy succinimide (NHS) (0.22 g) and N, N-diisopropylcarbodiimide (DIC) (0.95) were added to that mixture, mixing at room temperature to avoid light for 4 h. H_2N-PAMAM-Lys-Boc (5.992 g, 0.004 mol) was dissolved in 20 ml DMF and added into (e), the resulting mixture (f) stirred at room temperature for 24 h. After the end of the reaction, a large number of deionized water was added in the mixture, and a lot of white precipitate was separated out. A lot of white power solid (g) were obtain after centrifugal separation and Vacuum drying. The resulting mixture was concentrated and (h) was obtained after washed with Ether. Then (g) (1.075 g, 0.00031 mol)in 10 ml tetrahydrofuran (THF) was react with hydrochloric acid and 1,4-dioxane (10 ml) in the flask at 0 °C A rotary evaporator was used to remove the THF, hydrochloric acid and 1,4-

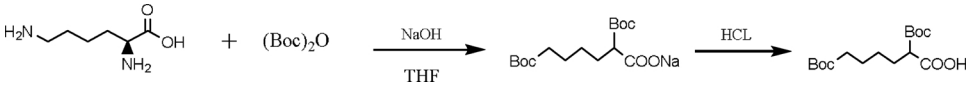

Fig. 2. The synthesis of Bis(N,N'-tert-butoxycarbonyl) Lysine Acid.

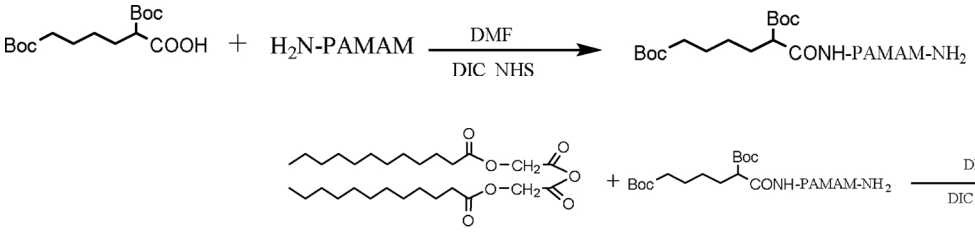

Fig. 3. The synthesis of H2N-PAMAM-Lys-Boc.

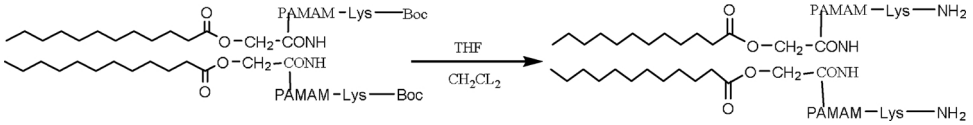

Fig. 4. The synthesis of Di lauroyl tartaric anhydride-PAMAM-Lys.

dioxane. Then Et_2O (40 ml) was applied to extract the unreacted. The mixture was dissolve in 40 ml deionized water and dialyze in a large number of deionized water. Then the residue was dried in a freeze drier for 48 h. The Fig. 4 show the synthesis of H2N-PAMAM-Lys-Boc.

2.7. Preparation of cationic liposomes

Liposomes were prepared through thin-film hydration method. Cationic lipids were often mixed with a neutral lipid dioleoylphosphatidylethanolamine (DOPE) to formulate into cationic liposome. (Balasubramaniam et al., 1996; Tang and Hughes, 1998; Kearns et al., 2010; Ren et al., 2000; Floch et al., 2000; Byk et al., 2000) lipid (1 g) was dissolved with dichloromethane and methanol (volume ratio = 2:1), and then vortexed. DOPE were taken in same molar ratios and dissolved in the above solution, solvent was slowly removed under vacuum. The resulting film was placed in vacuum oven (45 °C) overnight and then diluted with ddH_2O or phosphate buffered salinein (PBS). Hydration process continued for 4 h. The hydration solution was vortex-mixed or and sonicated to clarify. Cationic Liposomes were then extruded through filter with porosity of 0.22μm. The Fig. 5 show the synthesis of liposome.

2.8. Preparation of liposome/DNA complexes

The cationic liposomes diluted to 20 μg/mL with ddH_2O were mixed with pEGFP-C1 which diluted to the same concentration with ddH_2O. The mixture was then gently vortexed and incubated for 30 min at room temperature to form lipoplexes (cationic liposome/DNA).

2.9. Gel retardation assay

DNA–lipid complexes were formed by mixing plasmid DNA with varying amounts of cationic lipids so that the final lipid/DNA charge ratios were maintained at 0.5/1–15/1 in a total volume of 12ul. Complexes were incubated for 30 min at room temperature. The ethidium bromide intercalating agent was used as staining reagent. The complexes were electrophoresed on a 1.0% (W/V) agarose gel, with Tris-acetate-EDTA (TAE) running buffer at 120 V for 25 min.The gel images were taken by a UV light illuminator.

2.10. Measurement of size distribution and zeta-potential of liposome/DNA complexes

Proper size and zeta potential are important factors for liposome/DNA complexes (lipoplexes) used as gene vectors. The liposomes were mixed with DNA according to different weight ratio (5:1, 10:1, 15:1, 20:1, 25:1, 30:1, 40:1) in ultra-pure water. The particle sizes of different lipoplexes were measured for 3 times by particle size analyzer.

2.11. Cytotoxicity assay

The cytotoxicity of optimized formulation of cationic liposomes was also investigated with Hela and HepG2 cells. Cells were seeded in 96-well plate at a density of 1 *10^4cells/well and were incubated for 24 h. Then 100 μL of cell culture medium containing different concentration of the liposomes was added to replace the cell culture medium and incubated for an additional 4 h. Liposome was abandoned and replaced with RPMI1640 continued into the incubator cultivate 40–48 h. Then, 100 μL of MTT (5 mg/ml) was added into each well. The cells were incubated for further 4 h. Then MTT was discarded, and each well was treated with 100μL of dimethyl sulfoxide (DMSO) to fully dissolve the reduced crystal violet. Finally the Bio-enzyme mark Rad instrument was used to determine the absorbance of the solution at 490 nm.

2.12. Transfection activity

HeLa and HepG2 cells were plated at 24-well plates at 100000cells/well, and incubated for 24 h. The liposome and DNA was mixed

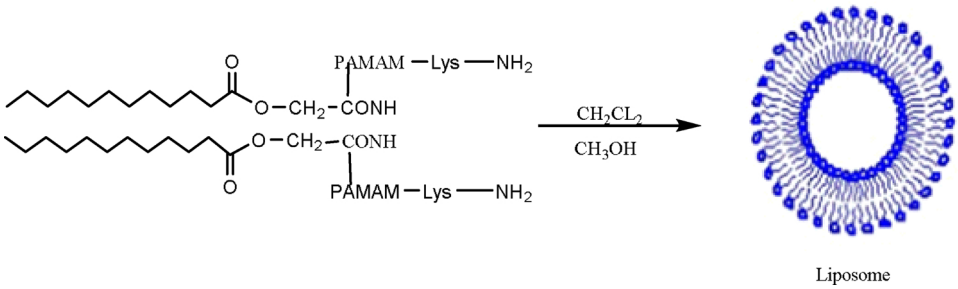

Fig. 5. The synthesis of liposome.

according to different weight ratios. After 24 h, cell cultures medium was discarded, and 500μL /well liposome/DNA were added to the cells. After incubation at 37 °C for 6 h, transfection media were removed, and replaced by 500μL RPMI1640 cell culture medium incubating another 48 h. Fluorescence microscopy was applied to examine GFP expression.

3. Results and discussion

3.1. Synthesis

According to the study of li jia[43], the best mole ratio of lauroyl chloride and tartaric acid is 2.2:1, which can improve product yield and save raw material. The reaction of Lauroyl chloride, anhydrous sodium carbonate and tartaric acid is exothermic reaction, so lauroyl chloride should dropped and reacted under the condition of ice bath and then transferred to the room temperature. The whole reaction should strictly control without water. In the synthesis of Bis(*N*,*N*′-tert-butoxycarbonyl) Lysine Acid, when the mole ratio of (Boc)2o and lysine is less than 2.0:1, it is not in conformity with the theory of reaction, and the products is the mixture of single amino and double, so the ratio from 2.0:1. With the increase of the mole ratio of (Boc)2o and lysine, the product yield also increased gradually. When the molar ratios increase from 2.1:1 to (2.2 ~ 2.3): 1, the increase of product yield is not obvious. So the appropriate mole ratio is 2.1:1. The activation of carboxylation should in the dark conditions, due to the activity of N-hydroxysuccinimide (NHS) and *N,N*-diisopropylcarbodiimide (DIC) is highly unstable. Tetrahydrofuran, DMF used in experiment should be strictly in addition to water. The lauryl chloride, tartaric acid and anhydrous sodium carbonate used in the synthesis of Di lauroyl tartaric anhydride should be react faster and in anhydrous conditions, so as not to absorb moisture in the air. Di-tert-butyl dicarbonate (BOC)2o is easy to decompose under the influence of water in neutral environment, so the tetrahydrofuran should remove the water in it, and be quickly accessed and to be slightly overdue to ensure that the two amino groups are fully protected.

3.2. Structural confirmation using IR spectra

As shown in Fig. 6, Di lauroyl tartaric anhydride has main characteristic peaks of 1724 cm^{-1}, the two peaks at 2927 cm^{-1} and 2933 cm^{-1}, which were attributed to the aliphatic carbonyl, the ester group, the methyl and methylene, respectively. The disappearance of

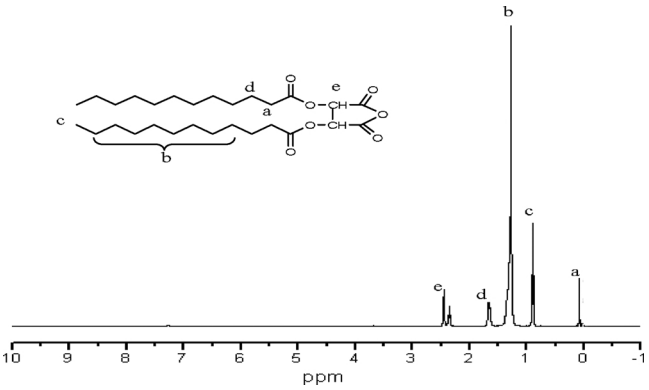

Fig. 7. ^{1}H-NMR spectra of Double lauric Acid Tartaric Acid.

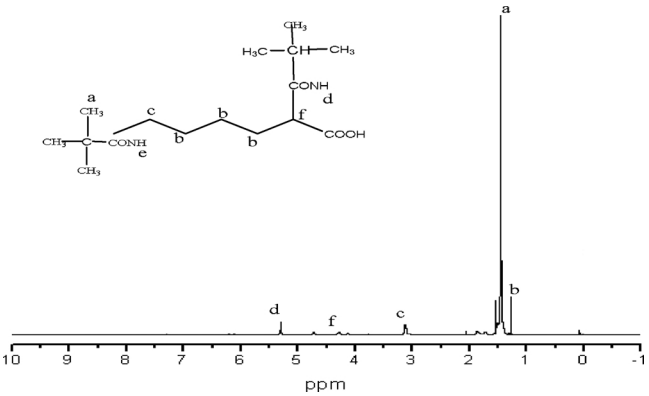

Fig. 8. ^{1}H-NMR spectra of Boc-Lys-Boc.

strong absorption peaks of tartaric acid indicates that lauroyl chloride has been attached to tartaric acid. The main characteristic peaks of 1721 cm^{-1}, 1528 $cm^{-1}$and 3352 cm^{-1}, 1167 $cm^{-1}$and the two peaks at 2987 and 2931 $cm^{-1}$of Bis(N,N′-tert-butoxycarbonyl) Lysine Acid were attributed to the amide bond, the hydroxyl of carboxyl group, the methyl and methylene, respectively. The weak and slightly sharp peak at 3500 ~ 3300 cm^{-1} of the lysine disappear, indicating that the double-ended amino of lysine has been protected. The main characteristic peaks of 3300 $cm^{-1}$and 1562, 1328 cm^{-1}were attributed to the

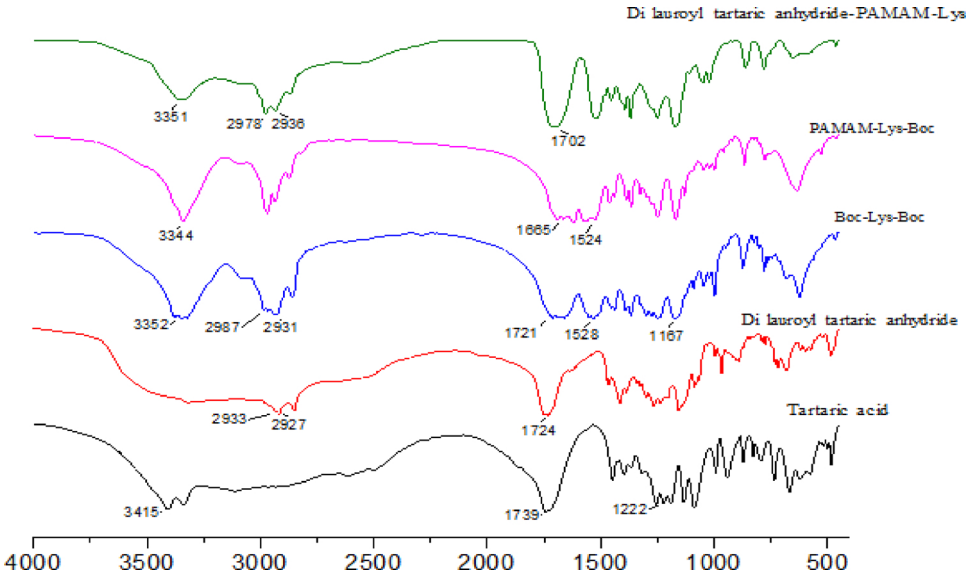

Fig. 6. IR spectra of various synthetic compounds.
The Fig.6 show the IRspectra of each synthetic compounds,and marked the special functional group of them.

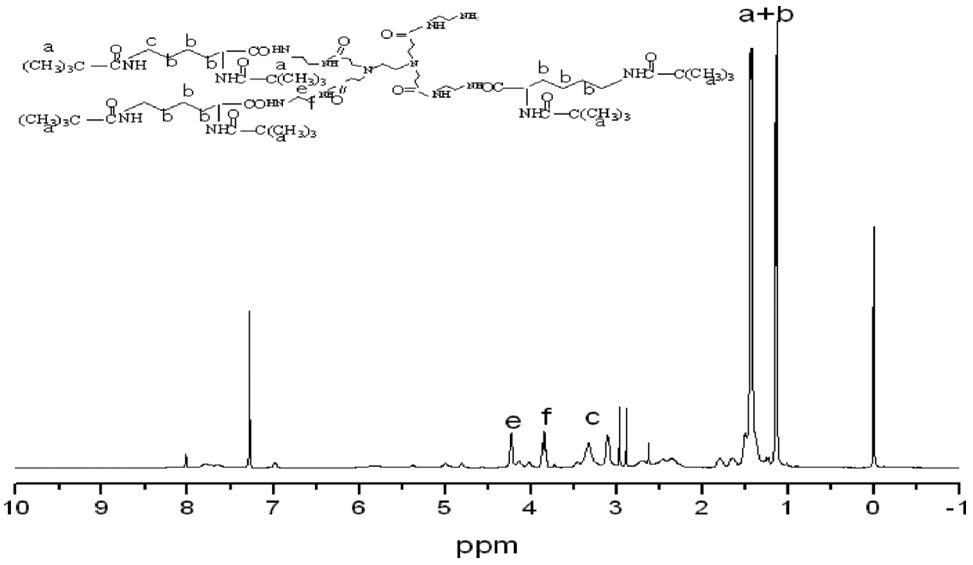

Fig. 9. ^{1}H-NMR spectra of PAMAM-Lys-Boc.

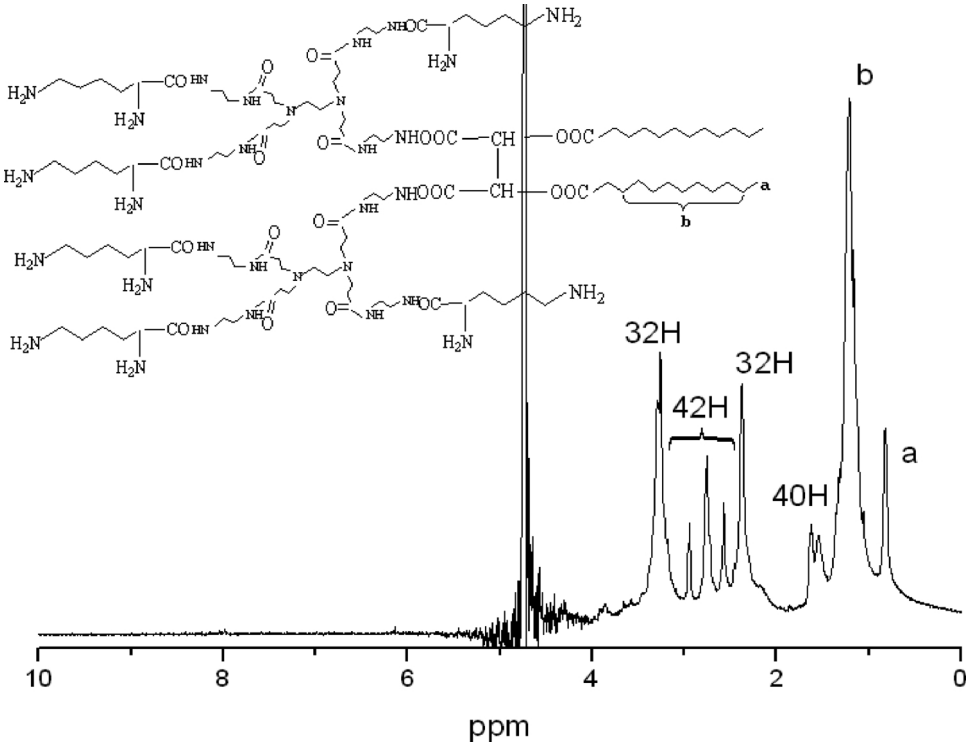

Fig. 10. ^{1}H-NMR spectra of Double lauric Acid Tartaric Acid Ester Cationic Lipid.

primary amine of PAMAM, the carbonyl of amido bond of PAMAM. The peaks at 1665, 1524 cm^{-1}, and the sharp peaks at 3344 cm^{-1} of H_2N-PAMAM-Lys-Boc were attributed to the amido bond, the primary amine. The main characteristic peaks of 1553 − 1642 cm^{-1} and 3299 cm^{-1} and the peaks at 2852 − 2923 cm^{-1} of Di lauroyl tartaric anhydride-PAMAM-Lys were attributed to the amino bond, the primary amine and the carbon chain respectively.

3.3. Structural confirmation using ^{1}H-NMR spectra

The ^{1}H NMR spectra of Double lauric Acid Tartaric Acid,Boc-Lys-Boc, PAMAM-Lys-Boc, Double lauric Acid Tartaric Acid Ester Cationic Lipid were evaluated, and the ^{1}H-NMR spectrum is shown (Figs. 7–10).

3.4. Agarose gel retardation assay

Liposomes should have the ability to bind to DNA and compress it to transport DNA to the corresponding target cells. Agarose gel retardation assay was carried out to evaluate the binding interactions between cationic liposomes and pDNA at different N/P ratios. Stable cationic liposome/DNA complexes were not able to penetrate into the agarose gel, on contrast, free DNA or DNA not fully combined to cationic liposomes could penetrate into the agarose gel.

As shown in Fig. 11, with the increase of the weight ratio of lipid/DNA, the DNA band moved to the positive pole weakened to disappear. Fig. 12 also shows that the DNA band moves toward the positive pole gradually weakened to disappear as the increase of weight ratio of liposome/DNA. It was found that the negative charge of DNA was

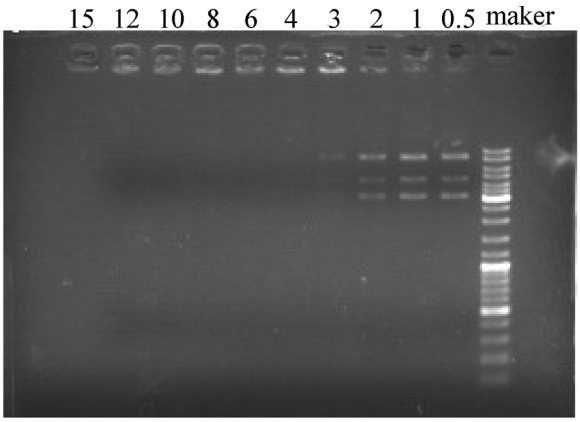

Fig. 11. Gel retardation of pDNA complexed to double lauric acid tartaric acid ester cationic.
lipids at the various charge ratios of 0.5:1, 1:1, 2:1, 3:1, 4:1, 6:1, 8:1, 10:1, 12:1,15:1 (n = 3).
The mark(0.5,1,2,3,4,6,8,10,12,15) in the Fig.11 indicates that the weight ratio of lipid and DNA is 0.5:1, 1:1, 2:1, 3:1, 4:1, 6:1, 8:1, 10:1, 12:1,15:1

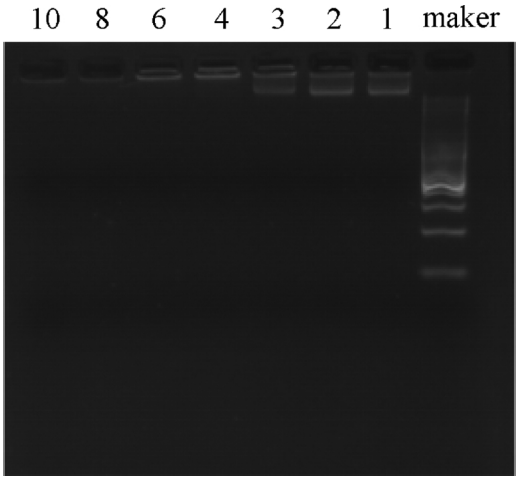

Fig. 12. Gel retardation of pDNA complexed to double lauric acid tartaric acid ester cationic liposomes at the various charge ratios of 1:1, 2:1, 3:1, 4:1, 6:1, 8:1, 10:1 (n = 3).
The mark(1,2,3,4,6,8,10) in the Fig.12 indicates that the weight ratio of lipid and DNA is 1:1, 2:1, 3:1, 4:1, 6:1, 8:1, 10:1.

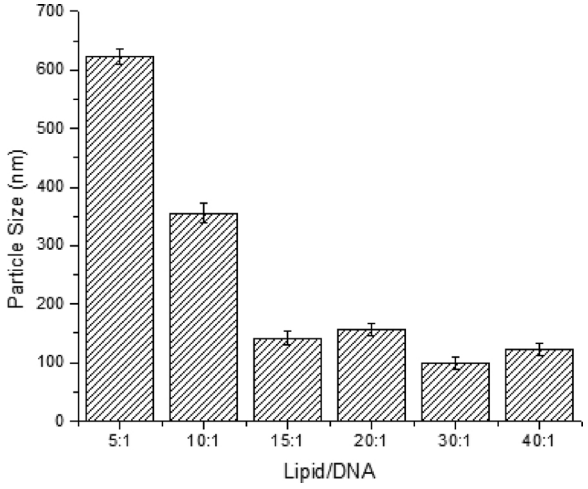

Fig. 13. The Particle size of pDNA complexed to double lauric acid tartaric acid ester cationic liposomes at the various weight ratio (n = 3).

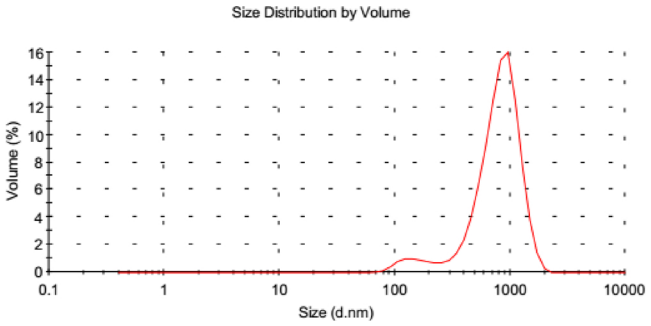

Fig. 14. Statistics Graph of pDNA complexed to double lauric acid tartaric acid ester cationic liposomes at weight ratio10.

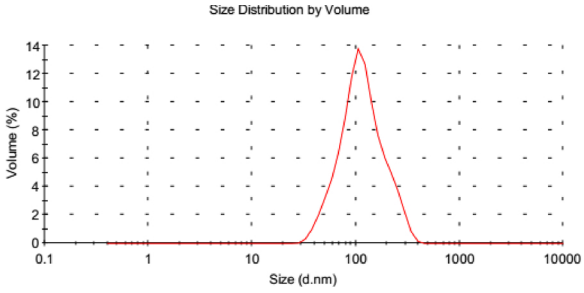

Fig. 15. Statistics Graph of pDNA complexed to double lauric acid tartaric acid ester cationic liposomes at weight ratio30.

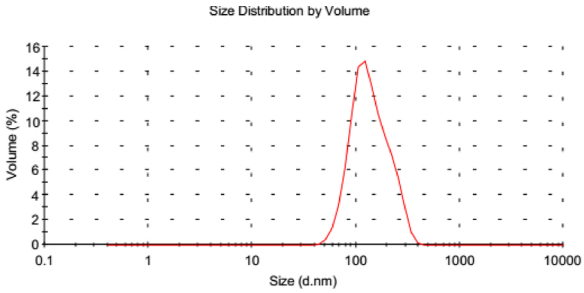

Fig. 16. Statistics Graph of pDNA complexed to double lauric acid tartaric acid ester cationic liposomes at weight ratio 40.

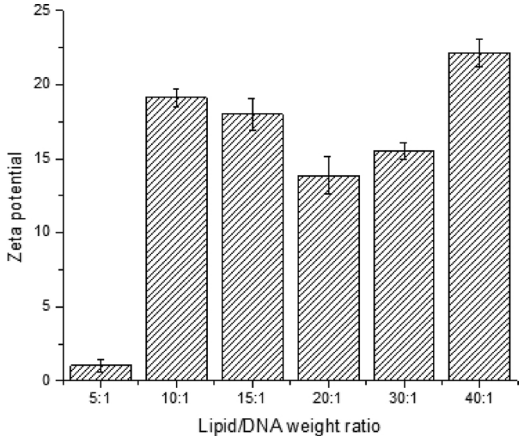

Fig. 17. Zeta Potential Comparison of pDNA complexed to double lauric acid tartaric acid ester cationic liposomes at the various weight ratio (n = 3).

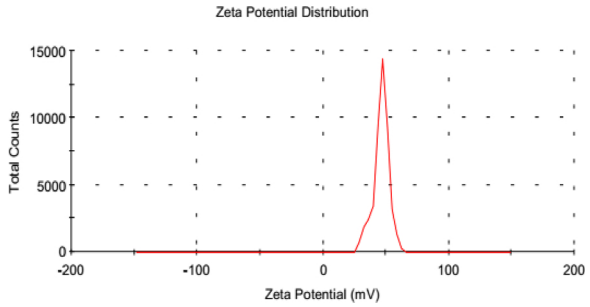

Fig. 18. Statistics Graph of pDNA complexed to double lauric acid tartaric acid ester cationic liposomes at weight ratio 20.

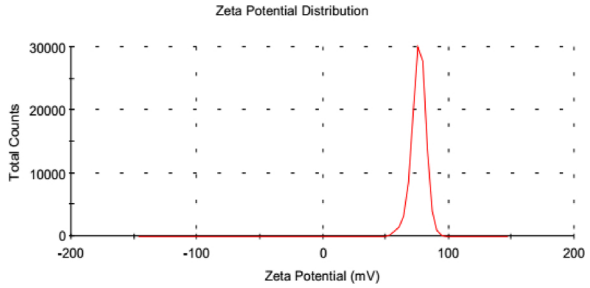

Fig. 19. Statistics Graph of pDNA complexed to double lauric acid tartaric acid ester cationic liposomes at weight ratio 30.

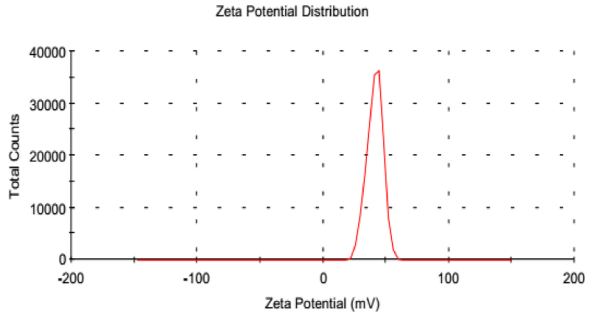

Fig. 20. Statistics Graph of pDNA complexed to double lauric acid tartaric acid ester cationic liposomes at weight ratio 40.

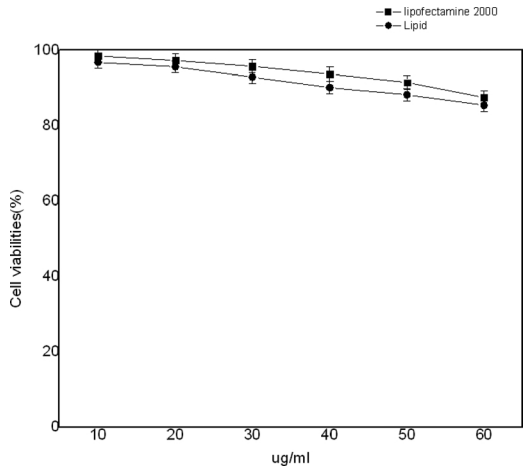

Fig. 21. Cell viabilities of double lauric acid tartaric acid ester cationic liposomes at various concentrations in HepG2 cell (n = 3).

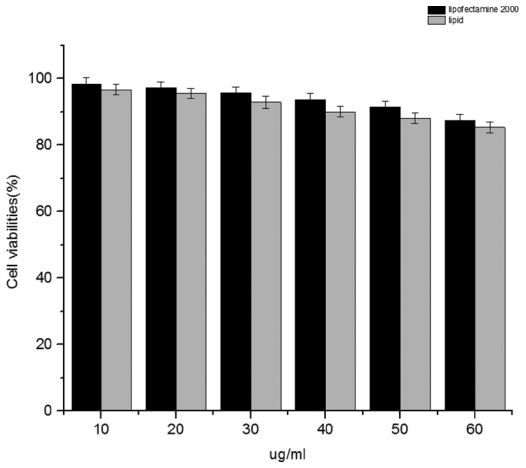

Fig. 22. Cell viabilities of double lauric acid tartaric acid ester cationic liposomes at various concentrations in HepG2 cell (n = 3).

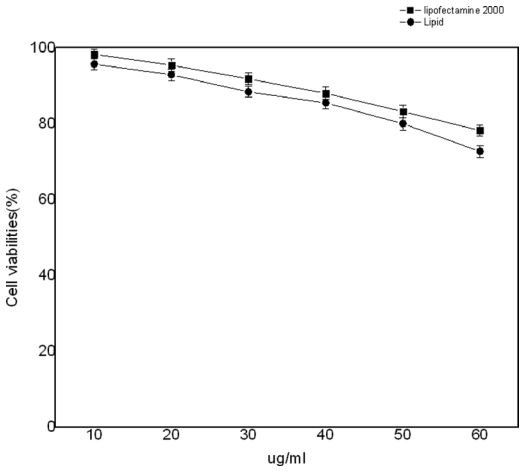

Fig. 23. Cell viabilities of double lauric acid tartaric acid ester cationic liposomes at various concentrations in HeLa cell (n = 3).

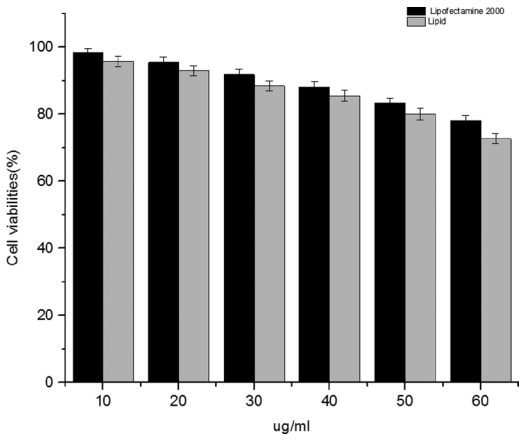

Fig. 24. Cell viabilities of double lauric acid tartaric acid ester cationic liposomes at various concentrations in HeLa cell (n = 3).

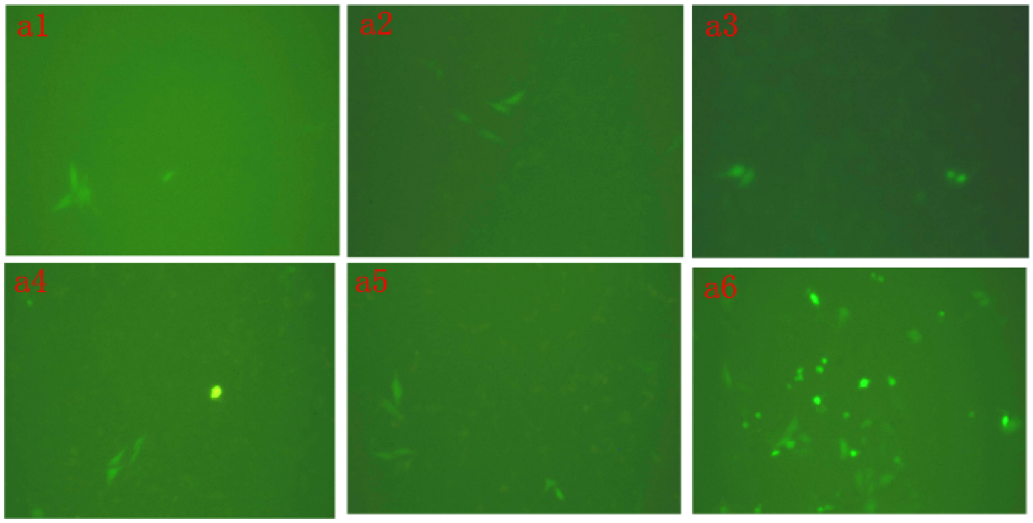

Fig. 25. Transfection results of the different weight ratios of double lauric acid tartaric acid ester cationic liposomes/DNA without serum in HeLa cell. a1-a5 showed the weight ratios: 2:1,4:1,8:1,10:1,15:1. a6 showed the weight ratios 2:1 of lipofectamine 2000 /DNA (n = 3).

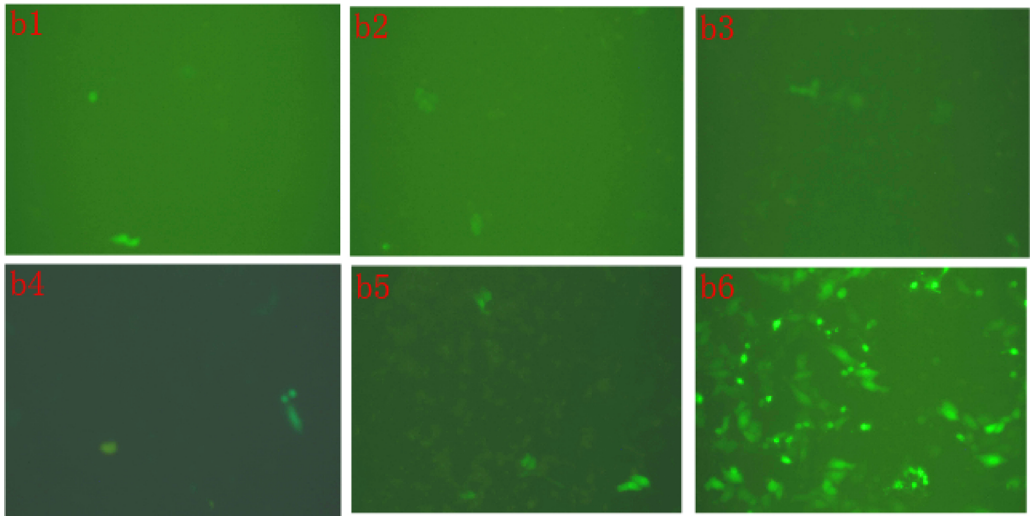

Fig. 26. Transfection results of the different weight ratios of double lauric acid tartaric acid ester cationic liposomes/DNA without serum in HepG2. b1-b5 showed the weight ratios: 2:1,4:1,8:1,10:1,15:1. 6b showed the weight ratios 2:1 of lipofectamine 2000/DNA(n = 3).

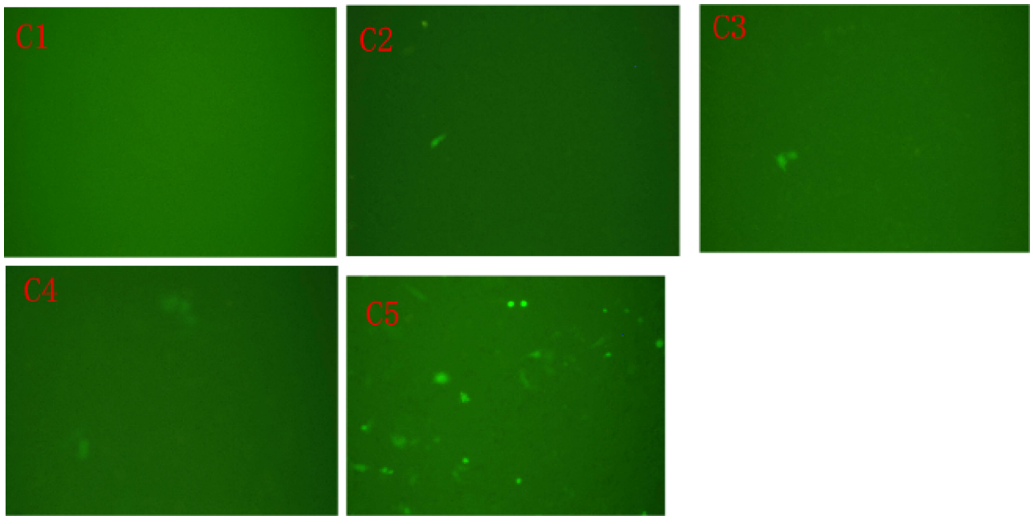

Fig. 27. Transfection results of the different weight ratios of double lauric acid tartaric acid ester cationic liposomes/DNA with serum in Hela cell. c1-c5 showed the weight ratios: 2:1,4:1,8:1,10:1. c5 showed the weight ratios 2:1 of lipofectamine2000 /DNA (n = 3).

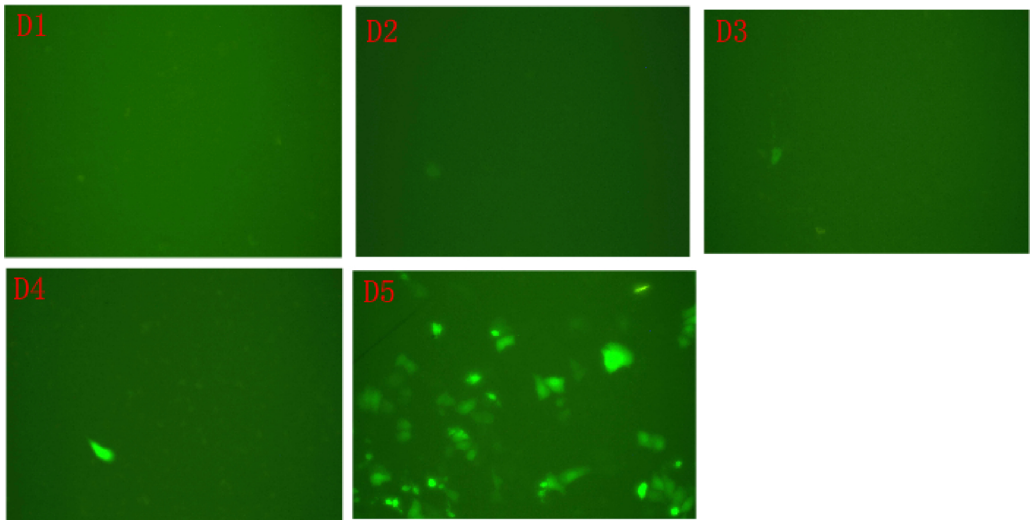

Fig. 28. Transfection results of the different weight ratios of double lauric acid tartaric acid ester cationic liposomes/DNA with serumin HepG2. D1-D4 showed the weight ratios: 2:1,4:1,8:1,10:1. D5 showed the weight ratios 2:1 of lipofectamine2000/DNA(n = 3).

neutralized by the positive charge of the liposome, causing the liposome / DNA complex to slow down in agarose gel electrophoresis. As can be seen from Fig. 12, the ability to bind and compression DNA was stronger with the addition of lipids DOPE(dioleoyl phosphatidylethanolamine). In addition, when N/P ratio was reach 4:1, DNA was entirely compacted and protected in the cationic liposomes, the fluorescent in the corresponding lanes was absence because the staining reagent was inaccessible to stain DNA.

3.5. Measurement of size distribution and zeta-potential of cationic liposomes

The gene transfection efficiency in vitro is influenced by many factors, including NP size, surface charge. Cell endocytosis is a major way that carriers to carry DNA into cells. Large particles cannot enter the cells. Therefore, the size of the carrier is one of the important conditions to carry the target gene into the cells to achieve transfection efficiency.Studies have shown that when the particle size of the complex is less than 200 nm, the complex can enter the cell smoothly to achieve transfection. As shown in Fig. 13, the particle size of the liposomes/DNA complex decreases with the increase of the weight ratio. When the weight ratio is greater than 15, the liposomal/DNA complex has a particle size of about 170 nm and less than 200 nm. May be the role of Bis-lauroyl tartaric acid-PAMAM-Lys liposomes in the presence of positively charged and liposomal bilayers. The positive charge of the liposomes can neutralize the negatively charged DNA and reduce the degree of particle aggregation, as the weight ratio increases, the amount of positively charged liposomes increases and the particle size of the liposomes/DNA complex decreases and gradually tend to be stable. The particle size distribution of liposomes/DNA complexes at a weight ratio of 10, 30 and 40 is shown in Figs. 14–16

Only the complex has positive charge to neutralize the negative charge on the cell surface, can it into the cell and to achieve transfection. But studies have shown that, too high positive charge will lead to greater cytotoxicity, so it is play an important role that through the potential measurement to select the appropriate potential. Fig. 17 shows the Zeta Potential Comparison of pDNA complexed to double lauric acid tartaric acid ester cationic liposomes at weight ratio: 5: 1, 10: 1, 15: 1, 20: 1, 25: 1, 30: 1. It shows that the zeta potential increases as the weight ratio increases. The Zeta Potential distribution of liposomes/DNA complexes at a weight ratio of 20, 30 and 40 is shown in (Figs. 18–20).

3.6. Cytotoxicity

It is well known that cationic liposome/pDNA complex with positive surface charge is ready to bind to negatively charged cell membrane, leading to cell membrane damage and high cytotoxicity. To investigate the cytotoxicity of the cationic liposomes, MTT cell viability assays were executed on HeLa and HepG2 cells, and Lipofectamine 2000 was used as the positive control. MTT assay was performed to determine the in vitro cytotoxicity of different concentration in HeLa, and HepG2 cells. As shown in Figs. 21–24, the dependence of double lauric acid tartaric acid ester cationic liposomes on cells was not very strong and the cytotoxicity increased with the increase of concentrations. When the concentration of liposomes reached 60 μg/mL, cell viabilities were above 75% in the HeLa and HepG2 cells. And it show that the cytotoxicity of liposome is lower than the same concentrations of commercial Lipofectamine 2000, is a promising candidates for gene delivery.

3.7. Transfection activity

Achievement of high gene transfection efficiency is essential for the development of new gene carriers. The gene transfection efficiency of different weight ratios of cationic liposomes/DNA, in comparison with commercially available lipofectamine 2000/DNA, was estimated by comparing the fluorescence intensities in fluorescence images of transfected HeLa and HepG2 cells.

As shown in Figs. 25 and 26, the transfection efficiency increased with the increase of liposome/DNA weight ratio in serum-free conditions, but the transfection efficiency was far from that of commercial liposomes. The balance of the hydrophilic head, the linker and the hydrophobic tail of the terminal peptide liposome. This may be related to the balance of the hydrophilic head, the linker and the hydrophobic tail of the terminal peptide liposome.

According to previous studies, the transfection efficiency of cationic lipid is determined by the hydrophilic head, the connection bond and the hydrophobic tail. It is not the more the head of the amino, the higher the efficiency of transfection, and more emphasis on the balance of connection key, amino head and hydrophobic tail. Further study should be taken to prepare more safe, high transfection efficiency cationic lipids. In this study, cell culture with fetal bovine serum (FBS) which usually contains growth factors, cofactors and other additives and have different effects on the growth of different cells. Some of the proteins in the serum carry negatively charged and affect the stability of

positively charged liposome/DNA complexes, reduced the transfection efficiency. As shown in Figs. 27 and 28, the transfection efficiency decreased obviously in Hela cell and HepG2 cell under serum conditions, so cationic liposomes are still need to be studied further on antiserum as a gene vector.

4. Conclusion

In summary, we have designed and synthesized the new cationic terminal peptide which Bis lauryl was used as the hydrophobic chain, tartaric acid as the skeleton and lysine-modified 0-generation PAMAM in head-groups. The results showed that Bis-lauroyl tartrate-PAMAM-Lys liposomes were successfully synthesized by 1H-NMR and IR. The results of gel electrophoresis, transfection activities, and cytotoxicity of cationic liposomes showed that the tartaric acid based cationic lipids are promising candidates for gene delivery. Result of the gel electrophoresis show that the liposome can bind and compress DNA well and form stable complex particles. When the weight ratio of liposome/DNA is greater than 15, the particle size is in the range of 200 nm and the surface potential is between14-23 mV, and is suitable as a gene transfection carrier. Studies on the cytotoxicity show that liposomes had no significant dependence on different cells and the toxicity of different cells were less than the commercial liposome lipofectamine 2000. However, the transfection efficiency of liposomes/DNA complexes in different cells was lower than that of commercial liposomes 2000. This may be related to the balance of the hydrophilic head, the linker and the hydrophobic tail of the terminal peptide liposomes, so the specific influencing factors of transfection efficiency need further study.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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