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Development and Evaluation of a Novel Nano-Scale Vector for siRNA

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ABSTRACT

To synthesize a lipid-cationic polymer (LCP) containing brassidic acid side chain and to investigate its transfection efficiency and characteristics as a siRNA gene vector. The LCP was chemically synthesized and its nucleic acid binding capacity was determined by gel electrophoresis. HeLa-EGFP and TH1080-EGFP cell lines were transfected with siRNA against enhanced green fluorescent protein (EGFP) gene using a LCP to investigate the transfection efficiency. An MTT assay was performed to evaluate the cellular toxicity of the LCP vector. Its degradability and stability under acidic conditions were also investigated. The LCP vector possessed high DNA binding capacity. More than 73% of the cellular fluorescence was inhibited by the LCP-mediated transfection of siRNA against EGFP gene, indicating that vector had high transfection efficiency. Cellular viability was about 95% at the optimum transfection efficiency of LCP, suggesting that the cellular toxicity of LCP was very low. The LCP was also observed to be degradable; moreover, it could be easily stored at normal temperature. A gene vector used for the transfection of siRNA was successfully fabricated from synthesized LCP. Its numerous excellent properties entitle values for further scientific research. J. Cell. Biochem. 111: 881–888, 2010.

KEY WORDS: LIPID-CATIONIC POLYMER; siRNA; GENE VECTOR; TRANSFECTION

In recent years, ribonucleic acid interference (RNAi) has become the frontier topic for international life science research as it plays very important roles in the fields of gene therapy, drug screening, and functional gene research [Fountaine et al., 2005; Morrissey et al., 2005]. A key point in RNAi research is the challenge of successfully shipping into target cells and inhibiting the expression of target genes using small inhibitory RNA (siRNA). There are two kinds of vectors for siRNA transportation: viral and non-viral vector. Viral vectors have attracted attention due to its high efficiency, but the complex process of preparation limits its applications [Verma and Somia, 1997; Marshall, 1999; Descamps and Benihoud, 2009]. Although the efficiency of non-viral vectors is low, it has attracted attention as well due to its better biosafety [Guo et al., 2009; Zhao et al., 2009]. Most commonly used non-viral vectors are synthesized through chemical methods, and they can be divided into cationic lipids and cationic polymers according to their structures. Cationic lipid gene vectors have a very high transfection efficiency when used for transfection of plasmids (large molecular weight), genetic materials, and oligonucleotides (e.g., antisense oligonucleotide, chemical-synthesized siRNA), but it has deficiencies, such as poor biocompatibility and cytotoxicity [Zhdanov et al., 2002; Ewert et al., 2004, 2005]. In contrast, cationic polymer gene vectors have better biocompatibility and lower cytotoxicity. It has high efficiency in the transfection of plasmids or genetic materials [Chen 2003; Fountaine et al., 2005; Huang et al., 2009; Wilschut et al., 2009], except in the case of siRNA. In this study, we designed and synthesized a degradable nano-scale cationic polymer with a brassidic acid side chain, called the lipid-cationic polymer (LCP). It was used for the transfection of nucleotides, especially those with small molecular weights. In this article, we studied the physical and chemical characteristics of LCP and further performed a transfection assay to investigate its transfection efficiency, cytotoxicity, serum-biocompatability, and degradability.

Guang-Jun Jing and Zuo-Gen Fu contributed equally to this work.

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EXPERIMENTAL PART

BIOMATERIALS

Plasmid pEGFPN1 was purchased from Clontech Company. HeLa-EGFP and TH1080-EGFP cell lines were from the American Type Culture Collection (ATCC). Cationic lipid Lipofectamine 2000 and cationic polymer Cytopure was purchased from Invitrogen and Qbiogene, respectively. Fetal calf serum, Dulbecco's modified Eagle medium (DMEM), and Opti-MEM® were all from Gibco. All the reagents used for the synthesis of LCP were analytically pure and provided by Xiamen Luyin Co. (China).

CONSTRUCTION OF THE LCP GENE VECTOR

Briefly, 200 ml CH_2Cl_2, 150 μl *N*,*N*-dimethylformamide, and 11 g brassidic acid were combined in a reaction vessel and cooled to 0°C. Then, 13.5 ml oxalyl chloride was added. When the mixture reached room temperature, 150 ml toluene and 30 g aluminum chloride were added. The mixture was heated in a water bath at 56°C. The menstruum was vacuum evaporated at this temperature. The residue was dissolved in CH_2Cl_2 and cooled to 0°C. Diethanolamine, 4-dimethylaminopyridine, and triethylamine were mixed with the residue and stirred overnight. Then, 150 ml CH_2Cl_2 was added into the mixture and washed with 1 mmol/L HCl solution. The organic layer was washed with 0.02 mol/L $NaHCO_3$ and then with water until it reached a neutral pH. After adding 25 g Na_2SO_4, the organic layer was dried overnight and vacuum concentrated at 70°C. The concentrated product was purified with silica gel column chromatography (60–100 mesh, 30 mm × 300 mm; eluate:ethyl acetate/methanol = 10/1), and 18 g intermediate was recovered. The intermediate (10 g) was mixed with 10 g triethylamine, 1 g 4-dimethylaminopyridine, 250 ml CH_2Cl_2 in a reaction vessel. After dissolving, the gas mixture (nitrogen and argon) was aerated for 50 min and the solution was cooled to 0°C. A solution of 1% propylene chloride (1 g propylene chloride dissolved in 100 ml CH_2Cl_2 solution) was added to the mixture, which was stirred overnight at room temperature. After being diluted with 200 ml CH_2Cl_2, the mixture was washed with 0.02 mol/L $NaHCO_3$ and then in water until it reached a neutral pH. After layering, the organic layer was dried overnight with Na_2SO_4. The dried solution was vacuum concentrated and purified by silica gel column chromatography (60–100 mesh, 30 mm × 300 mm; eluate:ethyl acetate/hexane = 1/4). Finally, the LCP with a brassidic acid side chain was successfully produced.

Work solution I for the gene vector consisted of 50 ml physiological saline, 49 ml DMSO, and 1 ml glycerol. Work solution I (10 ml) was mixed with 50 mg LCP. After being dissolved in a water bath heated at 56°C, the mixture was filtered with a 0.2 μm filter. The LCP-siRNA gene vector I was then used in an in vitro assay.

IN VITRO ANALYSIS OF THE CHARACTERISTICS OF THE LCP GENE VECTOR

Production of the LCP/siRNA and its diameter and zeta potential analysis. The siRNA (39.9 μg) against EGFP (21 bp, synthesized by Chinese Military Academy of Medical Sciences) was diluted in 200 μl opti-MEM®. A 5 mg/ml solution of the LCP gene vector (0, 4, 8, 16, 32, 64, 128, and 256 μl) was diluted in the Opti-MEM culture medium to a final volume of 2,000 μl. The LCP gene dilution was mixed with the siRNA dilution to a final volume of 4,000 μl. The mass ratios of LCP to siRNA were 0:1, 0.5:1, 1:1, 2:1, 4:1, 8:1, 16:1, and 32:1. After vortex oscillation and incubation for 15 min at room temperature, the mixture was dropped onto a copper mesh coated with carbon. After being stained with uranyl acetate for 30 s and dried in a vacuum desiccator for 3 h, the configuration and size of the complex was observed using transmission electron microscopy (TEM). The diameter and zeta potential were determined by a laser particle sizer (LPS), and their distributions were analyzed in an automatic mode.

Nucleotide binding capacity of the LCP gene vector. Five micrograms of siRNA (anti-EGFP, 22 bp) was diluted in 1,000 μl Opti-MEM. A 5 mg/ml solution of LCP gene vector (0, 4, 8, 16, 32, 64, 128, and 256 μl) was diluted in the Opti-MEM culture medium to a final volume of 1,000 μl. The dilutions of LCP gene vectors were then mixed with the siRNA dilutions. The mass ratios of LCP gene vector to RNA were 0:1, 0.5:1, 1:1, 2:1, 4:1, 8:1, 16:1, and 32:1. After incubating at room temperature for 15 min, the mixtures were analyzed by polyacrylamide gel electrophoresis (15% PAGE, 7 mol/L urea) and visualized under the UV light.

Buffer capacity of the LCP gene vector. A 20 ml solution of LCP gene vector (5 mg/ml, pH 8.0) was created by diluting LCP gene vector solution with 150 mM NaCl. Then 25 μl hydrochloric acid (0.1 mol/L) was continuously added and the pH recorded at each addition. The PBS solution (pH 8.0, 0.1 M) was taken as the control.

GENE TRANSFECTION ASSAY WITH THE LCP GENE VECTOR

HeLa-EGFP and HT1080-EGFP cells were seeded at 1.5×10^4 cells/well in 96-well plates. The cells were incubated for 24 h at 37°C and 5% CO_2 in DMEM medium with 10% fetal bovine serum until it reached 40–50% cell confluency.

At this point, 7.5 μl LCP gene vector was mixed with 7.5 μl 20 pmol siRNA (sense: 5′-GCAAGCUGACCCUGAAGUUCAU; antisense: 5′-GAACUUCAGGGUCAGCUUGCCG) against enhanced green fluorescent protein (EGFP) gene and incubated at room temperature for 15 min. HeLa-EGFP and HT1080-EGFP cells were maintained in the culture medium containing 15 μl LCP/siRNA complex for 48 h. The fluorescence intensity of the EGFP was observed under a fluorescence microscope and quantified with a microplate reader (FLX800). The cationic liposome [Lipofectamine 2000] and cationic polymer (Cytopure) were taken as positive controls, and the transfection with siRNA (no vector) was taken as the negative control.

ANTI-NUCLEASE ASSAY OF THE LCP/siRNA COMPLEX

The LCP/siRNA solution containing 3 μg siRNA against EGFP was made with the mass ratio of 18:1 at a final volume of 150 μl. LCP/siRNA was mixed with 75 μl nuclease I (100 U/ml) and incubated at 37°C for 90 min. The, 75 μl block solution (pH 8.0, 400 mmol/L NaCl, and 100 mmol/L EDTA) was added. The LCP/siRNA complexes, both pre- and post-digested with nuclease I, were used to transfect HT1080-EGFP and HeLa-EGFP, respectively. The fluorescence intensity of EGFP was observed under a fluorescence microscope and quantified with a microplate reader (FLX800).

CYTOTOXICITY ANALYSIS OF THE LCP GENE VECTOR

MTT assay was performed to measure the survival rate of HeLa-EGFP cells 48 h after being transfected with LCP/siRNA. The untransfected cells were taken as control (OD = 100%). Cellular survival rate was calculated after transfection.

DEGRADABILITY OF THE LCP GENE VECTOR

A solution of 500 mg each of LCP, PEI 25K, and PEI600 (Polysciences, Inc) in PBS (pH of 7.3) was prepared. The solution was then kept at 37°C for 2, 4, 8, 16, and 24 h, respectively. The samples before and after heat preservation were then mixed with oligonucleotides (5′-TAACATATGAAAGGATCTGTCCGCGCGC-3′, synthesized and labeled by Chinese Military Academy of Medical Sciences) conjugated with fluorescein isothiocyanate (FITC), and the fluorescence intensity was measured.

STABILITY ANALYSIS OF LCP

LCP was dissolved in PBS (pH = 7.2) and kept at 4°C for 14 months. Every 2 months, the LCP kept at 4°C was used to transfect HT1080-EGFP cells with siRNA following the methods described in Gene Transfection Assay With the LCP Gene Vector Section, and the inhibition rate of EGFP expression was determined.

COMPATIBILITY WITH SERUM OF THE LCP GENE VECTOR

The HeLa-EGFP cell was maintained and LCP/siRNA complex was produced according to the methods presented in this article. Two different transfection protocols were performed. First, following common gene transfection manuals, the cells were incubated at 37°C with 5% CO_2 for 16–24 h, and the final cell density was 40–50%. After washing with PBS, the cells were cultured for 4 h in the serum-free medium with LCP/siRNA and maintained in the medium with serum for 48 h. The transfection effect was also evaluated. In the second protocol, cells were incubated at 37°C with 5% CO_2 for 16–24 h, and the final cell density was 40–50%. The cells were maintained in the normal medium with LCP/siRNA. After being cultured for 48 h, the transfection effect was analyzed.

STATISTICAL ANALYSIS

All experiments in present study were repeated in triplicate. For quantification of the relative fluorescence intensity in EGFP-expressed cells, four to seven random fields of vision were selected, and all results were shown in mean ± SD.

RESULTS

FORMATION, DIAMETER, AND ZETA POTENTIAL OF THE LCP/siRNA COMPLEXES

The diameter of the LCP/siRNA complex gradually decreased with the increase of the proportion of LCP gene vector. When the mass ratio of LCP to siRNA was ≥4:1, LCP can fully compress siRNA to form complexes with diameters of 116.4–123.6 nm acquired by LPS (Fig. 1A). The zeta potential of the LCP/siRNA complex rose following the increase of mass ratio, which ranged from −7.3 to +48.1 mV (Fig. 1B). The TEM analysis revealed that the LCP/siRNA had a spherical morphology with an average diameter of 120 nm (Fig. 1C), which was close to the results of LPS.

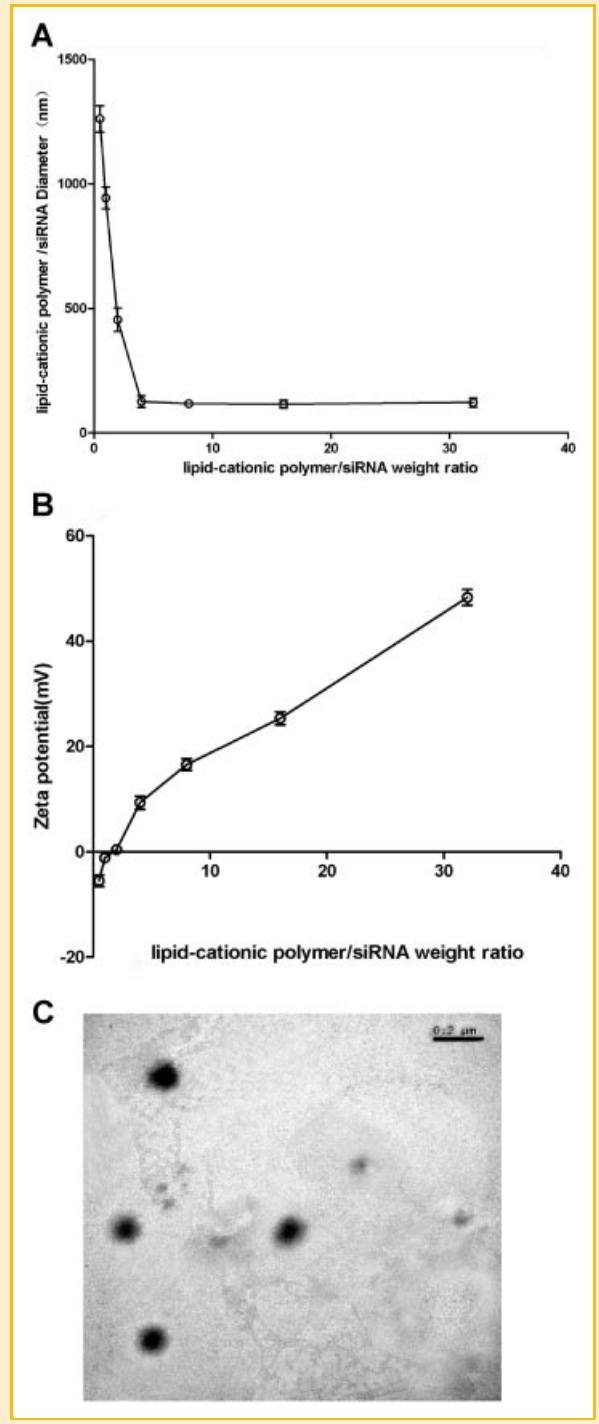

Fig. 1. Diameter and zeta potential of the LCP/siRNA complexes. A: Sizes of the LCP/siRNA complex with different LCP/siRNA weight ratios by laser particle sizer. B: Zeta potentials of LCP/siRNA complex with different LCP/siRNA weight ratios. C: Transmission electron microscope profiles of LCP/siRNA complex with a ratio of 4:1.

NUCLEOTIDE BINDING CAPACITY OF THE LCP GENE VECTOR (RNA RETARDATION ASSAY)

A polyacrylamide gel electrophoresis was performed to investigate the siRNA-binding capacity of the LCP gene vector. After LCP

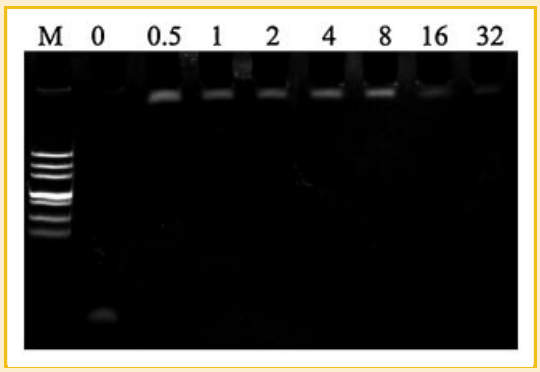

Fig. 2. Polyacrylamide gel electrophoresis of lipid-cationic polymer /siRNA complexes. The numbers 0, 0.5, 1, 2, 4, 8, 16, and 32 indicate the weight ratio of Lipid-cationic polymer and siRNA. 0 means there is no lipid-cationic polymer. M is for DNA marker.

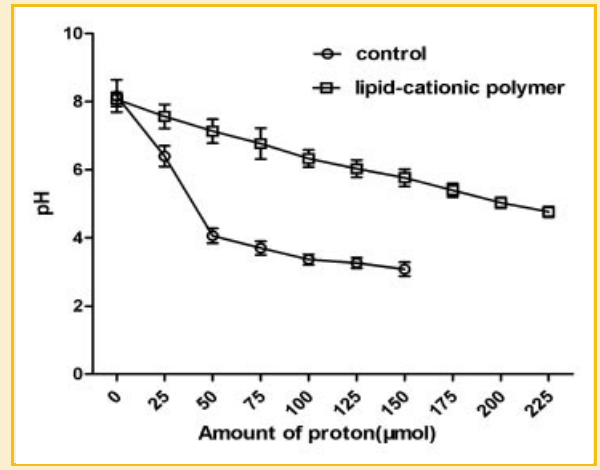

Fig. 3. Acid titration curve of lipid-cationic polymer gene carrier. Control is PBS (0.1 mol/L). The experiment was performed in triplicate. The dispersion bar showed SD values ($P < 0.05$).

binding with siRNA, the negative charge of the LCP/RNA complex was weakened and its molecular weight increased. The migration of the complex in the electric field became slow or ceased. As displayed in Figure 2, the electrophoresis of siRNA without LCP gene vector resulted in a typical small RNA band. When the mass ratio of LCP to DNA was 0.5:1, the electrophoresis was obviously delayed, and only an obscure RNA vestige was visualized. When the mass ratio of LCP to siRNA was more than one, almost all the RNA was integrated with LCP and the migration ceased. The results confirmed the high small RNA-binding capacity of the LCP gene vector, indicating that LCP possessed high siRNA-binding ability.

ANALYSIS OF THE BUFFER CAPACITY OF THE LCP GENE VECTOR

The buffer capacity of 5 mg/ml LCP gene vector solution (pH = 8.0) was determined by 0.1 mol/L HCl titration. The pH value of the LCP solution steadily decreased. When the amount of hydrochloric acid reached 225 μmol, the pH value of the LCP gene vector solution decreased to 4.9. On the other hand, in the control solution (0.1 mol/L PBS, pH = 8.0), the pH value rapidly decreased to 4.2 when the amount of HCl was 50 μmol, and the pH value rapidly declined to 3.3 when the total amount of hydrochloric acid was 150 μmol (Fig. 3). The results confirmed that the LCP had a high buffer capacity in acid.

EVALUATION OF siRNA TRANSFECTION USING THE LCP GENE VECTOR

HeLa-EGFP and HT1080-EGFP cell lines were transfected with siRNA against EGFP gene using the LCP gene vector. At 48 h after transfection, the change in EGFP signal was observed under a fluorescence microscope. The fluorescence intensity (RLU) of non-transfected cells was set as 100%. The fluorescence intensity after siRNA-transfection was negatively correlated with the siRNA-transfection efficiency.

The EGFP intensity in the HeLa-EGFP cells was extremely strong. After the HeLa-EGFP cells were transfected with siRNA using Lipofectamine 2000 or LCP, the fluorescence intensity became weaker than that of the untransfected cells. The fluorescence signal in the cells transfected with siRNA using LCP was the weakest, with a fluorescence inhibition rate of about 72%, while the inhibition rate of Lipofectamine 2000 was about 67%. The cationic polymer Cytopure, which worked well in transfection with plasmid, almost had no effect in siRNA-transfection (Fig. 4). The change of inhabitation rate in HT1080 cells was very similar to that in HeLa-EGFP cells. The inhibition rate was about 73% in the siRNA-transfection of HT1080-EGFP cells using LCP. While the inhibition rate of Lipofectamine 2000 was about 53%. Cytopure also almost had no effect in siRNA-transfection (data not shown). The siRNA-transfection efficiency of LCP was the highest across the samples, and it could strongly suppress the expression of the target gene.

ANTI-NUCLEASE ABILITY OF LCP/DNA COMPLEXES

After being digested with nuclease I at 37°C for 90 min, the LCP/siRNA complex was used for transfection. The fluorescence intensities of HT1080-EGFP and HeLa-EGFP were set at 100%, and the inhibition rates of the fluorescence in HT1080-EGFP and HeLa-EGFP cells were 71% and 72%, respectively, which was equivalent to that of undigested LCP/siRNA complex. There was no significant difference (Fig. 5), indicating that LCP could protect siRNA from digestion of nuclease I through binding with siRNA.

CELLULAR CYTOTOXIC ANALYSIS OF LCP/siRNA GENE VECTOR

MTT assay was performed to investigate the survival rate of HeLa-EGFP cells after transfection for 48 h. The untransfected cells were taken as the blank control (survival rate = 100%). The survival rate of cells transfected with LCP was about 95% and those with Lipofectamine 2000 was about 65% (Fig. 6), indicating that the LCP gene vector had an extremely low cellular cytotoxicity in comparison with Lipofectamine 2000.

DEGRADATION ANALYSIS

The fluorescence intensity of the dissociative oligonucleotide was set as 100%. After binding with LCP gene vector the fluorescence intensity of FITC-labeled oligonucleotides dramatically decreased due to steric hindrance; so the inhabitation of fluorescence of LCP was high before degradation. Once the LCP gene vector was

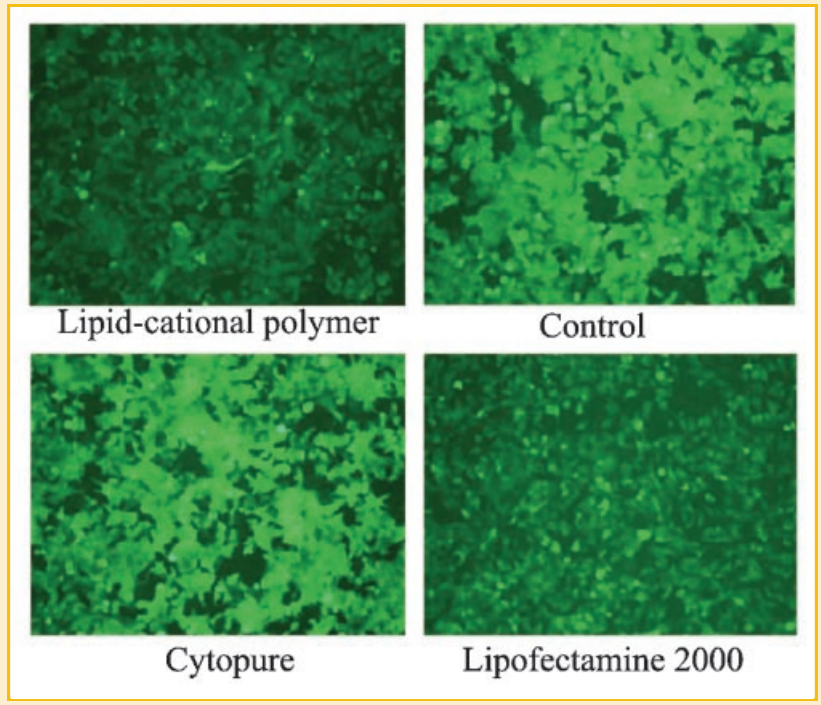

Fig. 4. Representative pictures of siRNA transfection of HeLa-EGFP using different gene vectors. The fluorescence intensity of the EGFP was observed under a fluorescence microscope and quantified with a microplate reader. This experiment was performed in triplicate. The fluorescent signal in the cells transfected with siRNA using LCP was the weakest compared with the control, with a fluorescence inhibition rate of about 72%, while the inhibition rate of Lipofectamine 2000 was about 67%. Cytopure had no effect in siRNA-transfection. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

degraded, the FITC-labeled oligonucleotides were released and the fluorescence intensity gradually increased, therefore the inhibition rate of fluorescence decreased. The fluorescence intensity decreased after polymer binding with oligonucleotide, reaching 65% in the LCP before degradation or sample binding with PEI25K. The fluorescence inhibition rate of PEI600 was only about 15% due to its weak ability to bind with the nucleotide. The fluorescence inhibition rate of PEI25K before and after heat preservation had no significant difference due to its weak degradability. The ability of LCP to inhibit fluorescence began to decline at 4 h and was at the same or even lower level with PEI600 at 8 h, indicating that LCP was

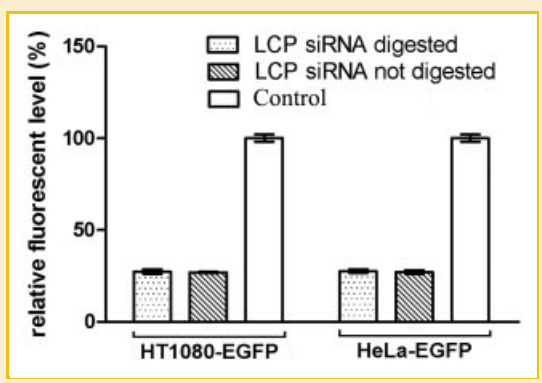

Fig. 5. Comparison of transfection efficiency of LCP/siRNA complex before and after being digested with nuclease I. There was no significant difference on the fluorescence inhibition of cells transfected with the LCP/siRNA or the nuclease I-digested LCP/siRNA, indicating that nuclease I had no effect on the LCP/siRNA complex and that LCP could protect siRNA from the degradation of nuclease I. The experiment was independently performed three times ($P < 0.05$). Control: group untransfected with LCP/siRNA.

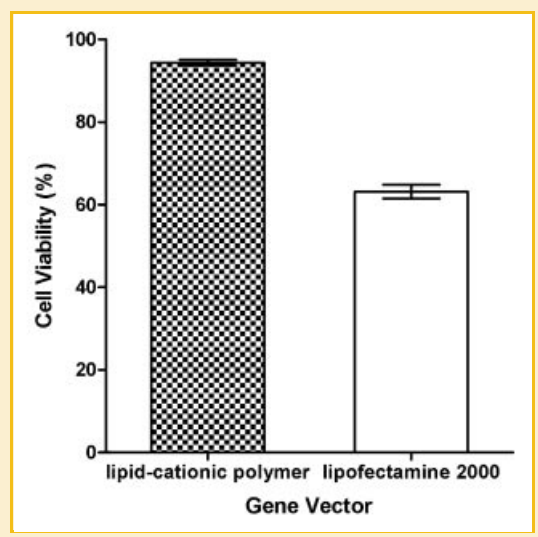

Fig. 6. HeLa-EGFP cell viability using different gene carriers. The survival rate of cells transfected with LCP (95%) was markedly higher than that of Lipofectamine 2000 (65%), indicating that the LCP gene vector had an extremely low cellular cytotoxicity in comparison with Lipofectamine 2000. The experiment was independently performed three times ($P < 0.05$).

almost degraded and the dissociative oligonucleotide was released (Fig. 7).

STABILITY ANALYSIS

HT1080-EGFP cells were transfected with siRNA against EGFP using LCP stored for a variable period at 4°C. The fluorescence intensity of the transfected cells changed in comparison with that of the untransfected cells (100%). After being preserved at 4°C for 14 months, the transfection efficiency of the LCP gene vectors had no significant changes (Fig. 8), indicating that it was stable at during storage. Thus, the shelf life of the product was over 1 year at 4°C.

ANTI-SERUM CHARACTERISTIC OF LCP GENE VECTOR

A traditional genetic protocol (serum-free, replacement of culture medium) and a modified procedure (no replacement of culture medium and with serum) were performed to transfect HeLa-EGFP cells with siRNA against EGFP using LCP gene vector. At 48 h after transfection, the inhibition rate of EGFP was 72% and 73% for the traditional and modified procedure, respectively (Fig. 9). Since there was also no significant difference, the LCP gene vector was confirmed to be compatible with the serum.

DISCUSSION

MECHANISM OF TRANSFECTION WITH siRNA USING LCP

At present, most gene vectors for siRNA are lipid-cationic. Of these, Lipofectamine 2000 (Invitrogen) is the most common multi-functional gene vector [Clements et al., 2007; Zhang et al., 2009]. However, lipid-cationic vectors are not compatible with serum. The transfection protocol is complicated and it may induce intense anti-inflammatory responses and lead to high toxicity. These deficiencies limit the application of lipid-cationic [Son et al., 2000; Wyatt and Giorgio, 2004; Hirashima et al., 2007; Zhong et al., 2008]. A cationic polymer is currently the main product used due to its high transfection efficiency, low cytotoxicity, and better serum-compatibility [Masago et al., 2007; Gao et al., 2009; Wilschut et al., 2009]. However, the transfection efficiency of cationic polymers with siRNA is not ideal. In comparison with the structures of our designed LCP, the disadvantages of the lipid-cationic vector and cationic polymer are as follows: (i) the cationic polymer had low affinity to the cell membrane; (ii) siRNA could not be detached from the cationic polymer/siRNA complex because of the high binding capacity of cationic polymer with siRNA.

The basic framework of LCP is similar to a cationic polymer, and brassidic acid was crosslinked on the side chain to give combined

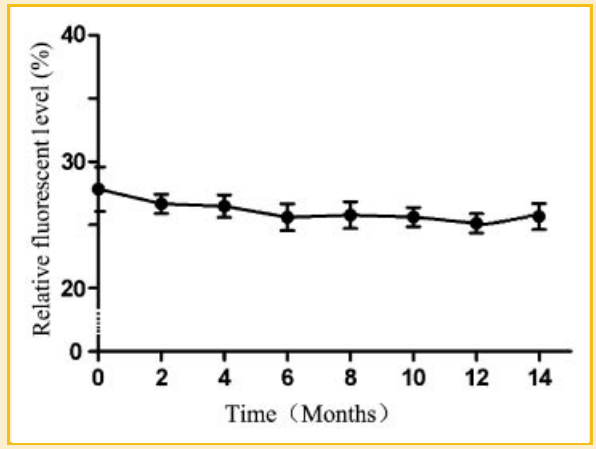

Fig. 8. The effect of LCP stored at 4°C for different intervals on fluorescence intensity of the transfected HT1080-EGFP cells (x-axis represented the hold time at 4°C, y-axis represented the relative fluorescence intensity of HT1080-EGFP cells transfected with siRNA against EGFP using LCP. After transfection, the relative fluorescence intensity remained unchanged, indicating that the LCP vector was stable.).

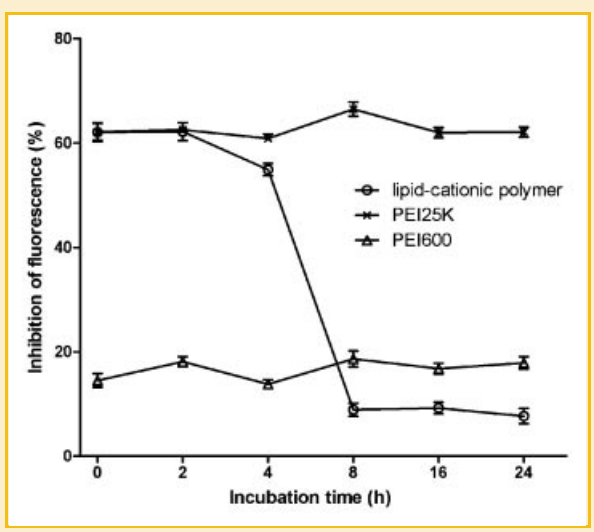

Fig. 7. Fluorescence inhibition rates of different samples. The fluorescence inhibition rate of LCP began to decline at 4 h and was at the same or even lower level with PEI600 at 8 h, indicating that LCP was almost degraded and the dissociative oligonucleotide was released. All procedures were independently performed three times ($P < 0.05$).

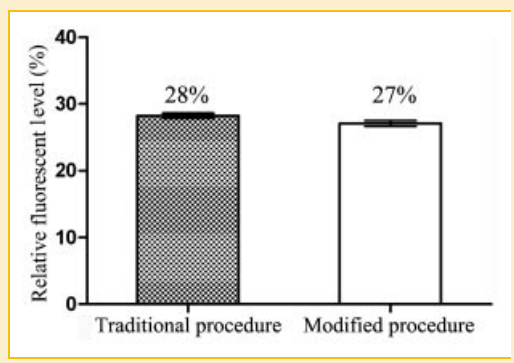

Fig. 9. Comparison of EGFP fluorescence intensity in HeLa-EGFP cells transfected using LCP gene vectors with different transfection protocols. At 48 h after transfection, the relative fluorescence intensity of EGFP was 28% and 27% for the traditional and modified procedure, respectively, indicating there was no significant difference and the LCP gene vector was compatible with the serum. This experiment was independently performed three times ($P < 0.05$).

characteristics of cationic polymers and lipid-cationic vectors. The mechanism of LCP transporting siRNA might be as follows: (i) LCP formed a complex with siRNA. The surface of LCP has high positive potential, while siRNA is negatively charged, so a complex can be formed through electrostatic interactions; (ii) interaction between LCP/siRNA and cells—the LCP/siRNA complex could be absorbed through the negative-charged cellular membrane, and the siRNA could be transported into the cells through membrane fusion, endocytosis, or osmosis. siRNA was then released from the endosome into the cytoplasm before finally reaching the nucleus; (iii) release of siRNA—the binding capacity of LCP was weakened after modification with brassidic acid. Moreover, the complex was degraded after being transported into the cells for 8–24 h. All these characteristics enabled the release of siRNA.

PHYSICAL PROPERTIES OF LCP/siRNA

The diameter of LCP/siRNA complex was affected by the electric charge proportion between LCP and siRNA. The compound diameter gradually decreased along with the enhanced ratio of LCP gene vector. When the mass ratio was equal to or over 4:1, LCP could thoroughly compress siRNA and form complexes with diameters of 120 nm. The existing research indicated that the complex with diameters of 100–200 nm could easily enter the cells by cytophagy [Midoux and Monsigny, 1999]. This might be one of the reasons for the high transfection efficiency of the LCP/siRNA complex.

BUFFER CAPACITY AND ANTI-NUCLEASE ABILITY OF LCP

During the delivery of siRNA from the external environment to the nucleus, the LCP/siRNA went through variable acidic conditions at a pH range of 5.0–7.3. The LCP/siRNA complex had a strong buffer capacity when it was placed in various acidic conditions (pH 4.0–8.0) and, as such, could protect siRNA from damage. This might be one of the key factors for the high transfection efficiency of LCP.

Nuclease I is the main nuclease in the cytoplasm and blood plasma, so success of gene vectors depends on whether they could protect siRNA from the degradation of nuclease I. It was also a key factor affecting transfection efficiency. When the mass ratio of LCP to siRNA ranged from 4:1 to 32:1, the fluorescence intensity in HT1080-EGFP and HeLa-EGFP cells decreased by 71% and 69%, respectively by the LCP/siRNA complex which was digested with nuclease I for 90 min, indicating that the LCP/siRNA complex could effectively protect siRNA from the degradation of nuclease I and maintain the integrity of the structure and function of siRNA.

CELLULAR TRANSFECTION CHARACTERISTICS

LCP, a novel compound, overcame both the poor biocompatibility and high toxicity of the lipid-cationic vector. It facilitated the biological function of siRNA through enhancing lipid solubility and promoting the release of siRNA in target cells.

Strong nucleic acid binding capacity is the requisite condition for gene vectors. Our research showed that the electrophoresis velocity of plasmid DNA was obviously delayed when the mass ratio of LCP to DNA was 0.5, indicating that the polymer had extreme nucleic acid binding capacity. siRNA against EGFP was transfected into HeLa-EGFP and HT1080-EGFP cells using LCP. The inhibition rate of fluorescence intensity was 72% and 73%, while in the cells transfected with siRNA using Lipofectamine 2000, the inhibition rate was 67% and 53%, respectively. Thus, LCP had good siRNA transfection efficiency. The cationic polymer Cytopure, which fit well for plasmid transfection, almost had no effect on siRNA transfection.

The cytotoxicity of gene vectors was another important parameter. Vectors with high cytotoxicity can cause the death of transfected cells. We compared the cellular viability of HeLa-EGFP and TH1080-EGFP cells transfected using different gene vectors at their highest transfection efficiency. The cellular viability in LCP-transfected cells was 95%, indicating low cytotoxicity. The cellular viability of TH1080-EGFP and HeLa-EGFP cells transfected with Lipofectamine 2000 was about 65%, presenting higher cytotoxicity of Lipofectamine 2000. The high viability in LCP-transfected cells might be related to the degradation of LCP in cells. The degradability of LCP both promoted the release of siRNA and decreased its accumulation and toxicity.

The commonly used lipid-cationic vector was easily eliminated by serum and could only be used in serum-free medium. The transfection procedure was also very complicated. It was not suitable for in vivo application due to its poor biocompatibility. Data in present study showed that LCP had better serum-compatibility, which simplified the transfection procedure and provided the basis for in vivo application of LCP.

In summary, the nano-scale LCP synthesized in this article for siRNA transfection had high transfection efficiency, degradability, low cytotoxicity, good serum compatibility, and easy storage. This study could be very valuable for further scientific research and medical applications.

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