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Influence of lipid components on gene delivery by polycation liposomes: Transfection efficiency, intracellular kinetics and *in vivo* tumor inhibition

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ABSTRACT

Transfection efficiency of non-viral gene vectors is influenced by many factors, including chemical makeup, cellular uptake pathway and intracellular delivery. To investigate the effect of lipid saturation on transfection efficiency of polycation liposomes (PCLs), a soybean phospholipids (SPL), egg phospholipids (EPL) and hydrogenated soybean phosphatidylcholine (HSPC) series was used to prepare PCLs. Testing these PCLs in a luciferase assay indicated that with increasing saturation (SPL < EPL < HSPC), the ability to confer gene expression decreased. The effect of protamine combined with these PCLs was also studied in different cell lines. Improved transfection because of protamine incorporation was dependent on lipid saturation and on the cell line tested. The kinetics of cellular uptake and intracellular distribution was studied using flow cytometry and laser scanning confocal microscope, which showed that naked oligonucleotide (ODN) and PCLs/ODN complexes became equilibrium after 4 h incubation. PCLs containing SPL (PCLs-S) and 1,2-dieleoyl-*sn*-glycero-3-phosphoethanolamine (PCLs-D) increased uptake rates by 2.20- and 5.45-fold, respectively. Furthermore, pCMV-IL-12 transfection mediated by PCLs-D showed excellent tumor inhibition efficiency compared with control and naked pCMV-IL-12 treatments *in vivo*.

1. Introduction

Liposomes are nanosized vesicles consisting of an inner aqueous core surrounded by a lipid bilayer that have been successfully used for over two decades in treatment of various cancers and fungal infections (Schwendener, 2007). They have several advantages over other gene carriers, including low immune response, straightforward preparation and relatively high transfection efficiency. Recently, they have been widely applied as gene delivery systems in laboratory settings and in clinical trials (Karmali and Chaudhuri, 2007; Schroeder et al., 2010). However, several barriers exist that prevent genetic material from the intended targets in the cytoplasm or nucleus (Kamiya et al., 2001; Wang et al., 2010), which affected the final therapy efficiency. Thus, understanding intracellular dynamics affecting intracellular transport of genetic materials is important for improvement of liposomes as gene vectors.

Some studies on pharmacokinetics of gene delivery, including systemic and intracellular pharmacokinetics, have been reported (Forsha et al., 2010; Kamiya et al., 2003; Parra-Guillen et al., 2010). It is clear that intracellular delivery of genes is a multi-step process, the endosomal membrane and the nuclear membrane are the two main barriers. Li (Li and Huang, 2008) and Pichon (Pichon et al., 2010) previously summarized the mechanisms of uptake and intracellular trafficking of nanoparticles, indicating that the chemical and physical properties of nanoparticles are important factors in determining pharmacokinetics and biodistribution. For liposomes, lipid components are very important for their physicochemical properties, even they could determine the internalization ability, endosomal ability and transfection efficiency of liposomes (Torchilin, 2005). Positively charged lipid derivatives could condense DNA efficiently and easy to internalization; incorporation of some fusogenic lipids could destabilize cell or endosomal membrane, especially 1,2-dieleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) which could transit from the lamellar lipoplex phase into a non-lamellar structure under acidic pH and enhance the endosomal escape of liposomes; cholesterol could change the phase transition temperature of lipid bilayer resulting to change of liposomes stability.

In previous work, we successfully developed polycation liposomes (PCLs) (Chen et al., 2007, 2009) and polycation liposomes/protamine/DNA complexes (Chen et al., 2011) as novel gene transfection vectors to overcome intracellular barriers and achieve

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high transfection efficiency. In this study, we investigated the effect of different lipid components, including lipids with varying levels of saturation and the fusogenic lipid DOPE, on transfection efficiency, kinetics of intracellular uptake, and tumor growth inhibition *in vivo*.

2. Materials and methods

2.1. Materials

DOPE, cholesterol (Chol) and protamine sulfate salt from Salmon were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Soybean phospholipids (SPL), egg phospholipids (EPL) and hydrogenated soybean phosphatidylcholine (HSPC) were purchased from Lipoid GmbH (Ludwigshafen, Germany). Polyethylenimine–cholesterol (PEI 800-Chol) was synthesized as previously reported (Chen et al., 2007). Doxorubicin hydrochloride (DOX) was kindly provided by Hisun Pharmaceutical Co. Ltd. (Taizhou, Zhejiang, China). The luciferase assay system was purchased from Promega (Madison, WI, USA). The bicinchoninic acid (BCA) protein assay kit was purchased from the Beyotime Institute of Biotechnology (Haimen, Jiangsu, China). Phosphorothioate oligonucleotide (ODN) with a 15-mer random sequence was synthesized by the Shanghai Sangon Bio-engineering Technology Company (Shanghai, China) and the 5′-end was labeled with FAM. Dulbecco's Modified Eagle's Medium (DMEM), trypsin and fetal bovine serum (FBS) were purchased from Gibco BRL (Gaithersberg, MD, USA). All other chemical reagents were of analytical grade and obtained from Sinopharm Chemical Reagent Company (Shanghai, China).

2.2. Cell lines and animals

Human lung adenocarcinoma cells (A549) and human breast adenocarcinoma cells (MCF-7) were kindly provided by Sir Run Run Shaw Hospital, Zhejiang University (Hangzhou, Zhejiang, China). Human cervical adenocarcinoma cells (HeLa) and mouse sarcoma S180 cells (S180) were obtained from Institutes of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences (Shanghai, China). The cells were cultured in DMEM medium containing 10% FBS at 37 °C and 5% CO_2.

Female ICR mice (4–6 weeks old) were obtained from the Laboratory Animals Central of Zhejiang University and were maintained under a 12 h light/12 h dark photoperiod for *in vivo* tumor inhibition studies.

2.3. Plasmid DNA

The pGL3-promoter plasmid was kindly provided by the Institute of Biochemical Pharmaceutics, Zhejiang University (Hangzhou, Zhejiang, China). The pCMV-IL-12 plasmid was kindly provided by Osaka University (Osaka, Japan). All the plasmids were propagated and purified by Biocolor BioScience & Technology Company (Shanghai, China).

2.4. Preparation of PCLs and PCLs/protamine/DNA complexes

PCLs containing SPL (PCLs-S) were prepared using a previously reported film dispersion method (Chen et al., 2007). Briefly, a lipid mixture of SPL, Chol and PEI 800-Chol (50:45:5 molar ratio) in chloroform was dried under vacuum to form a lipid film, and then hydrated using a 5% glucose solution. The PCLs-S were then extruded through a 0.22 μm filter after probe ultrasonication. PCLs containing EPL (PCLs-E, with EPL:Chol:PEI 800-Chol of 50:45:5), HSPC (PCLs-H, with HSPC:Chol:PEI 800-Chol of 50:45:5) or DOPE (PCLs-D, with DOPE:PEI 800-Chol, 50:50) were prepared using the same method.

PCLs/DNA complexes were prepared by mixing equal volumes of PCLs and plasmid DNA at various N/P ratios (1 mol PEI 800-Chol nitrogen per mol DNA phosphate), and incubated for 30 min at room temperature.

For preparation of PCLs/protamine/DNA complexes, protamine solution was added to an equal volume of DNA solution (protamine:DNA mass ratio = 1:1) to form nanoparticles, and then the nanoparticles and PCLs were mixed and incubated for 30 min at room temperature.

2.5. In vitro transfection

For transfection, A549 cells, HeLa cells or MCF-7 cells (1×10^5 cells/well) were seed in 24-well plate and incubated in DMEM medium with 10% FBS for 24 h before transfection. After the medium was removed and replaced with DMEM medium without FBS, various PCLs/DNA complexes (1 μg DNA/well) or PCLs/protamine/DNA complexes were added to cells for 6 h. Thereafter, the medium was replaced with DMEM containing 10% FBS, and the cells were incubated for another 18 h at 37 °C with 5% CO_2. The transfection efficiency was evaluated by luciferase assay.

2.6. Luciferase assay

Following transfection, cells were washed with phosphate buffered saline (PBS) and 200 μL of lysis buffer was added to each well. Cells were harvested, transferred to eppendorf tubes and centrifuged ($12{,}000 \times g$) for 2 min at 4 °C. Supernatants were stored in fresh tubes at −80 °C until use. Luciferase activity was measured in terms of relative light units (RLU) using a Luminometer (20/20; Turner). Protein concentrations in corresponding supernatants were determined using a BCA protein assay kit. The final transfection efficiency is reported in terms of RLU/mg protein.

2.7. Cellular uptake kinetics of PCLs

A549 cells were seeded at 2×10^5 cells/well in a 24-well plate and incubated at 37 °C with 5% CO_2 overnight. ODN or PCLs/ODN complexes (ODN 1 μg/well) were added after transferring cells to serum-free DMEM. At various times (0, 5, 15, 30, 60, 120, 240, 360 min), cells were collected, washed with PBS and fixed with 4% (w/v) paraformaldehyde. Flow cytometry (FACSCAL, BD, USA) was used to analyze cellular uptake kinetics.

2.8. Confocal microscopy of intracellular trafficking of PCLs

A549 cells were seeded into 24-well plates at 1×10^5 cells/well and grown for 24 h before treatment in DMEM containing 10% FBS. Before treatment, the medium was replaced with serum-free DMEM, and PCLs/ODN complexes (PCLs labeled with rhodamine) were then added to the cells. At specific intervals (0.5, 1, 2, 4, and 6 h), medium was removed and the cells were washed three times with PBS and fixed for 20 min in 4% (w/v) paraformaldehyde. Following three more PBS washes, nuclei were stained by incubating cells with Hochest 33342 (2 mg/mL) for 10 min. After a further PBS wash, the cells were permeabilized with 0.5% (v/v) Triton X-100. The intracellular distribution of FAM-ODN was analyzed using a laser scanning confocal microscope (LSM510 META; Carl Zeiss, Germany).

2.9. Tumor growth inhibition study

Female ICR mice (4–6 weeks old) were subcutaneously injected in the right forelimb armpit with S180 cells (1×10^7 cells/mL, 0.1 mL/mouse) suspended in PBS. After 7 days, mice bearing S180 tumors of approximately 100 mm^3 were randomized into

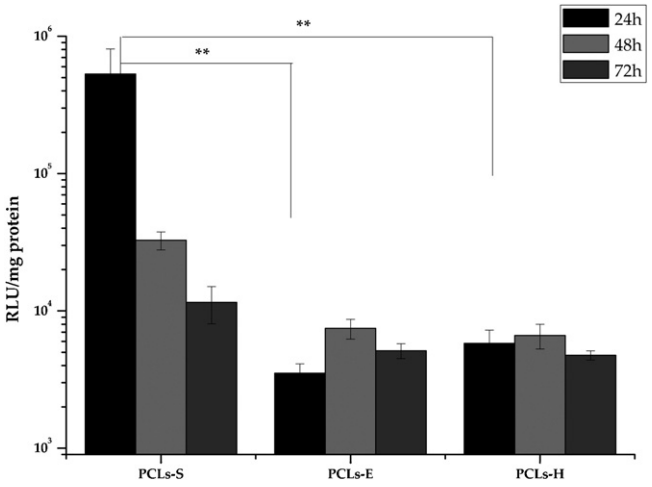


Fig. 1. The transfection ability of PCLs with different lipid saturations in A549 cells at different transfection times. The N/P ratio of PCLs and DNA was 10. The DNA dosage was 1.0 μg. PCLs-S, SPL/Chol/PEI 800-Chol liposomes; PCLs-E, EPL/Chol/PEI 800-Chol liposomes; PCLs-H, HSPC/Chol/PEI 800-Chol liposomes. Error bars represent standard deviation, $n = 3$. $^{**}p < 0.01$.

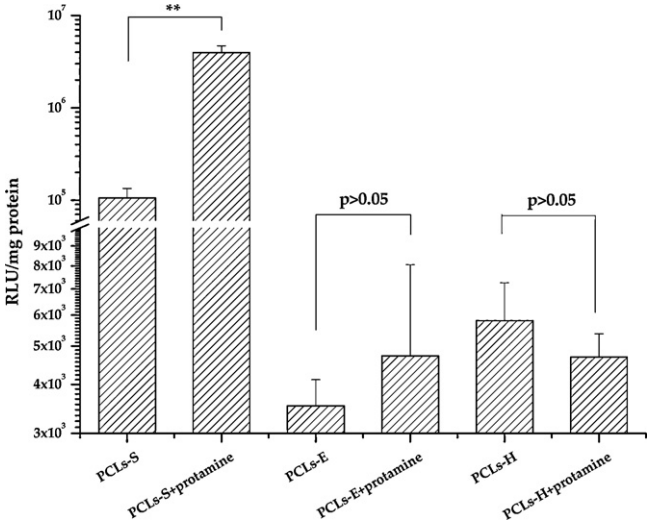


Fig. 2. The effect of protamine in transfection of PCLs with different lipid saturations after 24 h transfection (A549 cells). The DNA dosage was 1.0 μg. The mass ratio of protamine and DNA was 1, and the N/P ratio of PCLs and DNA was 10. Error bars represent standard deviation, $n = 3$. $^{**}p < 0.01$.

eight groups to receive an intratumoral injection (100 μL/mouse, five times every other day) with PBS, naked pCMV-IL-12 (5 μg, 10 μg and 30 μg/mouse), DOX (5 mg/kg), or PCLs-D alone or PCLs-D/pCMV-IL-12 complexes (5 μg and 10 μg/mouse, pCMV-IL-12). The mice were weighed and their tumor volume measured every other day after the first drug administration. Tumor volume was calculated according to the formula: $V = 0.5 \times ab^2$, where a is the maximum and b is the minimum diameter of the tumor.

2.10. Statistical analyses

Statistical analyses were performed using Student's t-test.

3. Results

3.1. Influence of lipid saturation on transfection efficiency of PCLs

The transfection efficiency of PCLs containing three lipids with varying degrees of saturation was evaluated in A549 cells. The results indicated that the lipid component would influence the final transfection ability of the PCLs (Fig. 1). We found that as lipid saturation increased, transfection efficiency decreased. As shown in Fig. 1, gene expression with PCLs-S (least saturated) was approximately 150-fold higher than that with PCLs-E (highly saturated) and approximately 90-fold higher than PCLs-H (fully saturated). However, no significant difference was observed between PCLs-E and PCLs-H ($p > 0.05$). As the transfection time increased, the transfection efficiency of PCLs-S clearly decreased. The transfection efficiency of PCLs-S at 72 h was only about 2% of that of PCLs-S at 24 h, but was still greater than the other two PCLs tested.

3.2. The effect of protamine in transfection

The effect of protamine on transfection of PCLs with different lipid saturations, PCLs-S, PCLs-E and PCLs-H, was then investigated. The results showed protamine effectively increased transfection efficiency when combined with PCLs-S, (approximately 38-fold increase), but had little effect when combined with PCLs-E or PCLs-H (Fig. 2).

PCLs-S and PCLs-S combined with protamine were then used to transfect A549, HeLa and MCF-7 cells. The results showed that the transfection efficiencies with the same carrier were different in these three cell lines. The levels of gene expression in A549 and HeLa cells transfected by PCLs-S were higher than that in MCF-7 cells (Fig. 3). The transfection efficiency in all three cell lines was enhanced when PCLs-S were combined with protamine. The largest improvement was observed in A549 and MCF-7 cells ($p < 0.01$), while the enhancement was lower in HeLa cells ($p > 0.05$). These results indicate that both transfection efficiency and the effect of protamine vary depending on the target cell line.

3.3. The cellular uptake kinetics of PCLs

To investigate the uptake kinetics of PCLs, naked ODN and PCLs/ODN complexes were transfected into A549 cells and the intracellular fluorescence intensity at different time points was determined using flow cytometry. The total fluorescence intensity over time was then plotted (Fig. 4). As expected, the intracellular fluorescence intensity increased over time, and reached equilibrium after 4 h incubation. The intracellular levels of ODN transfected using PCLs were obviously greater than for naked ODN

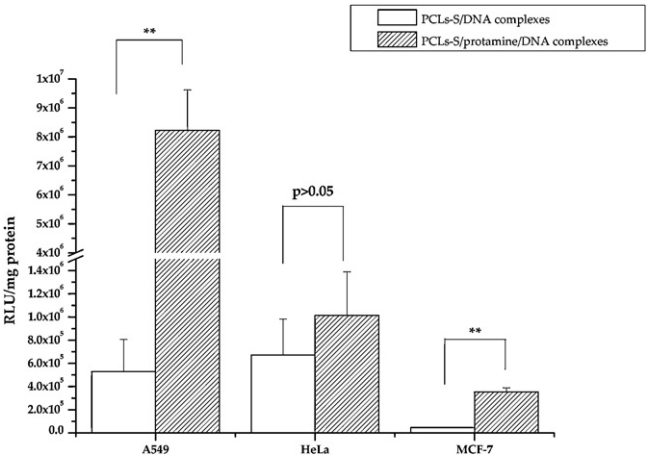


Fig. 3. The effect of protamine in transfection of PCLs-S against different cell lines after 24 h transfection (A549, HeLa and MCF-7 cells). The DNA dosage was 1.0 μg. The mass ratio of protamine and DNA was 1, and the N/P ratio of PCLs and DNA was 10. Error bars represent standard deviation, $n = 3$. $^{**}p < 0.01$.

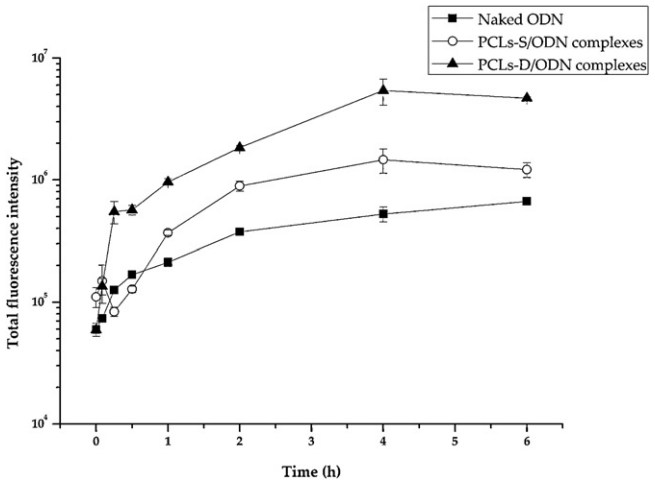
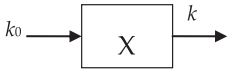


Fig. 4. Kinetics of uptake of naked ODN, PCLs-S/ODN complexes and PCLs-D/ODN complexes in A549 cells.

over an equivalent incubation time. PCLs-D in particular showed the greatest ability to enhance internalization.

The concentration of ODN in DMEM medium was much higher than the intracellular concentration, thus we hypothesized that cells uptake ODN at a constant rate, k_0. At the same time, intracellular ODN was eliminated via a first-order process (k). The kinetic model for cellular uptake is shown below:



As the incubation time increases, the ODN elimination rate and intracellular amount of ODN (represented by total fluorescence intensity) also increase proportionally.

$$\frac{dX}{dt} = k_0 - kX$$

The final equation was obtained by Laplace transform:

$$X = \frac{k_0}{k}(1 - e^{-kt})$$

This equation represented the relationship between intracellular amount of ODN (X) and incubation time (t). Origin® software was used to fit a nonlinear curve to calculate k_0 and k (Table 1). The uptake rates of the PCLs-S/ODN and PCLs-D/ODN complexes were 2.20 and 5.45-fold higher than that of naked ODN, respectively. On the other hand, the elimination rate of the PCLs-S/ODN complex was similar to that of naked ODN, which was about two-fold greater than that of the PCLs-D/ODN complex.

3.4. Confocal microscopic imaging of intracellular trafficking of PCLs

Rhodamine labeled PCLs (red fluorescence) containing FAM-ODN (green fluorescence) were prepared for an intracellular trafficking investigation using a laser scanning confocal microscope. Our previous study found that cell uptake of free FAM-ODN was immediate, and green fluorescence was obvious after 0.5 h of incubation of the naked FAM-ODN with cells in this study (data not shown). The two PCLs complexes showed different rates and extents of internalization (Fig. 5). Little red fluorescence was observed during the 2 h incubation with PCLs-S. For PCLs-D, intense fluorescence was observed within 30 min of transfection, which indicates that the PCLs-D were easily internalized by the cells. No visible red fluorescence was observed inside the nucleus, which suggests that the PCLs do not enter the nucleus. More FAM-ODN seemed to enter the nucleus when transported by PCLs-D compared to PCLs-S. However, notable levels of FAM-ODN remained in the cytoplasm bound to PCLs (yellow fluorescence).

Table 1
k_0 and k values for cellular uptake and elimination of naked ODN, PCLs-S/ODN complexes and PCLs-D/ODN complexes in A549 cells.

	k_0 (TFI/h)	k (h^{-1})	R^2
Naked ODN	2.77×10^5	0.39	0.9539
PCLs–S/ODN complexes	6.09×10^5	0.40	0.9144
PCLs–D/ODN complexes	1.51×10^6	0.20	0.9034

3.5. Tumor growth inhibition study

PCLs containing DOPE (PCLs-D) were next selected as a pCMV-IL-12 carrier for an *in vivo* tumor inhibition study because of their optimal internalization kinetics and transfection efficiency *in vitro*. In this model, the therapeutic effect was evaluated by monitoring xenografted tumor growth in an animal model after intratumoral injection of PCLs carrying pCMV-IL-12 or naked pCMV-IL-12.

When compared with the positive control group treated with DOX no tumor inhibition was observed in any of the three naked pCMV-IL-12 groups, even at the highest dosage tested (Fig. 6A). As shown in Fig. 6B, PCLs-D/pCMV-IL-12 complexes strongly inhibited tumor growth. At low dosage (5 μg), tumor growth slowed after 4 days administration, but tumors grew rapidly when administration ended. The tumor growth rate in the higher dosage group (10 μg) was significantly slower than that in PBS group or blank PCLs-D group, and was even slower than treatment with DOX. After 12 days of treatment, the relative mean tumor volumes of the 5 μg PCLs-D/pCMV-IL-12, 10 μg PCLs-D/pCMV-IL-12, and the DOX groups were reduced to 96.2%, 24.2% and 45.5%, respectively, compared with the PBS group.

4. Discussion

Non-viral vectors have attracted increasing attention as a relatively safe gene delivery system. However, transfection efficiency is a large obstacle to their widespread use. Endosomal escape and nuclear entry are thought to be the two main barriers for successful gene transfer (Parra-Guillen et al., 2010). Fusogenicity of lipids is thought to play an important role in cytoplasmic gene delivery. Liposomes that incorporate lipids in the reversed hexagonal phase (H_{II}), such as DOPE, are more fusogenic while those incorporating lipids in the lamellar phase (L_α) are more stable. A low phase transition temperature between these two phases indicates a lower energy barrier to formation of the fusogenic phase (Heyes et al., 2005).

Three lipids with varying levels of saturation were used to prepare PCLs and their resulting transfection efficiencies were determined. PCLs-S showed the highest gene expression compared with PCLs-E and PCLs-H (Fig. 1). The only difference between these three PCLs was the lipid composition. SPL and EPL were used in their natural form with low and high saturation, respectively, while the double bonds in alkyl chains of HSPC were completely hydrogenated, leading to full saturation. Reducing the number of double bonds in a lipid's alkyl chain increases the saturation and subsequently the L_α phase formation ability (Szule et al., 2002). Therefore, lower saturation lipids such as SPL possess a lower phase transition temperature, which results in greater flexibility and fluidity when these liposomes enter cells. A more rigid bilayer because of higher lipid saturation may lead to less DNA release from liposomes into the cytoplasm. Our results indicate that lipid saturation is a factor that affects transfection efficiency, with relatively lower

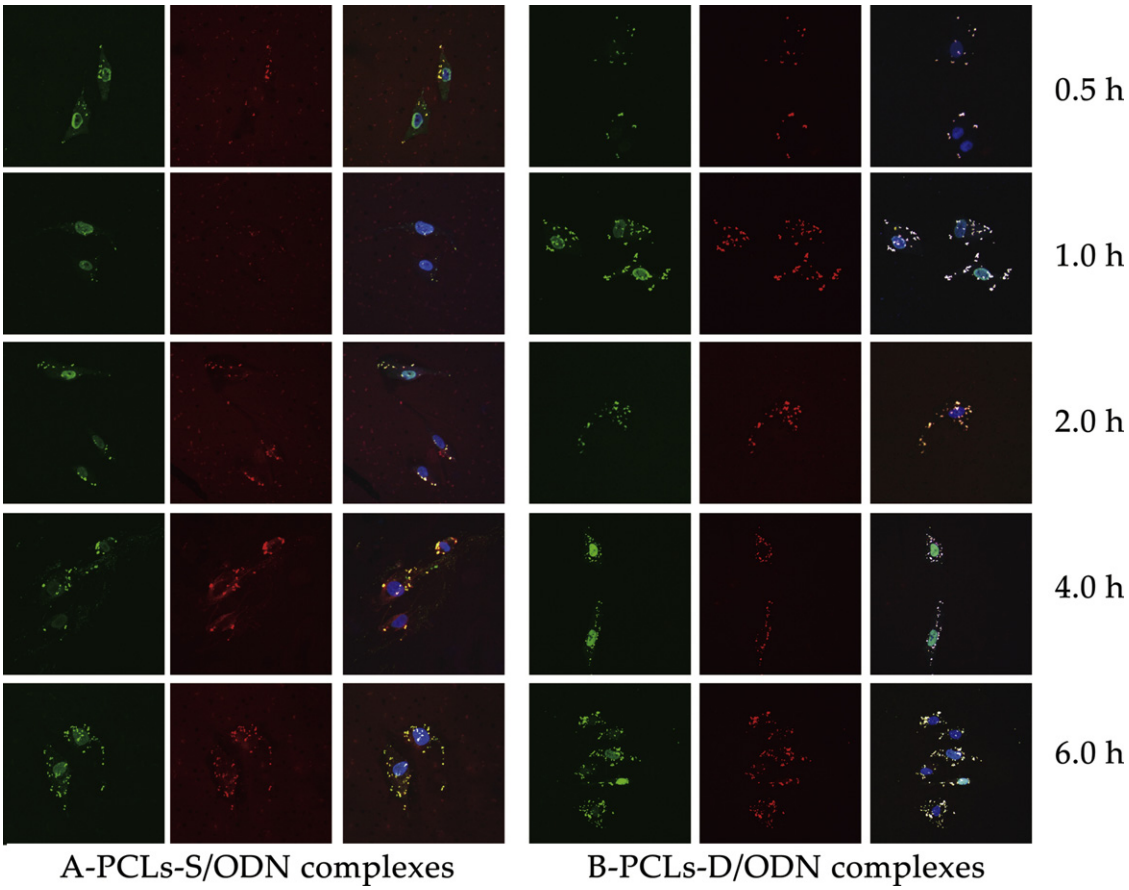


Fig. 5. Confocal microscopy of intracellular trafficking of PCLs-S/ODN and PCLs-D/ODN in A549 cells. ODN labeled by FAM showed green fluorescence, rhodamine labeled PCLs showed red fluorescence, and Hochest 33342 stained nuclei showed blue fluorescence. Red fluorescence was only observed in the cytoplasm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

saturation lipids being more suitable for gene transfer and higher saturation lipids being more stable (Nara et al., 1997).

PCLs combined with protamine have previously been shown to enhance the transfection efficiency of PCLs (Chen et al., 2011). Incorporation of protamine usually could improve the transfection efficiency of vectors because of it containing a nuclear localization signal, which directs the complex to the nucleus. In this study enhancement because of protamine was found to be dependent on the saturation of lipids in the liposomes. When combined with PCLs-S, protamine could improve the transfection ability to a large degree (approximately 38-fold), while little improvement was seen when protamine was combined with PCLs-E or PCLs-H. These findings also suggested that the mobility of PCLs in the cytoplasm is an important factor for the following nuclear entry. More protamine/DNA complexes transferred to the surrounding nucleus area, more DNA might enter into the nucleus by protamine

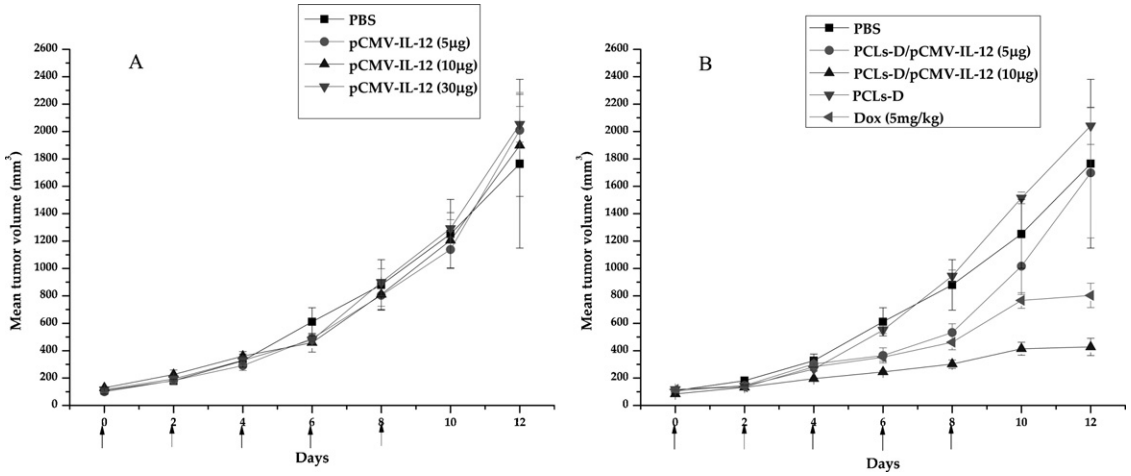


Fig. 6. *In vivo* gene therapy of established subcutaneous S180 tumors with different dosages of naked pCMV-IL-12 (A) and PCLs-D/pCMV-IL-12 complexes (B). PBS and PCLs-D were set as negative control groups, and DOX was set as the positive control group. The arrows show the injection days. Mean tumor volumes ± s.e. are shown for five mice per group.

to generate higher transfection efficiency. Here the mobility of PCLs was major determined by the lipid saturation of PCLs.

Transfection efficiency with PCLs also appeared to vary amongst different cell lines. Levels of gene expression in A549 and HeLa cells after transfection with PCLs-S were higher than in MCF-7 cells (Fig. 3). This phenomenon has also been observed in other reports. Douglas et al. suggested that cell physiology may affect the internalization pathways of non-viral vectors, leading to cell line-dependent transfection efficiency (Douglas et al., 2008). Interestingly, when combined with protamine, notable improvements in the transfection efficiency were found in A549 and MCF-7 cells, whereas little improvement was seen in HeLa cells (Fig. 3).

Mathematical modeling of intracellular processing of non-viral vectors represents a useful strategy for optimizing intracellular trafficking for gene delivery (Kamiya et al., 2003). A three-compartment model (milieu, endosome and cytoplasm) was first proposed by Ledley to describe the kinetics of internalized DNA (Ledley and Ledley, 1994). There are some mathematical models to characterize cellular uptake and intracellular processes; however, none of them could describe these processes precisely. Certain processes, such as degradation of endosomal or intracellular DNA, can be described by conventional first-order kinetics. A kinetic model was applied to quantitatively describe the uptake and elimination process of naked ODN and PCLs/ODN complexes in A549 cells. The calculated kinetic parameters suggested that the uptake rate of ODN can be increased by PCLs-S and PCLs-D (Table 1). Meanwhile, PCLs-D could slow the elimination rate of intracellular ODN to approximately half that of naked ODN or PCLs-S, resulting in retention of more ODN in the cell. Though it is a simple kinetic model, more precise description might need some other more complex models, these results were similar with that obtained by confocal microscopy (Fig. 5). These images showed that many FAM-ODNs remained in the cytoplasm and remained bound to PCLs during the entire transfection processes. DNA release from vectors to enter the nucleus has been proposed as an important step to achieve high transfection efficiency (Bertschinger et al., 2006). Therefore, nuclear targeting components could be incorporated into PCLs to target required gene transcripts to the nucleus. However, these PCLs may already be suitable for those genes whose therapeutic effect is produced in the cytoplasm, such as anti-ODN or siRNA.

Naked plasmid has been shown to be unstable *in vivo,* which is probably because of rapid degradation by nucleases. Thus, intratumoral injection of naked plasmid is still limited and somewhat controversial. In this study, direct intratumoral injection of naked pCMV-IL-12 failed to show any tumor inhibition (Fig. 6A), which is in good agreement with previous reports (Diez et al., 2009) (Suzuki et al., 2010). Given that PCLs-D increased the rate of gene internalization, slowed the elimination rate, and showed high transfection efficiency, pCMV-IL-12 transfected by PCLs-D may effectively suppress xenografted tumor growth. Experimental results showed this to be the case (Fig. 6B). Effective tumor growth suppression was observed and the inhibitory rate was about 75%, which could be compared to 50% following DOX treatment. Additionally, no decrease in the body weight of the mice was found during the whole therapeutic period (data not shown). These results suggest that PCLs-D may be a promising non-viral vector for gene delivery *in vivo*.

5. Conclusion

The results of this study suggest that lipid components can influence the transfection efficiency and the intracellular kinetics of PCLs. Relatively low saturation lipids are more suitable for gene transfer, and improvement of the transfection ability of protamine might be dependent on the saturation of lipids and cell lines. A kinetics model was applied to quantitatively describe the ODN uptake and elimination process. PCLs-D proved to be useful gene carriers for cancer therapy. These findings will be useful in the design of non-viral gene delivery systems.

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