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Pharmaceutical Nanotechnology

Spray-drying preparation of microparticles containing cationic PLGA nanospheres as gene carriers for avoiding aggregation of nanospheres

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Abstract

Preparation of nano-sized particles using lyophilization, which is a standard drying technique for high-molecular-weight compounds such as bioactive peptides, proteins, plasmid DNA and siRNA, often results in particle aggregation. In this study, spray-drying was applied for preparation of cationic PLGA nanospheres as gene delivery vectors in order to minimize aggregation and loss of gene transfection efficiency. PLGA nanoparticle emulsions were prepared by dropping an acetone/methanol mixture (2/1) containing PLGA and a cationic material, such as PEI, DOTMA, DC-Chol or CTAB, into distilled water with constant stirring. The PLGA nanosphere emulsion was dried with mannitol by spray-drying, and mannitol microparticles containing PLGA nanospheres were obtained. Mean particle diameter of spray dried PLGA particles was 100–250 nm, which was similar to that of the nano-emulsion before drying, whereas the lyophilized PLGA particles showed increased particle diameter due to particle aggregation. PEI, DOTMA and DC-Chol were useful for maintaining nanoparticle size and conferring positive charge to nanospheres. Transfection of pDNA (pCMV-Luc) using these spray-dried cationic PLGA nanospheres yielded high luciferase activity in COS-7 cells, particularly with PLGA/PEI nanospheres. The present spray-drying technique is able to provide cationic PLGA nanospheres, and may improve redispersal and handling properties.

Keywords: Non-viral vectors; Poly(D,L-lactic/glycolic acid) (PLGA); Nanospheres; Spray-drying; Polyethyleneimine (PEI); Mannitol microspheres

1. Introduction

Gene delivery systems for DNA, oligonucleotides and small interfering RNA (siRNA) have been widely investigated in basic research and in the pre-clinical/clinical gene therapy field. Gene delivery is limited by intracellular uptake and susceptibility to degradation following uptake by endocytosis, resulting in low gene expression efficiency. Various viral and non-viral vectors have been used to enhance transfection and gene expression efficiencies. Although retrovirus and adenovirus vectors are associated with efficient intracellular delivery and high gene expression, they are also associated with immunogenicity and toxicity, and a lack of tissue specificity. Non-viral vectors, such as cationic lipids, liposomes (Kawakami et al., 2000, 2002; Nishikawa et al., 2000; Simoes et al., 2003), cationic polymers (Kakizawa and Kataoka, 2002; Otsuka et al., 2003) and dendrimers (Arima et al., 2001; Kihara et al., 2002; Manunta et al., 2004), are advantageous for their low toxicity, low immunogenicity, ease and scale of production, low tissue-specific targeting and good handling properties. Although these non-viral vectors have been proposed as alternatives to viral vectors, their low transfection and gene expression efficiencies must be improved.

Upon mucosal DNA vaccination, induction of both humoral and cellular immune responses is essential. O'Hagan et al. reported that cationic PLGA microspheres containing CTAB are useful non-viral vectors for intranasal DNA vaccination (Briones et al., 2001; Singh et al., 2000, 2002; Vajdy and O'Hagan, 2001). These microparticles comprising encapsulated pDNA elicited good IgG and IgM responses, and a modest IgA response (Chen et al., 1998; Jones et al., 1997). Nanoparticles are useful for mucosal administration due to their increased accumulation of mucosal membranes on the surface, and they facilitate transcellular penetration of genes through the cell membrane, thus

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allowing efficient gene delivery (Akagi et al., 2003; Brooking et al., 2001; Hawley et al., 1997a,b; Kawashima et al., 2000). Therefore, development of nano-sized non-viral vectors such as nanoparticles is important for mucosal DNA vaccination.

A number of researchers have attempted to provide biodegradable nanoparticle vectors formulated with poly(D,L-lactic/glycolic acid) (PLGA). PLGA is a biocompatible and biodegradable polymer that can provide sustained release of encapsulated therapeutic agents, such as bioactive peptides, etc. (Hawley et al., 1997a,b; Kwon et al., 2001; Mathiowitz et al., 1997; Okada, 1997; Okada and Toguchi, 1995). Most PLGA nanoparticles are prepared by using a double emulsion-solvent evaporation technique and are finally collected by lyophilization. However, nanoparticles often form aggregates due to their large surface area and strong interparticle adhesion, and it is difficult to prevent this aggregation. Lyophilization often facilitates particle aggregation as the ice press adheres to individual particles. The aggregation of nano-sized particles causes problems such as poor redispersal, which results in low reproducibility and low gene delivery efficiency. Generally, solid preparations are highly desirable from the standpoint of preserving stability, and ease of handling and transportation.

Our objective is to establish a preparation method for nano-sized non-viral vectors possessing good reproducibility and high efficiency for mucosal membrane pDNA vaccination. Mannitol is already used for osmotic adjustment, as well as an excipient, in pharmaceutical preparations such as injection products, and would be a useful additive to disperse nanospheres without inducing aggregation. In the present study, a spray-drying preparation method to obtain PLGA nanospheres without aggregation was investigated and compared with a lyophilization method. We designed micro-sized mannitol particles in which cationic PLGA nanospheres were uniformly dispersed.

2. Materials and methods

2.1. Materials

Plasmid DNA (pCMV-Luc), which comprised a subcloned luciferase cDNA fragment at the Hind III and BamHI sites of pcDNA3.1, was amplified in *E. coli* (DH5α) and purified using an Endfree Plasmid Maxi kit (QIAGEN, USA), followed by ethanol precipitation and dilution in Tris/EDTA buffer. DNA concentration was measured based on UV absorption at 260 nm. A Label IT nucleic acid labeling kit (MURUS label IT® Cy3 labeling kit, TAKARA BIO Inc., Japan) was used for fluorescent labeling of pDNA. In addition, pEGFP-N1 (4.7 kb; BD Biosciences Clontech, USA), which codes for green fluorescence protein, was used for fluorescence microscope observation.

PLGA having a lactic/glycolic acid ratio of 75/25 and a molecular weight of 14,400, and mannitol were purchased from WAKO (Japan). Polyethylenimine (PEI, $(C_6H_{21}N_5)n$, Mw 750 kDa; Sigma–Aldrich Co., USA), trimethyl [2,3-(dioleyloxy) propyl]-ammonium chloride (DOTMA, $C_{42}H_{84}ClNO_2$, Mw 670.57; Tokyo Kasei Kogyo Co., Japan), cholesteryl 3β-*N*-(dimethylaminomethyl) carbamate (DC-Chol, $C_{32}H_{57}N_2O_2Cl$, Mw 537.27; Avanti Polar Lipid, USA) and cetyl trimethylammonium bromide (CTAB, $CH_8(CH_2)_{15}N(Br)(CH_8)_8$, Mw 364.45; WAKO, Japan) were used as cationic materials. Fugene 6® (Roche Diagnostics K.K., Japan) and Lipofectamine® (Invitrogen Japan K.K., Japan) were used as transfection reagents. The Luciferase Assay system (Promega Co., Ltd., USA) was used for determining luciferase activity.

Table 1
Operating conditions for spray-drying

Feed concentration (w/v%)	1.3
Inlet temperature (°C)	60.0
Outlet temperature (°C)	36.0
Feed rate (g/min)	5.0
Spray pressure (kgf/cm^2)	3.0
Drying air volume (m^3/h)	24.0

2.2. Preparation of cationic PLGA nanospheres

Cationic PLGA nanospheres were prepared by the oil-in-water emulsion solvent-evaporation method, as reported previously, with some modification (Horisawa et al., 2002). One hundred twenty milliliters of acetone/methanol mixture (2/1) containing 2 g of PLGA and cationic material (0.04–4 g of PEI, or 1 g of DOTMA, DC-Chol and CTAB) was dropped into 1000 mL of distilled water with constant stirring. After 5 to 25 g of mannitol (0.45–2.23%) was dissolved in this emulsion, spray-drying or freeze-drying was carried out using a spray dryer (Pulvis Mini-Spray GA32, YAMATO Co., Japan) or freeze dryer (FRD-82M, ASAHI TECHNO GLASS Co., Japan). PLGA nanospheres containing cationic materials and dispersed in mannitol microparticles were obtained by spray-drying. Table 1 shows the operating conditions for spray-drying.

2.3. Preparation of PLGA/PEI microspheres

The two phases, consisting of distilled water (25 mL) and 3 mL of methylene chloride/methanol (2/1) containing PLGA (4 g) and PEI (2 g), were emulsified using a homogenizer (Polytron PT3100, Kinematica AG, Switzerland) at 9000 rpm for 3 min. This emulsion was added to 250 mL of distilled water and homogenized. The resulting double emulsion was then stirred for 3 h to allow the evaporation of the organic solvent. After adding 4 g of mannitol, the PLGA/PEI microspheres, which were dispersed in the mannitol microparticles, were finally dried and collected by spray-drying.

2.4. Particle size and zeta-potential of PLGA nanospheres

The PLGA nanospheres in the mannitol microparticles were dispersed into distilled water. The particle size distribution of PLGA nanospheres was then analyzed by using a super dynamic light scattering spectrophotometer (DLS-7000, Otsuka Electronics Co., Ltd., Japan), and mean particle diameter (D_{50}) was defined as the median diameter on the cumulative curve of scattering intensity-based particle size distribution.

The zeta-potential of PLGA nanospheres was analyzed by laser zeta electrometer (LASER ZEE model 501, Pen Kem, Inc., USA).

2.5. Observation of PLGA nanospheres

The mannitol microparticles containing PLGA nanospheres were placed on a polycarbonate membrane filter (pore size, 0.2 μm), and were dissolved by dropping distilled water. The PLGA nanospheres were then observed using a scanning electron microscope (SEM, S-2250N, Hitachi Ltd., Japan).

2.6. Complex formation potential of cationic PLGA nanospheres and pDNA

pDNA-PLGA/PEI nanosphere complexes were prepared by combining 2 μg of pDNA and 20 mg of PLGA nanospheres in 500 μL of TE buffer for 12 h at 4 °C. The supernatant was collected after centrifugation at 30,000 rpm for 3 h and was subjected to agarose gel electrophoresis (1% agarose, TBE buffer, 100 V, 55 min, ethidium bromide staining for 15 min) in order to confirm complex formation. The pDNA in TE buffer and pDNA-PLGA nanosphere complexes without cationic materials were also analyzed as controls.

2.7. DNase I protection assay

Complexes were prepared by combining 25 μg of pDNA and 1.875 mg of PLGA/PEI nanospheres. pDNA alone (25 μg) and complexes were respectively incubated with 100 μL of aqueous solution containing 5 μL of DNase I and 25 μL of reaction buffer at 37 °C for 3 min. Following digestion, samples were analyzed by 1% agarose gel electrophoresis for DNA fragments.

2.8. Cell culture

COS-7 cells (African green monkey kidney epithelial-like cells) were maintained at 37 °C and 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS (GIBCO) and 1% penicillin/streptomycin (stock 10,000 U/mL, 10,000 μg/mL, GIBCO). Cells (2×10^5) suspended in 30 mL DMEM were then placed in a culture flask and were grown for experiments.

2.9. In vitro transfection study

Complexes were prepared by combining PLGA/PEI nanospheres and pDNA in serum-free Opti-MEM medium (GIBCO) at 4 °C for 12 h. The weight ratio ranged from 5/1 to 100/1. Control complexes of pDNA (2 μg) and optimal amounts of other transfection reagents (PEI 20 μg, Fugene 6® 6 μL, or Lipofectamine® 25 μL) were prepared under the same conditions.

COS-7 cells were seeded onto 6-well plates at a density of 2×10^5 cells per well (2 mL DMEM), and were transfected after 70–80% confluence was reached (∼24 h). Cells were washed twice with phosphate buffered saline (PBS). pDNA (2 μg) or complexes containing 2 μg of pDNA were added to each well with 2 mL of Opti-MEM medium. After 6 h incubation at 37 °C in a humidified 5% CO_2 atmosphere, cells were washed twice with PBS, 2 mL of fresh DMEM containing 10% FBS was added, and cells were incubated.

After 48 h, cells were washed three times with PBS, lysed by addition of 100 μL of lysis buffer per well, and left to stand for 15 min at room temperature. Cell lysates were then collected and centrifuged at 15,000 rpm for 3 min. After addition of 100 μL of luciferase substrate solution to 50 μL of cell lysate, luciferase activity was measured using a chemiluminescence instrument (LumiCounter 1000, MICROTECH). Protein quantification was performed on the same cell lysate using a standard BioRad protein assay kit (BioRad, Hercules, USA). A 5-μL aliquot of cell lysate diluted 160 times with ultrapure water was then reacted with 40 μL of dye reagent for 1 h at room temperature, and absorbance was measured at 595 nm using a microplate reader (SAFIRE, TECAN). The protein concentration of the cell lysate was calculated using a calibration curve for BSA standards (2 mg/mL). The results are shown as relative light units (RLU) per mg of protein.

2.10. Cellular uptake and gene expression of pEGFP-cationic PLGA nanosphere complex

COS-7 cells were seeded onto cover glasses at a density of 2×10^5 cells per 2 mL of DMEM, and were incubated for 24 h at 37 °C in a humidified 5% CO_2 atmosphere. To determine the size-dependent cationic PLGA particle uptake and gene expression, cells were incubated with Cy-3 labeled pEGFP complexed PLGA/PEI nanospheres and with PLGA/PEI microspheres in Opti-MEM medium for 30 h under the same conditions. Cells were washed twice with PBS at different time intervals and were then fixed on cover glasses with 2% paraformaldehyde for 30 min. Mounting medium was dropped after washing and drying cover glasses. pEGFP uptake and GFP expression efficiency were observed by fluorescence microscopy.

2.11. In vivo transfection study

Six- to eight-week-old Balb/c female mice were purchased from Japan SLC Inc. Mice were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) before all procedures.

The complex of PLGA/PEI nanospheres and pCMV-Luc (50 μg/100 μL) was injected intravenously. The heart, lung, liver, kidney and spleen were removed at 12 h after injection. Tissues were washed twice with cold PBS and weighed, and were then homogenized with 4 mL of lysis buffer (0.05% Triton X, 2 mM EDTA, 0.1 M Tris) per 1 g of tissue. After 3 freeze–thawing cycles, tissue lysates were centrifuged at 15,000 rpm and 4 °C for 15 min. Supernatants were collected and analyzed for luciferase activity.

Furthermore, the pCMV-Luc complex suspension with PLGA/PEI nanospheres or microspheres was administered into the lung through a tracheal incision. The lung was removed at 9 h after instillation and was analyzed for luciferase activity, as described above.

Table 2
Mean particle diameter (D_{50}) of nanospheres spray-dried with different amount of mannitol to o/w suspension consisting of 1.79% PLGA and 0.83% PEI

Mannitol addition (%)	D_{50} (nm)
0.00	297.4
0.45	199.2
0.89	158.5
1.34	138.7
1.79	121.8
2.23	122.9

Table 4
Mean particle diameter (D_{50}) and zeta potential of spray-dried PLGA/PEI nanospheres prepared with different amounts of PEI in oil phase of o/w suspension

PEI (%)	D_{50} (nm)	Zeta potential (mV)
0.03	1661.3	38.4
0.17	420.5	47.1
0.33	196.1	54.8
0.83	124.1	57.2

3. Results and discussion

3.1. Effects of PEI and mannitol on particle size of PLGA/PEI nanospheres in mannitol

The mean particle diameter of PLGA/PEI nanospheres in o/w emulsion before drying was determined. Particles were prepared using 2 g of PLGA (1.67%) with 0.04–4 g of PEI (0.03–3.33%) dissolved in 100 mL of acetone/methanol solution. The nanospheres in o/w emulsion formulated at 0.17–0.83% PEI were approximately 100 nm in diameter, which is suitable for mucosal DNA vaccines. Table 2 shows the mean particle diameter of PLGA/PEI nanospheres consisting of 1.67% PLGA and 0.83% PEI spray-dried with various amounts of mannitol. Particle diameter of spray-dried nanospheres increased with decreasing mannitol concentration, and was smallest (ca. 120 nm) when mannitol was added at more than 1.79%, indicating that mannitol acts as an anti-aggregating agent. This indicates that mannitol efficiently suppresses particle aggregation because mannitol uniformly surrounds the PLGA/PEI nanospheres.

3.2. Particle size and zeta potential of nanoparticles by spray-drying and freeze-drying

Table 3 shows the mean particle diameter (D_{50}) of PLGA nanospheres with cationic materials before and after spray-drying or freeze-drying and zeta potential of spray-dried nanospheres. These nanospheres were also formulated from 1.67% PLGA, 0.83% cationic material (PEI, DOTMA, DC-Chol or CTAB) and 1.79% mannitol. In the case of spray-drying, the mean particle diameter of nanospheres with any cationic material was approximately equal to that of the nano-emulsion before drying. In the case of freeze-drying, particles showed larger particle diameters, possibly due to agglomeration of particles by compression during ice growth. The zeta potentials of PLGA nanospheres with PEI, DOTMA and DO-Chol confirmed higher positive charges. After spray-drying, cationic nanoparticles were obtained as mannitol microparticles having diameters of ca. 10 μm, as shown in Fig. 1A. The nanoparticles observed after dissolving mannitol with water on the filter were spherical in shape and were well dispersed, as shown in Fig. 1B–D. The PLGA/PEI nanospheres were smaller than the PLGA/DOTMA and PLGA/DC-Chol nanospheres.

Table 4 shows the mean diameter (D_{50}) and zeta potential of PLGA/PEI nanospheres prepared with different amounts of PEI. The mean diameter decreased with increasing amounts of PEI, while zeta potential gradually increased. PLGA/PEI nanoparticles with 0.83% PEI had the smallest diameter (124.1 nm). Stronger agitation during homogenization of the o/w emulsion might have provided finer nanospheres, even with smaller amounts of PEI. These results indicate that spray-drying is useful for preparation of cationic PLGA nanospheres without aggregation. The spray-drying method in this study allows improvements in handling properties, production efficiency and stability of nanoparticles without increasing size. The particle size of obtained nanoparticles corresponded to the respirable particle fraction, which can be delivered to the bronchioles and alveoli of the lung, and therefore mannitol microparticles containing PLGA nanospheres may be applicable to the dry powder inhaler (DPI) and pulmonary administration of DNA, siRNA and poorly-soluble compounds.

3.3. In vitro complex formation potential and DNase resistance of pDNA and PLGA/PEI nanospheres

Fig. 2 depicts the results of agarose gel electrophoresis of the supernatants collected by centrifuging TE buffer containing pDNA and nanospheres in order to evaluate the complex formation ability of cationic nanospheres. pDNA was undetectable in the supernatant if the complex was formed. Free pDNA was detected in the supernatant of mixture of pDNA and PLGA nanospheres without PEI, indicating no complexation with negatively charged PLGA nanospheres (lane 3). In contrast, pDNA was not present in the supernatant containing

Table 3
Mean particle diameter (D_{50}) of PLGA nanospheres with cationic materials before and after freeze-drying or spray-drying, and zeta potential after spray-drying

Cationic materials	Before drying, D_{50} (nm)	Freeze-dried, D_{50} (nm)	Spray-dried, D_{50} (nm)	Zeta potential (mV)
PEI	99.8	342.6	124.1	57.2
DOTMA	183.8	442.7	189.1	50.8
DC-Choi	263.4	784.2	278.3	52.4
CTAB	216.8	1281.2	219.1	−7.5

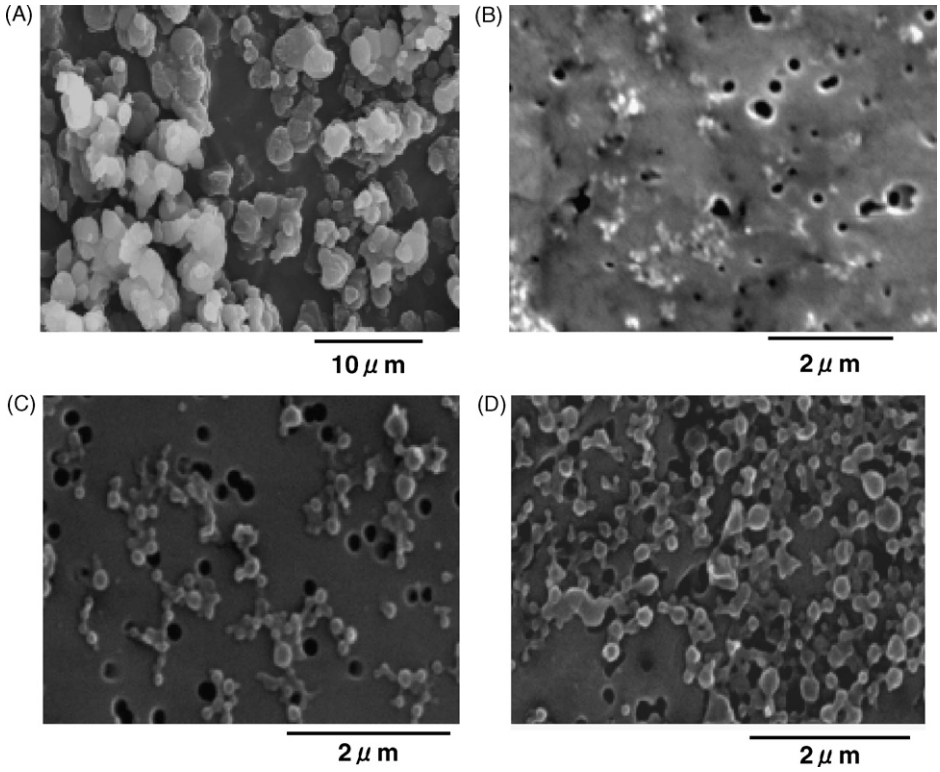

Fig. 1. SEM photographs of (A) mannitol microparticles including nanospheres, (B) PLGA/PEI nanospheres, (C) PLGA/DOTMA nanospheres and (D) PLGA/DO-Chol nanospheres in mannitol microparticles.

PLGA/PEI nanospheres, indicating that PLGA/PEI nanospheres were bound to pDNA (lane 4). Fig. 3 shows the results of DNase protection assay *in vitro*. Although the naked pDNA (lane 3) was completely degraded rapidly after treatment with DNase at 37 °C for 3 min, pDNA complexed with PLGA/PEI nanospheres (lane 5) remained intact. This suggests that the pDNA complexed with PLGA/PEI nanospheres would be stable against nucleases in the plasma during intravenous injection of the complexes.

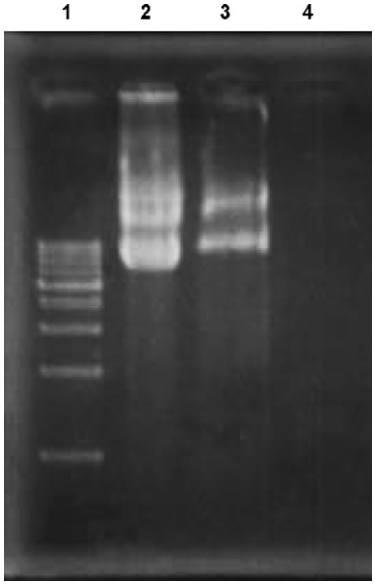

Fig. 2. Electrophoresis for pDNA uncomplexed with PLGA nanospheres. Lane 1 is molecular weight marker. Lane 2 shows naked pDNA. Lane 3 is supernatant from the mixture of pDNA and PLGA nanospheres without PEI. Lane 4 is supernatant from the mixture of pDNA and PLGA/PEI nanospheres. Supernatants were analyzed by 1% agarose gel electrophoresis. pDNA was not detected if pDNA complexes were formed with PLGA nanospheres.

Fig. 3. Resistance of pDNA-PLGA/PEI nanosphere complex to DNase I degradation. Lane 1 is molecular weight marker. Lane 2 is naked pDNA. Lane 3 is digestion of naked pDNA with DNase I. Lane 4 is pDNA-PLGA/PEI nanosphere complexes. Lane 5 is digestion of pDNA-PLGA/PEI nanosphere complexes with DNase I.

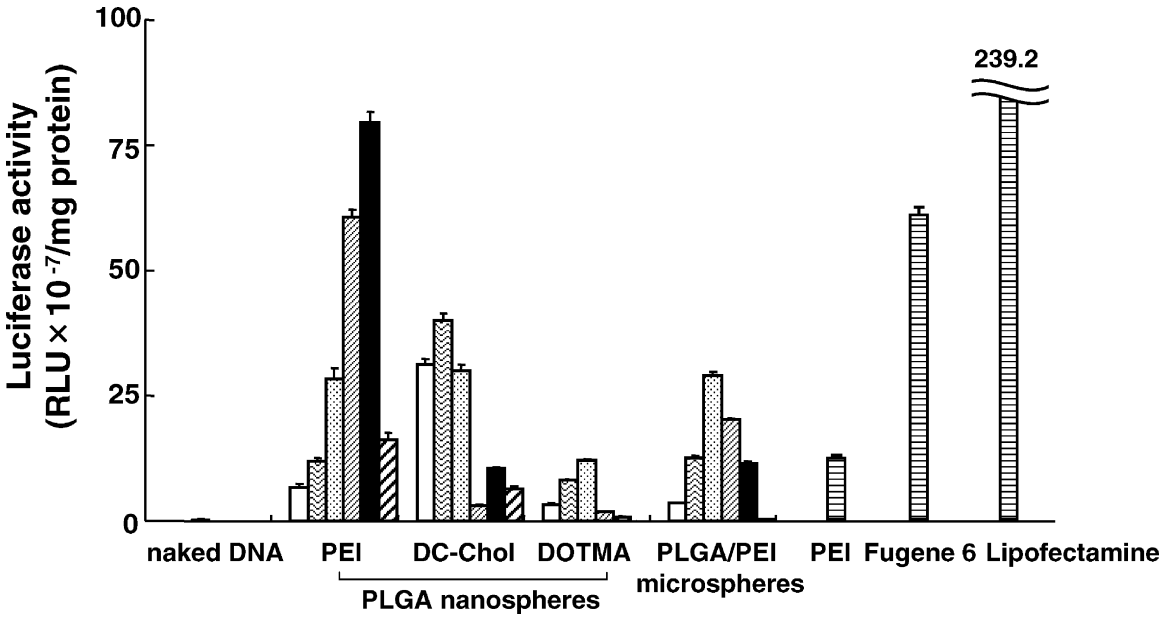

Fig. 4. Effects of cationic vector/pDNAratio on transfection efficiency in COS-7 cells. Vector/pDNA ratio: □, 5/1; ▨, 10/1; ⬚, 25/1; ▨, 50/1; ■, 75/1; ◪, 100/1. Each bar represents the mean ± S.D. ($n = 5$).

3.4. In vitro transfection activity of pDNA-nanosphere complexes

The transfection activities of cationic PLGA nanospheres were determined using COS-7 cells. As shown in Fig. 4, pDNA-PLGA/PEI nanospheres (50/1 and 75/1, PLGA/PEI) showed markedly higher luciferase activity than complexes with PLGA nanospheres containing DO-Chol and DOTMA. For 75/1 PLGA/PEI nanospheres, luciferase activity was particularly high when compared with other transfection reagents (PEI, Fugene 6), but was lower than Lipofectamine. Furthermore, PLGA/PEI nanospheres showed significantly higher gene expression than PLGA/PEI microspheres (∼1 μm in diameter), indicating that particle size affects transfection activity and that nano-sized particles are easily transfected into COS-7 cells. Fig. 5 shows fluorescence microscopy images of COS-7 cells transfected with Cy-3-labeled pEGFP complexed with PLGA/PEI nanospheres and PLGA/PEI microspheres. At 5 h after transfection, cellular localization by PLGA/PEI nanosphere complexes was markedly greater than that by PLGA/PEI microsphere complexes (red color), suggesting that intracellular uptake was improved by using nanospheres having diameters of approximately 100 nm. At 30 h after transfection, GFP (green color) was more strongly expressed when pDNA was transfected with PLGA/PEI nanospheres when compared with microspheres. These results indicate that cationic PLGA particles should be of nanosize in order to maximize gene expression, and that the present spray-dry technique with mannitol to give cationic nanospheres results in good transfection potential of pDNA.

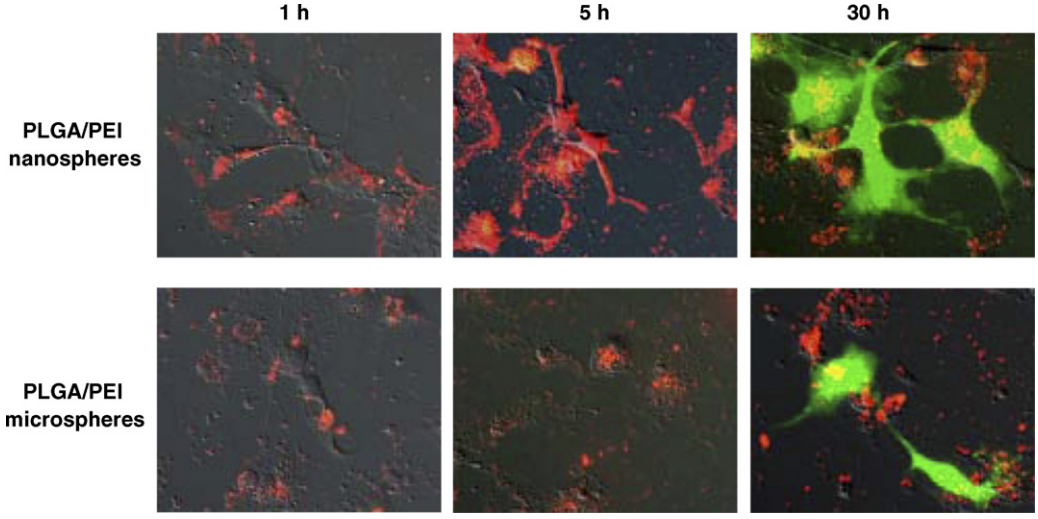

Fig. 5. Fluorescence microscopy of COS-7 cells transfected with Cy3-labeled pEGFP complexed with PLGA/PEI nanospheres or PLGA/PEI microspheres. Red: Cy-3-labeled pEGFP; green: expressed GFP.

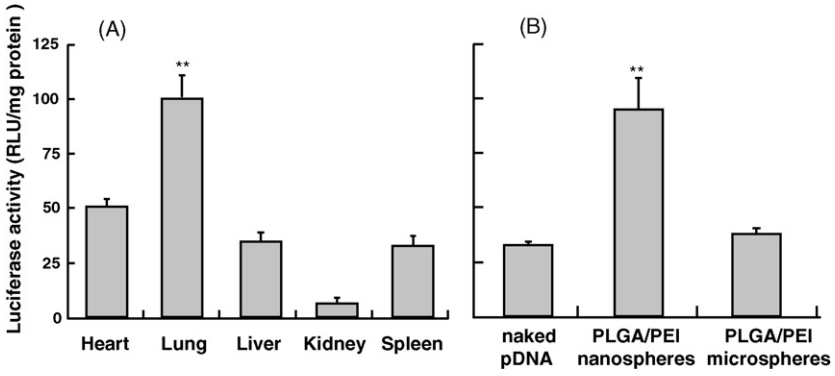

Fig. 6. Luciferase activity in mouse tissues after i.v. injection of pDNA-PLGA/PEI nanosphere complexes (A) and in lung after intratracheal administration of PLGA/PEI nanospheres and microspheres (B). (A) i.v. administration (50 μg pDNA/100 μL TE buffer, after 12 h). $^{**}P < 0.01$ compared with heart, liver, kidney and spleen. (B) Intratracheal administration (25 μg pDNA/50 μL TE buffer, after 9 h). $^{**}P < 0.01$ compared with naked pDNA.

3.5. *In vivo* gene expression study

Fig. 6 shows the luciferase activity following *in vivo* transfection with pDNA using PLGA/PEI nanospheres as a vector. Fig. 6A and B show the gene expression in various mouse tissues after i.v. administration and in the lung after intratracheal administration, respectively. Luciferase activity was highest in the lung tissue, as shown in Fig. 6A. This was due to the fact that particle size of the complexes increased by interaction between pDNA and serum protein, and therefore the complexes were temporarily trapped in the capillary bed of the lung. As shown in Fig. 6B, luciferase activity in the lung was higher with PLGA/PEI nanospheres than with PLGA/PEI microspheres, indicating that particle size affects gene expression efficiency *in vivo*. This suggests that suppressing increases in particle size during preparation is important in obtaining higher gene expression *in vivo*.

4. Conclusion

In this study, we investigated methods for the preparation of cationic PLGA nanoparticles as non-viral vectors. The spray-drying method was useful for preparing cationic PLGA nanospheres designed to enhance the electrostatic interaction between pDNA and the cell membrane. PLGA and cationic materials were dissolved in an acetone-based organic solvent containing methanol in order to dissolve the water-soluble cationic materials. By dropping the organic solution into distilled water, a PLGA nanosphere emulsion was obtained as a result of spontaneous ionic interaction between the basic imino groups of the cationic materials and terminal carboxylic anions of PLGA. By spray-drying the emulsion with mannitol, cationic PLGA nanospheres were collected without aggregation as mannitol microparticles, which have good handling properties. The mean particle diameter of nanospheres was smallest when PEI was used as a cationic material. The obtained cationic PLGA/PEI nanospheres formed strong complexes with pDNA, and pDNA was thus protected from nucleases such as DNase. In an *in vitro* transfection study using COS-7 cells, PLGA/PEI nanospheres complexed with pDNA gave significant luciferase expression when compared with other cationic PLGA nanospheres as a result of good uptake into COS-7 cells. This technology would allow the preparation of nano-sized particles as gene carriers without aggregation or loss of gene transfection potential, and may enable the large-scale production of such particles.

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