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ORIGINAL ARTICLE

Aerosol delivery of programmed cell death protein 4 using polysorbitol-based gene delivery system for lung cancer therapy

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Abstract

The development of a safe and effective gene delivery system is the most challenging obstacle to the broad application of gene therapy in the clinic. In this study, we report the development of a polysorbitol-based gene delivery system as an alternative gene carrier for lung cancer therapy. The copolymer was prepared by a Michael addition reaction between sorbitol diacrylate (SD) and spermine (SPE); the SD–SPE copolymer effectively condenses with DNA on the nanoscale and protects it from nucleases. SD–SPE/DNA complexes showed excellent transfection with low toxicity both *in vitro* and *in vivo*, and aerosol delivery of SD-SPE complexes with programmed cell death protein 4 DNA significantly suppressed lung tumorigenesis in K-*ras*^{LA1} lung cancer model mice. These results demonstrate that SD–SPE has great potential as a gene delivery system based on its excellent biocompatibility and high gene delivery efficiency for lung cancer gene therapy.

Keywords

Accelerated transfection, aerosol delivery, gene therapy, non-viral vector, polysorbitol, polyspermine

History

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Introduction

Gene therapy is becoming a promising therapeutic method for treatment of various diseases including cancer [1]. Because naked gene therapeutics are vulnerable to enzymatic degradation, poor cellular uptake and inefficient escape from the endosomal compartments to the cytosol, the development of safe and effective gene delivery systems for carrying genetic therapeutics to target sites has made great progress in recent years [2–4]. To date, the most clinical gene therapy trials have used modified viruses as delivery vehicles; however, concerns such as toxicity, potential immunogenicity and large-scale production have led to develop non-viral vectors [5,6]. Non-viral vectors have attracted great interest because they are low in toxicity, easy preparation and modification, better stability and no limitation on vector size [7,8]. However, in contrast to viral vector, the main disadvantage is low transfection efficiency [3,9].

Polyethylenimine (PEI) is well known to be effective cationic non-viral vector due to its superior transfection efficiency in many cell lines [10–12]. With the increase of molecular weight of PEI, its transfection efficiency and cytotoxicity were increased [5,13]. Therefore, more safe and human endogenous amino compounds with high buffering capacities should be considered as a replacement for PEI.

As a biocompatible material, spermine (SPE) is found naturally in all eukaryotic cells and involved in cellular metabolism [14,15]. Furthermore, the tetra-amine of SPE, which is composed of two primary and two secondary amino groups, can be reacted with an acrylate group by a Michael addition reaction to make a polyspermine with high buffering capacity [16,17]. In a previous study, we also prepared polyspermine based on SPE and glycerol propoxylate triacrylate (GPT) used for a biocompatible alternative gene carrier [18]. This GPT–SPE copolymer showed low cytotoxicity and high serum stability, although its transfection efficiency remained low.

Aerosol delivery is a promising approach for the treatment of a broad spectrum of pulmonary disorders and offers numerous advantages over more invasive modes of delivery. In particular, aerosol administration allows for DNA deposition in the lungs at the pulmonary region, thereby bypassing systemic distribution, and prolonging DNA retention [19]. Therefore, in this work, we developed a polysorbitol-based accelerated gene delivery system (SD–SPE) using sorbitol diacrylate (SD) and SPE by a Michael addition reaction, as previous reports had suggested that transporters based on sorbitol could increase the

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transfection efficiency for aerosol lung cancer gene therapy [20,21]. In addition, sorbitol (D-glucitol) is widely produced in plants and is extensively used for the food industry because of its lack of any perceptible toxicity and good water solubility [21]. The physicochemical properties of the SD–SPE/DNA complexes were analyzed, and their cellular toxicity and transfection efficiency were performed. In adition, the *in vivo* toxicity and transfection efficiency after aerosol delivery were evaluated, and the therapeutic efficiency of SD–SPE complexes containing programmed cell death protein 4 (*Pdcd4*) DNA was measured in K-*ras*^{LA1} lung cancer mice model.

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Materials and methods

Materials

SD was purchased from Monomer-Polymer & Dajac Labs, Inc. (Trevose, PA). SPE, branched PEI (25 K), dimethyl sulfoxide (DMSO), 4′,6-diamidino-2-phenylindole dihydrochloride and bafilomycin A1 were purchased from Sigma-Aldrich (St. Louis, MO). The pGL3 (SV40 promoter, enhancer encoding firefly luciferase, 5.3 kb) vector and Cell Titer 96® A_{queous} One Solution Reagent were purchased from Promega (Madison, WI). The pEGFP-N_2 (CMV promoter, enhanced green fluorescent protein gene, 4.7 kb) vector was purchased from Clontech (Palo Alto, CA). All other chemicals were reagent grade or higher.

Preparation of SD–SPE

The SD–SPE copolymer was prepared according to a modified method [18]. Briefly, SD (2.5 M) and SPE (2.7 M) were separately dissolved in 2 mL of DMSO, and then the SD solution was slowly added to the SPE solution with stirring at 80 °C for 24 h. The obtained SD–SPE copolymer was dissolved in distilled water at 4 °C and then dialyzed using a Spectra/Por® membrane (MWCO = 3500) against distilled water at 4 °C for 24 h. After lyophilization, the SD–SPE copolymer was stored at –20 °C for subsequent use.

Characterizations of copolymer and SD–SPE/DNA complexes

The SD–SPE copolymer was characterized by ^{1}H nuclear magnetic resonance (^{1}H NMR) spectroscopy with an Avance™ 600 spectrometer (Bruker, Rheinstetten, Germany) to confirm the synthesis and composition of the synthesized SD–SPE. For NMR measurements, SD–SPE copolymers were dissolved in D_2O with a concentration of 10 mg/mL. Gel permeation chromatography (DAWN Eos, Wyatt, Santa Barbara, CA) was used for measurement of the SD–SPE copolymer molecular weight. The temperature of column was maintained at 25 °C with a flow rate of 0.5 mL/min and measured at a 690 nm laser wavelength.

All SD–SPE/DNA complexes were characterized using the same method of Jiang et al. [5]. SD–SPE/DNA complexes were prepared by adding a 150 μL DNA solution to equal volumes of SD–SPE copolymer solution with vortex and then incubated for 30 min at room temperature.

The DNA condensation and protection studies were performed using gel electrophoresis. The complexes were formed with various weight ratios ranging from 0.1 to 10. To evaluate the DNA protection ability, DNase I was used as a digestion enzyme. For DNase inactivation, all samples were treated with 4 μL of EDTA (250 mM) for 10 min and mixed with 1% sodium dodecyl sulfate, dissolved in 1 M NaOH (pH 7.2) at a final volume of 18 μL [5]. The SD–SPE/DNA complexes were loaded into agarose gels (1%) and run with Tris-acetate buffer at 50 V for 40 min. DNA retardation was observed by irradiation with UV light.

The SD–SPE/DNA complexes morphology was observed by energy-filtering transmission electron microscopy (EF-TEM, LIBRA 120, Carl Zeiss, Jena, Germany). Twenty microliter of the SD–SPE/DNA complexes was placed on a copper grid, and the complexes were stained by uranyl acetate solution (1%) for 10 s. The grid was allowed to incandescent lamp dry for another 10 min and then was measured by the EF-TEM.

The SD–SPE/DNA complexes sizes and surface charges were measured by an electrophoretic light scattering spectrophotometer (ELS8000, Otsuka Electronics, Osaka, Japan), with 90 and 20 scattering angles, respectively. The sample volume was 2 mL with 40 μg/mL of DNA concentration.

Cell lines, cell viability and transfection efficiency studies *in vitro*

Two cell lines, including A549 (human lung carcinoma) and 16HBE14o- (immortalized human bronchial epithelial cells), were chosen for the cellular studies. A549 and 16HBE14o- cells were cultured in RPMI 1640 culture media (Gibco BRL, Paris, France), supplemented with 10% heat-inactivated fetal bovine serum (HyClone, Logan, Utah), 100 U/mL of penicillin and 100 μg/mL of streptomycin. All cells were cultured in an incubator at 37 °C with 5% CO_2 and 95% relative humidity atmosphere.

In vitro cytotoxicity studies were performed according to a method previously reported [5]. Two-hundred microliter of RPMI 1640 culture media with 1×10^4 cells were seeded in 96-well plates and incubated for 24 h. Furthermore, the culture media were replaced with serum-free media containing various dilutions of polymers. The cells were cultured for an additional 24 or 72 h incubation period, the media were exchanged with culture media containing 20 μL of Cell Titer 96® A_{queous} One Solution Reagent. After an additional incubation for 3 h, the absorbance was measured by a microplate reader (GLR 1000, Genelabs Diagnostics, Singapore) at a wavelength of 570 nm.

For intracellular uptake study, FITC-labeled SD–SPE/DNA complexes were delivered and visualized using confocal microscopy. Briefly, cells were seeded in 24-well plates at an initial density of 10×10^4 cells/well. After incubation for 18 h, the media were replaced with serum-free media with FITC-labeled SD–SPE/DNA complexes and additionally incubated for 6 h. Then, the media were exchanged by fresh media and allowed to incubate for 24 h for confocal microscopy measurement.

For *in vitro* transfection efficiency study, 10×10^4 cells/well was seeded in 24-well plates in 1 mL of growth medium and grown for 18 h. And then, the polymer/pGL3 (1 μg)

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complexes with various weight ratios were added. N/P ratio 10 was used for PEI 25K. Six hours later, wells were rinsed with phosphate-buffered saline (PBS), and fresh media containing 10% serum media was added and allowed to incubate for 24 h. The luciferase activity assay was performed according to the manufacturer's protocol. A chemiluminometer (Autolumat, LB953; EG&G Berthold, Bad Wilbad, Germany) was used for the observation of relative light units, normalized to the estimated protein concentration in the cell extract using a BCA protein assay kit (Pierce Biotechnology, Rockford, IL).

To assay the buffering capacity of the polymers, bafilomycin A1 (200 nM) diluted in DMSO was placed in the 24-wells. After a 10-min pre-incubation, the transfection efficiency was evaluated as described above. The buffering capacity of SD–SPE was re-conformed by acid–base titration over the pH range from 10.0 to 3.0. The pH of the polymer solution was set at 10.0 and then was titrated with 0.1 M HCl.

For measurement of confocal laser scanning microscopy, 5×10^4 cells/well was seeded in 24-well plates in 1 mL of growth medium and grown for 18 h. Then the media were replaced with polymer/pEGFP-N_2 (1 μg) complexes. Six hours later, wells were rinsed with PBS, and fresh media containing 10% serum media was added and allowed to incubate for 24 h. Samples were viewed with a Zeiss LSM 510 confocal microscope (Carl Zeiss). For flow cytometry, transfected cells were prepared by the same method as for confocal microscopy. After transfection, cells were washed once with PBS and detached with 0.25% trypsin/EDTA. Transfection efficiency was evaluated by scoring the percentage of cells expressing GFP, using a FACS Calibrator System from Becton-Dickinson (San Jose, CA). Fluorescence parameters from 10 000 cells were acquired, and transfection was carried out in triplicate.

To further investigate the SD–SPE-mediated gene transfection mechanism, SC58236, a cyclooxygenase (COX)-2 inhibitor, was used to inhibit the osmotic activity of SD–SPE to determine the inhibitory effect on *in vitro* gene transfection. A 10 μM SC58236 solution was prepared by initial dissolving in DMSO and subsequent dilution in serum-free medium, and this solution was then added to each well (10×10^4 cells/well) of 24-well plates. After pre-incubation with SC58236 for 1 h, the A549 cells were transfected with SD-SPE/pGL3 and PEI 25K/pGL3 complexes. Luciferase activity was performed according to the same method described above. The cytotoxicity studies of bafilomycin and SC58236 were performed according to the same method described above.

Transfection efficiency and therapeutic studies *in vivo*

BALB/c mice (six-week-old, female, Breeding and Research Center, Seoul National University, Korea) were used according to the policy and regulations for the care and use of laboratory animals (Laboratory Animal Center, Seoul National University, Seoul, Korea). Animals were kept in a laboratory animal facility, the temperature and relative humidity of which were maintained at $23 \pm 2\,^{\circ}C$ and $50 \pm 20\%$, respectively, under a 12-h light/12-h dark cycle. For the gene transfection study, mice were placed in a nose-only exposure chamber and exposed to the aerosol according to previously reported methods [22]. BALB/c mice were randomly divided into four groups (four mice/group). Treatment groups were exposed to aerosols containing the GFP-expressing plasmid either with or without the SD–SPE and PEI 25K carriers, and the remaining four mice as controls were exposed to aerosols with distilled water. Forty-eight hours after aerosol delivery, all mice were sacrificed and lung tissues were collected. For measurement of the GFP signal, lungs were embedded in Tissue-Tek OCT compound (Sakura, Torrance, CA) at room temperature. Then, 10 μm of tissue cryosections were cut with a microtome (Leica, Nussloch, Germany) and mounted onto slides for measurement of the GFP signal.

K-*ras*^{LA1} mice (six-week-old, female), the human non-small cell lung cancer murine model, were obtained from the Human Cancer Consortium-National Cancer Institute (Frederick, MD). These mice were divided into five groups (four mice/group), including the control group that exposed to aerosol with distilled water, *Pdcd4* gene only, SD–SPE polymer only and the treated group that exposed to aerosol containing SD–SPE (8 mg) complexed to the *Pdcd4* gene (0.8 mg) and PEI 25K complexed to the *Pdcd4* gene (0.8 mg) with N/P ratio 10 in distilled water. Mice were treated with aerosol device eight times for four weeks (two times/week). Forty-eight hours after final aerosol delivery, all the mice were sacrificed, and neoplastic lesions on the lung surfaces were carefully counted under a microscope.

Statistical analysis

All values are presented as the mean ± standard deviation. The statistical significance of differences among the groups was determined using an unpaired *t*-test. p values < 0.05 indicated statistical significance, p values < 0.01 indicated high significance and p values < 0.001 indicated greater significance than the corresponding values.

Results and discussion

Synthesis and characterization of SD–SPE

The Michael addition reaction has gained considerable attention as a strategy for polymer synthesis [23]. In this work, ester-based SD–SPE copolymer was prepared by a Michael addition reaction between SPE and SD as a cross-linker (Figure 1A). The composition of the SD–SPE copolymer was characterized by ^{1}H NMR as shown in Figure 1(B). The NMR spectra of the reactant showed proton peaks for both SPE (–CH_2–) at $\delta = 1.6$–1.8 ppm and SD (–CH_2–) at $\delta = 4.2$–4.3 ppm, indicating that SD–SPE was well synthesized. DMSO, an anhydrous organic solvent, was used during the synthesis to avoid hydrolysis degradation of SD–SPE [24]. The molecular weight of SD–SPE copolymer was 4.52 kDa, with a polydispersity index of 1.23, as shown in Figure 1(C). The obtained SD–SPE was readily water-soluble due to its hydrophilic nature.

Characterizations of SD–SPE/DNA complexes

The DNA condensation capability of SD–SPE was measured by agarose gel electrophoresis. As shown in Figure 2(A),

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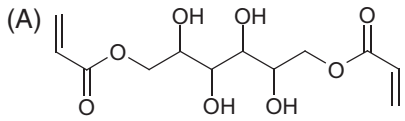
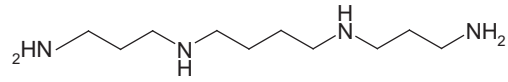
(A)



Sorbitol diacrylate (SD)

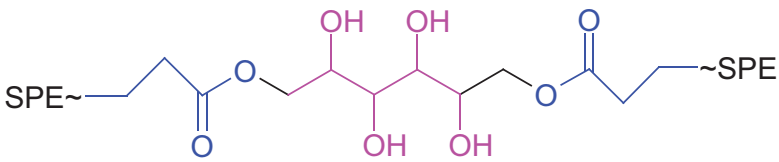
+



Spermine (SPE)

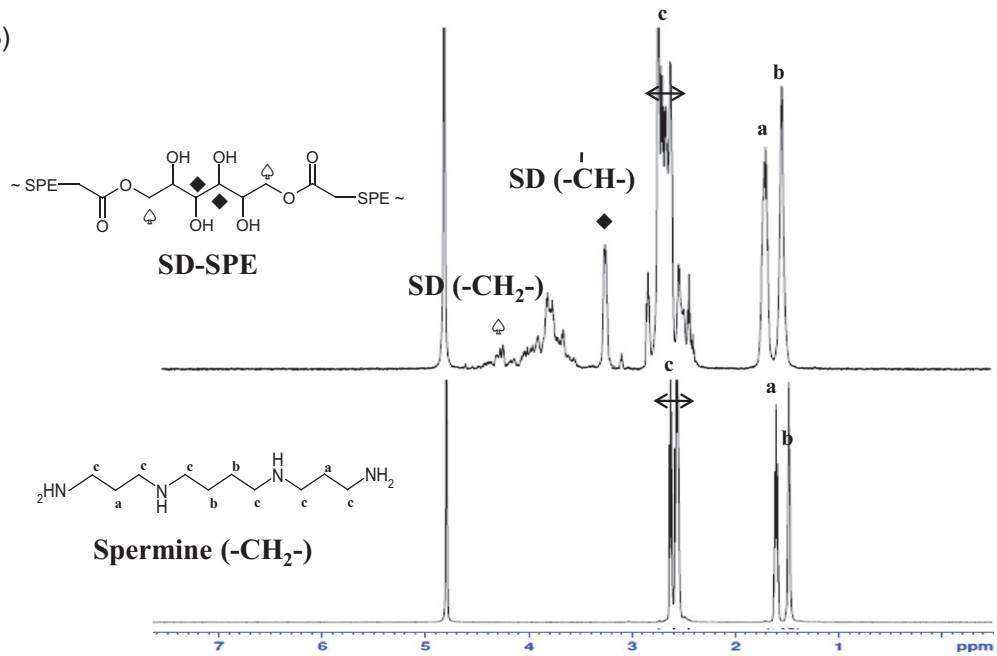
Michael Addition

DMSO



Amine-terminated polyspermine

(B)



(C)

	Mw	Mn	PDI
SD-SPE	4516	3672	1.23

Figure 1. SD–SPE copolymer synthesis and characterization. (A) Proposed reaction scheme for SD–SPE; (B) representative ^{1}H NMR spectra of SD–SPE and SPE in D_2O: $\delta = 1.6–1.8$ ppm (–CH_2–, spermine) and 4.2–4.3 ppm (–CH_2–, SD); and (C) GPC analysis.

the migration of DNA was retarded completely when the weight ratio between SD–SPE copolymer and DNA was approximately 5.

For effective gene expression, the DNA in the gene vehicle should be protected from degradation by enzymes in the cellular environment [5,25]. As shown in Figure 2(B), in a DNA protection experiment, the DNA in the SD–SPE/DNA complexes was protected from DNase I, whereas naked DNA was completely degraded by the DNase I. These results suggested that the integral DNA in complexes could be transferred to cells without degradation by enzymes after local or systemic treatment.

Figure 2(C) shows representative morphologies of SD–SPE/DNA complexes, which demonstrated compact structure with spherical shapes. The particle size of the complexes is an important parameter that affects the access and passage of the complexes to the targeting site [26]. The average particle size of the complexes measured by DLS

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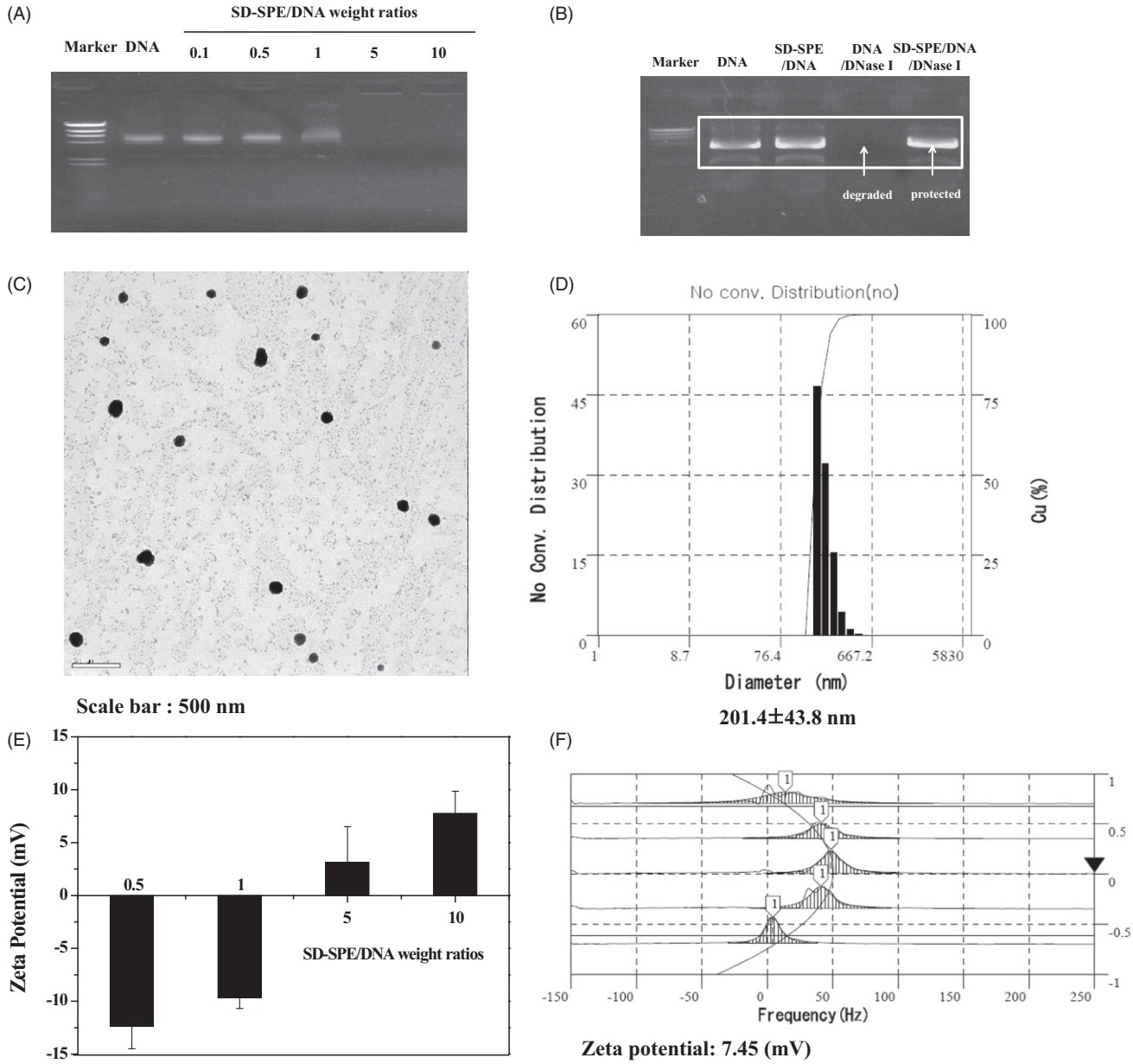

Figure 2. Characterization of the SD–SPE/DNA complexes. (A) Agarose gel electrophoresis of SD–SPE/DNA complexes with various weight ratios; (B) DNA protection and release assay; (C) EF-TEM images of SD–SPE/DNA complexes with a weight ratio of 10; (D) size distribution assay; (E) surface charges of SD–SPE/DNA complexes with various weight ratios; and (F) particle surface charges of SD-SPE/DNA complexes with a weight ratio of 10.

was 201.4 nm, and the homogenous size distribution was observed as shown in Figure 2(D).

It was reported that positive charged polyplexes could facilitates nanoparticle uptake by the cells due to interaction with anionic charged cells surfaces [27]. For weight ratios between 0.5 and 1, in which case the complexes could not form completely, the SD–SPE/DNA complexes were negative charged; however, with the increase of weight ratios, the SD–SPE/DNA complexes had positive zeta potential values (Figure 2E), indicating that the SD–SPE/DNA complexes were sufficiently cationic for binding with negatively charged cell membranes and subsequent endocytosis. At the weight ratio 10, the zeta potential of the SD–SPE/DNA complexes was 7.45 mV as shown in Figure 2(F).

Cell viability and transfection efficiency studies *in vitro*

Low cytotoxicity is an important parameter for the clinical application of gene therapy. Therefore, we performed a cytotoxicity study on two different cell lines with two different time periods. As shown in Figure 3(A–D), high cell viabilities were observed in SD–SPE-treated group (at 24 h: 75.9% in A549 cells and 69.0% in 16HBE14o- cells; at 72 h: 70.6% in A549 and 68.7% in 16HBE14o-), even at a high concentration (125 μg/mL), whereas at same concentration, the serious cytotoxicities were observed in PEI 25K-treated group (at 24 h: 9.9% in A549 and 8.4% in 16HBE14o-; at 72 h: 9.5% in A549 and 8.2% in 16HBE14o-), indicating that the SD-SPE copolymer has less cellular

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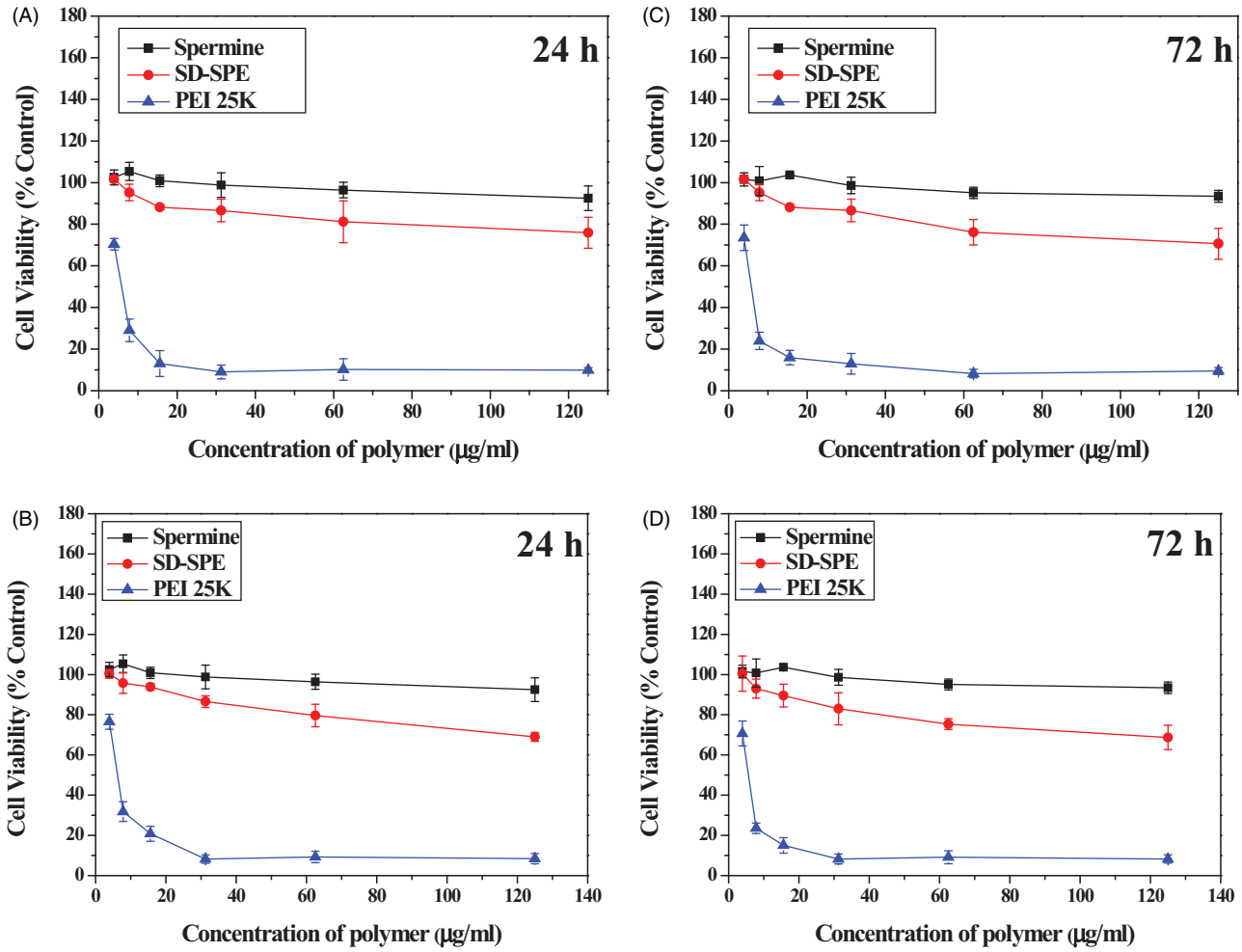

Figure 3. Cytotoxicity of the SD–SPE copolymer at various concentrations in two different cell lines (A549 and 16HBE14o-) after different periods of time (24 and 72 h). (A) A549 cells, 24 h; (B) 16HBE14o- cells, 24 h; (C) A549 cells, 72 h; and (D) 16HBE14o- cells, 72 h (mean ± SD, $n = 3$).

toxicity than that of the standard PEI 25K in both cell lines (A549 and 16HBE14o-) after different amounts of time (24 and 72 h). Moghimi et al. previously demonstrated that PEI and PEI/DNA complexes could induce mitochondria-mediated apoptosis [28,29]. In addition, it was also reported that PEI could induce relatively weak apoptotic and strong necrotic effect as well as genotoxic effect [30]. On the other hand, it has been reported that SPE is a safe monomer that occurs naturally in bodily tissues [15,17]. As expected, SPE showed good cell viability even at high concentrations as shown in Figure 3(A–D). In addition, cationic polymers with high charge densities showed high cell lytic and toxic properties; however, reducing the charge density of these polymers leaded to lower cell toxicity [3,31]. In this work, the zeta potential of the SD–SPE/DNA complexes (+7.45 mV) was lower than that of the PEI 25K/DNA complexes (+38.63 mV). Therefore, it is reasonable to assume that SD–SPE showed lower cytotoxicity than PEI 25K due to the biocompatible properties of SPE and the low surface charge of SD–SPE that arises from the shielding of the primary amines of SPE by the Michael reaction.

To confirm intracellular uptake of SD–SPE/DNA complexes, FITC-labeled SD–SPE/DNA complexes were delivered and visualized using confocal microscopy. As shown in Figure 4(A), significant fluorescence of labeled complexes was observed, indicating that high intracellular uptake occurred. Polymeric gene carriers have received considerable attention due to the advantages including their ease of synthesis, safety, low-immunogenicity as well as potential for large-scale production. However, to date, most of these carriers have demonstrated unsatisfactory transfection efficiency and have thus remained at the laboratory stage [32]. To investigate gene transfection efficiency of the SD–SPE copolymer, we performed luciferase activity assays *in vitro*. SD–SPE/pGL3 complexes were transfected into the A549 (Figure 4B) and 16HBE14o- (Figure 4C) cell lines, and the expressed luciferase activity was evaluated. The transfection efficiency of the SD–SPE/pGL3 complexes increased with an increasing weight ratio up to 10, and the luciferase activity of the SD–SPE/pGL3 complexes remained nearly constant at a higher weight ratio of 20. Interestingly, the transfection efficiency of the SD–SPE/pGL3 complexes was higher than that of the PEI 25K/pGL3 as well as Lipofectamine/pGL3 complexes in the A549 cell line, whereas the transfection efficiency of the SD–SPE/pGL3 complexes was reduced 38-fold in the 16HBE14o- normal cell line without cytotoxicity (Figure 4D and E). In addition, we reconfirmed the gene expression of the copolymer by confocal laser

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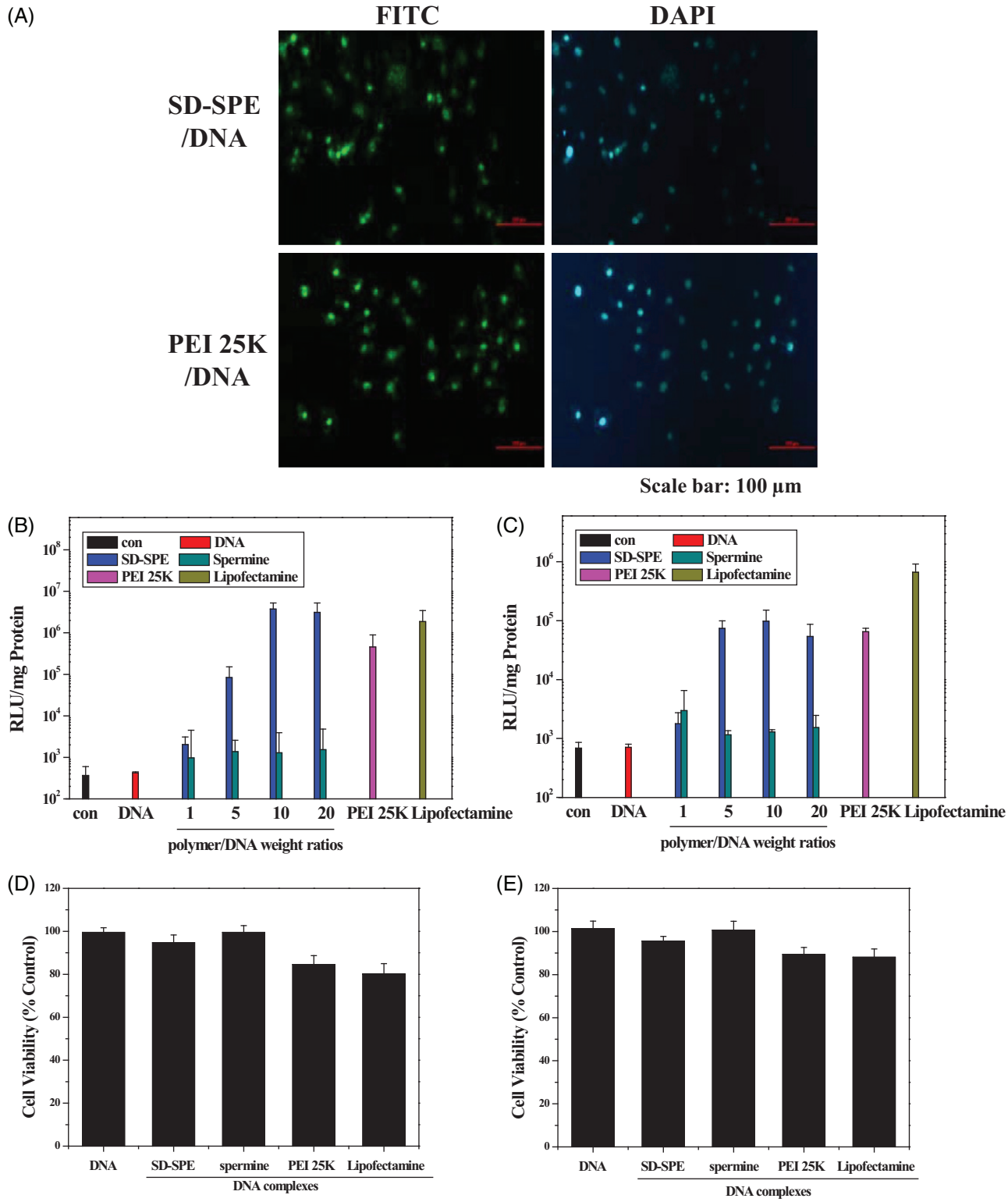

Figure 4. Cellular uptake and transfection studies *in vitro*. (A) Cellular uptake study; (B) transfection efficiency study of A549 cells by SD–SPE/pGL3 complexes at various weight ratios (mean ± SD, $n = 3$); (C) transfection efficiency study of 16HBE14o- cells by SD–SPE/pGL3 complexes at various weight ratios (mean ± SD, $n = 3$); cytotoxicity of the polymer/DNA complexes at functional ratios in A549 cells; (D) and in 16HBE14o- cells; (E) (mean ± SD, $n = 3$); (F) GFP expression analysis of A549 cells transfected with SD-SPE/GFP complexes. Confocal laser scanning microscopy (scale bar = 20 μm); and (G) FACS analysis of A549 cells transfected with SD-SPE/GFP complexes (mean ± SD, $n = 3$).

scanning microscopy as well as flow cytometry. As shown in Figure 4(F and G), more GFP expression was observed in the SD–SPE/GFP complexes than in the PEI 25K/GFP complexes, indicating that SD–SPE was more effective as a gene carrier.

To further elucidate the mechanism of gene transfection, bafilomycin A1, a specific vacuolar type H^+ ATPase inhibitor, was used to analyze the buffering capacity of the SD–SPE copolymer [15,33,34]. Similar to those of PEI 25K (52-fold decrease), the gene transfection level of the

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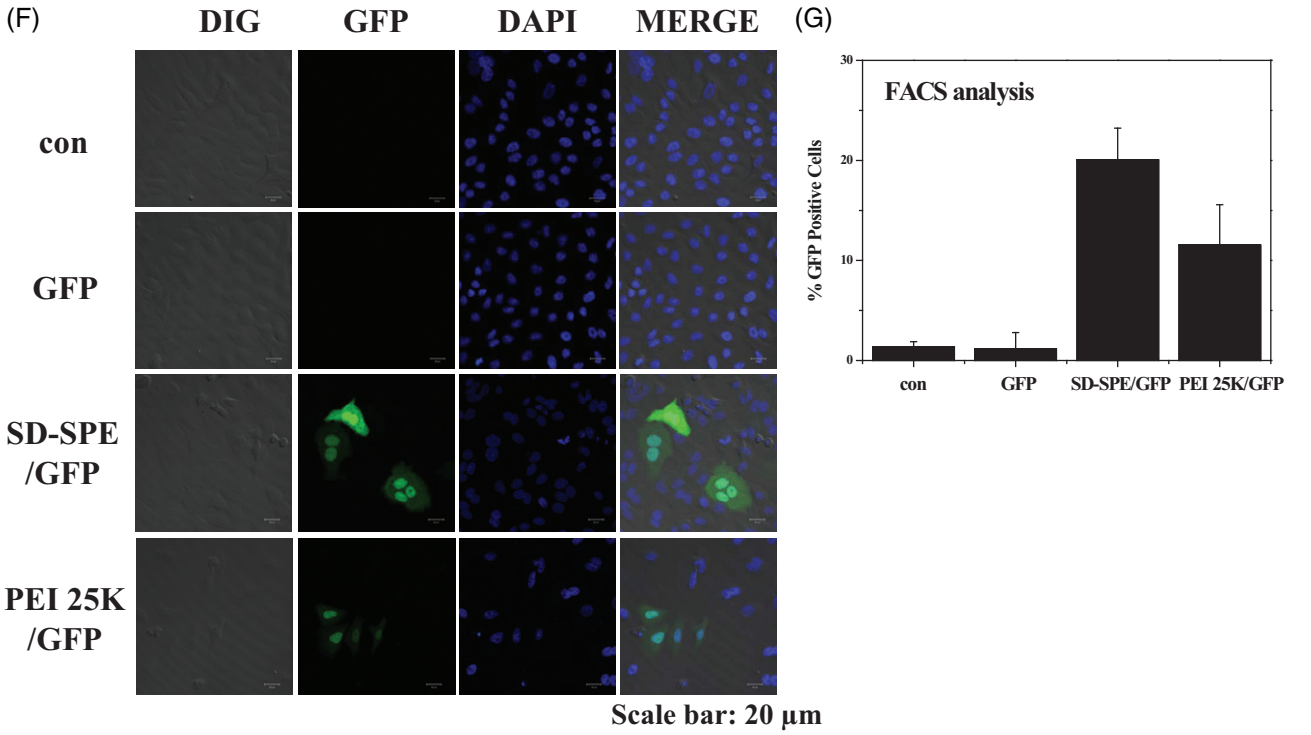
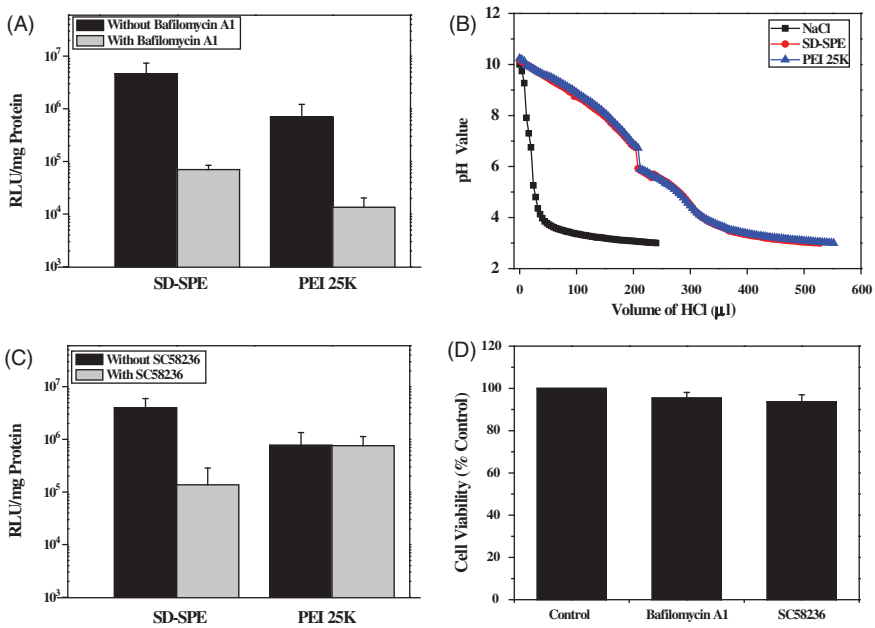


Figure 4. Continued.

Figure 5. Mechanism studies. (A) Effect of bafilomycin A1 on gene transfection in A549 cells (mean ± SD, $n = 3$). (C) Buffering capacity of SD-SPE. (D) Effect of polysorbitol activity inhibition by SC58236 on gene transfection. Cytotoxicity of bafilomycin A1 (B) and SC58236 (E) treated groups in A549 cells (mean ± SD, $n = 3$).



SD-SPE/pGL3 complexes was decreased 66-fold after bafilomycin A1 treatment (Figure 5A), which strongly suggested that SD–SPE copolymer had a high buffering capacity. Buffering capacity of SD–SPE copolymer was also re-confirmed by acid–base titration. Likewise, PEI 25K and SD–SPE copolymer show high buffering capacity (Figure 5B). In addition, after SC58236 [a COX-2-specific inhibitor] treatment, the transfection efficiency of the SD–SPE/pGL3 complexes was drastically decreased, whereas SC58236 had no effect on the transfection efficiency of PEI 25K/pGL3 complexes, as shown in Figure 5(C). It was previously reported that SC58236 potentially reduced osmolyte (such as inositol and sorbitol) accumulation in many cell lines [35]. Islam et al. also reported that the polysorbitol backbone could accelerate gene transfection efficiency through a polysorbitol-based transporter [32,36].

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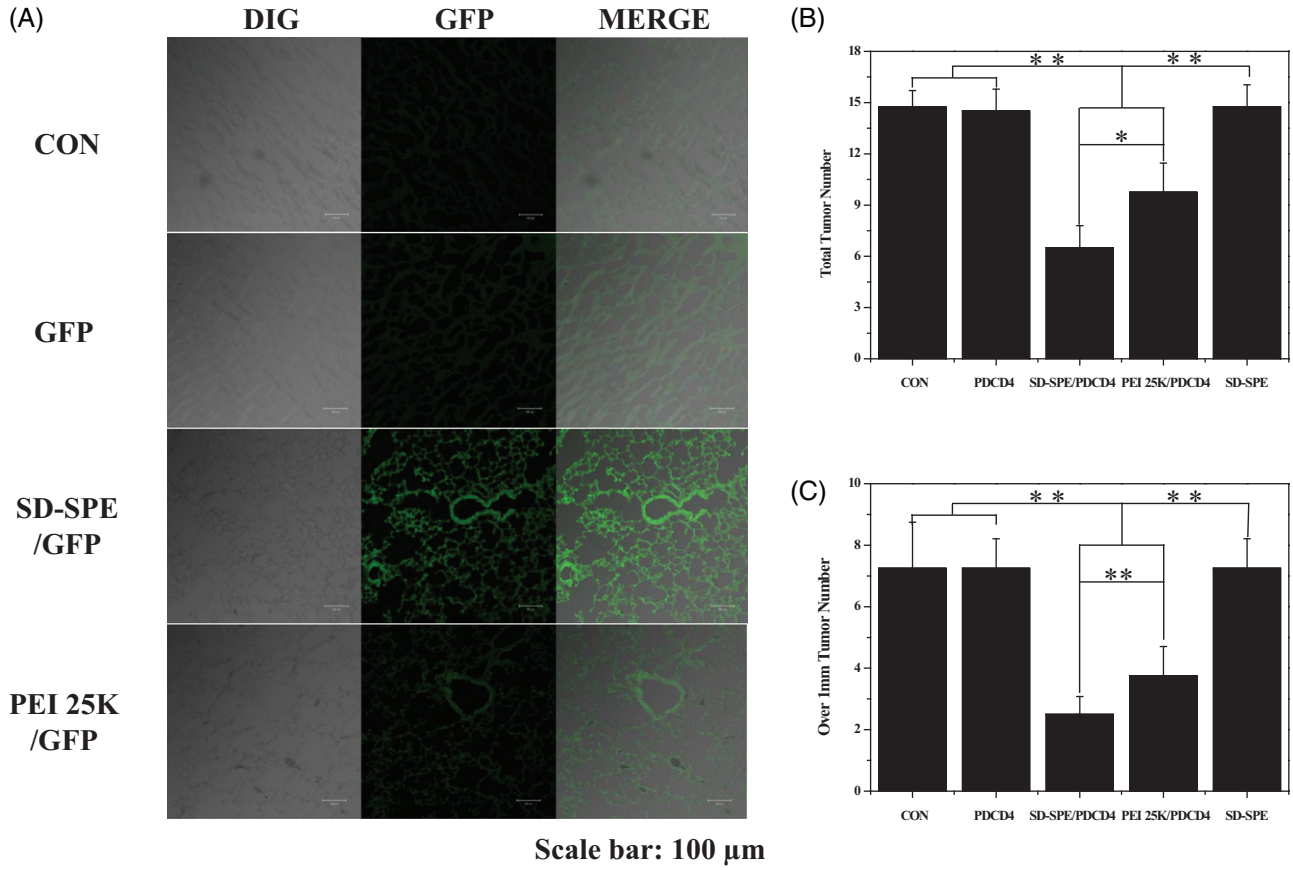

Figure 6. *In vivo* analysis following aerosol administration to the lungs. (A) Transfection efficiency study. GFP expression levels were measured after the aerosol delivery of SD-SPE/GFP complexes at a functional weight ratio in BALB/c mice (magnification: 200 ×, scale bar = 100 μm). Therapeutic efficiency study. The numbers of lung tumors were evaluated after the administration of SD-SPE/*Pdcd4* complexes at a functional weight ratio in lung-tumor-bearing K-*ras*^{LA1} mice. (B) Total number of tumors and (C) the number of lung tumors larger than 1 mm ($n = 4$, $^{*}p < 0.05$, $^{**}p < 0.01$).

Therefore, it was reasonable to assume that the high gene transfection efficiency of the SD–SPE copolymer was due to the synergistic effects of the high buffering capacity of polyspermine as well as the accelerated gene delivery capacity of the polysorbitol backbone. The treatment of bafilomycin A1 and SC58236 did not affect the cell viability (Figure 5D).

In vivo aerosol delivery of SD–SPE

The *in vitro* results showing the high transfection efficiency and low cytotoxicity of SD–SPE encouraged us to investigate the *in vivo* gene transfection efficiency following aerosol administration. Compared to the invasive administration, the aerosol delivery of genes represents a promising approach for the treatment of many pulmonary disorders because aerosol delivery allows genes directly deposition at the pulmonary region, thereby bypassing the systemic distribution, and prolonging DNA retention [19]. Aerosol delivery is also an efficient tool for delivery of a target gene to the respiratory system in a non-invasive manner, as demonstrated in our previous studies [18,22,37]. Following aerosol delivery of the complexes, the SD–SPE/GFP showed high GFP fluorescence signals compared to the control, or naked GFP, and PEI 25K/GFP-treated groups, as shown in Figure 6(A). This result indicated that SD–SPE acted as an effective gene carrier *in vivo*. To further confirm the therapeutic effect of SD–SPE for lung cancer gene therapy, *Pdcd4* was used as a therapeutic gene. As expected, the tumor size and number of SD-SPE/*Pdcd4* complexes were significantly decreased compared to the control group (Figure 6B and C), demonstrating that SD–SPE functioned efficiently as a gene carrier for lung cancer *in vivo*.

Conclusion

In this work, we successfully prepared and evaluated SD–SPE copolymer as a new gene carrier for lung cancer therapy. The SD–SPE copolymer demonstrated a strong ability for forming complexes with DNA and exhibited suitable physicochemical properties as a polymeric gene carrier. This polymer also exhibited low cytotoxicity and enhanced gene transfer efficiency *in vitro* as well as *in vivo*. Therefore, SD–SPE has the potential to become a safe and efficient gene carrier for lung cancer gene therapy.

Declaration of interest

The authors report no conflicts of interest in this work.

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