Plasmid-encapsulated polyethylene glycol-grafted polyethylenimine nanoparticles for gene delivery into rat mesenchymal stem cells

Xiao-Ai Chen1,5*
Li-Jun Zhang2*
Zhi-Jie He3
Wei-Wei Wang4
Bo Xu1
Qian Zhong1
Xin-Tao Shuai4
Li-Qun Yang4
Yu-Bin Deng1

1Department of Pathophysiology, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, China; 2Futian Affiliated Hospital, Guangdong Medical College, Shenzhen, China; 3Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China; 4Institute of Polymer Science, School of Chemistry and Chemical Engineering, BME Center, State Key Laboratory of Optoelectronic Materials and Technologies, Sun Yat-sen University, Guangzhou, China; 5Yunnan Cancer Hospital, The Third Affiliated Hospital, Kunming Medical College, Kunming, China

*Both authors contributed equally to this work

Background: Mesenchymal stem cell transplantation is a promising method in regenerative medicine. Gene-modified mesenchymal stem cells possess superior characteristics of specific tissue differentiation, resistance to apoptosis, and directional migration. Viral vectors have the disadvantages of potential immunogenicity, carcinogenicity, and complicated synthetic procedures. Polyethylene glycol-grafted polyethylenimine (PEG-PEI) holds promise in gene delivery because of easy preparation and potentially targeting modification.

Methods: A PEG8k-PEI25k graft copolymer was synthesized. Agarose gel retardation assay and dynamic light scattering were used to determine the properties of the nanoparticles. MTT reduction, wound and healing, and differentiation assays were used to test the cytobiological characteristics of rat mesenchymal stem cells, fluorescence microscopy and flow cytometry were used to determine transfection efficiency, and atomic force microscopy was used to evaluate the interaction between PEG-PEI/plasmid nanoparticles and mesenchymal stem cells.

Results: After incubation with the copolymer, the bionomics of mesenchymal stem cells showed no significant change. The mesenchymal stem cells still maintained high viability, resettled the wound area, and differentiated into adipocytes and osteoblasts. The PEG-PEI completely packed plasmid and condensed plasmid into stable nanoparticles of 100–150 nm diameter. After optimizing the N/P ratio, the PEG-PEI/plasmid microcapsules delivered plasmid into mesenchymal stem cells and obtained an optimum transfection efficiency of 15%–21%, which was higher than for cationic liposomes.

Conclusion: These data indicate that PEG-PEI is a valid gene delivery agent and has better transfection efficiency than cationic liposomes in mesenchymal stem cells.

Keywords: stem cells, gene delivery, nanoparticles, atomic force microscopy

Introduction

Stem cells are clonal precursors of nonhematopoietic tissue, and possess the capability of self-renewal and differentiation into various tissue cells, such as osteoblasts, chondrocytes, astrocytes, neurons, and adipocytes. Among several categories of stem cells, mesenchymal stem cells are used widely in regenerative medicine because of their multilineage differentiation, secretion of many cytokines and trophic factors, low immunogenicity, and freedom from ethical controversy. Mesenchymal stem cells can be safely introduced into the brain, migrate directionally towards injured tissue, and can promote functional recovery. Transplanted mesenchymal stem cells are currently under intensive investigation in regenerative medicine as potential cell-based therapies for neurological diseases or injuries. Gene-modified mesenchymal stem
cells possess superior characteristics of specific tissue differentiation,11 resistance to apoptosis,12 and directional migration.13

Viral gene delivery vectors, such as retroviruses, adenoviruses, and adeno-associated viruses, are extensively used for gene delivery and show superior transfection efficiency.14,15 However, viral vectors have shown the disadvantages of potential immunogenicity and carcinogenicity, and have a complicated synthesis procedure. Recently, nonviral gene vectors, including liposomes and cationic polymers, have received great attention because of their easy preparation, lack of immunogenicity, and ability to be modified for potential targeted delivery.16–18 Among the cationic polymers, polyethylenimine was reported to be an effective transfection reagent due to easy synthesis and multiple modifications.19,20 Polyethylenimine concentrates negatively charge DNA via electrostatic interaction and form polyethylenimine/DNA nanoparticles. They enter nuclei through several steps, including cellular uptake, escaping from nucleases, endosomal release, and DNA decondensation inside nuclei.21 However, excessive usage of polyethylenimine results in high cytotoxicity, which is the major limitation in polyethylenimine-mediated gene delivery.22 Furthermore, serum-free culture media is preferred in transfection experiments in vitro to avoid the interaction between polyethylenimine and serum protein. In addition, serum deprivation could induce apoptosis of mesenchymal stem cells.23 Therefore, covalent linkage with nonionic and hydrophilic polymers, such as polyethylene glycol, has been used to minimize the toxicity of polyethylenimine.24–26 Polyethylene glycol has also improved the solubility of polyethylene glycol-grafted polyethylenimine (PEG-PEI) complexes, minimized aggregation, and reduced nonspecific interactions with proteins in physiological fluids.27–29

To our knowledge, there are few studies reported on the use of PEG-PEI to prepare PEG-PEI/DNA nanoparticles for gene delivery into mesenchymal stem cells. In this work, PEG-PEI was synthesized, and its toxicity and biocompatibility were evaluated by MTT, wound and healing, and cell differentiation assays using mesenchymal stem cells. Plasmid-encapsulated PEG-PEI (PEG-PEI/plasmid) nanoparticles were prepared, and their properties were investigated by agarose gel retardation assay and dynamic light scattering analysis. The gene transfection efficiency was assessed by inverted fluorescence microscopy and flow cytometry. The interaction between plasmid-encapsulated PEG-PEI nanoparticles and rat mesenchymal stem cells was studied by atomic force microscopy.

Materials and methods

Materials

Polyethylenimine with a molecular weight of 25 kDa was bought from Sigma-Aldrich (St Louis, MO). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Dulbecco’s modified Eagle’s medium/F-12 (DMEM) were obtained from Gibco (Gibco, Carlsbad, CA). Adipogenic and osteogenic media were bought from Cyagen Biosciences, China. PureYield™ Plasmid Midi Prep System was bought from Promega (Madison, WI). Lipofectamine™ was bought from Beyotime Institute of Biotechnology, China.

Synthesis and characterization of PEG8k-PEI25k

Methoxy N-hydroxysuccinimide polyethylene glycol (mPEG2k-NHS) was synthesized according to our previous work.30 PEG8k-PEI25k was synthesized by adding 1.25 g of hyperbranched polyethylenimine and 0.5 g of mPEG-NHS to phosphate-buffered saline (pH 7.4), and the solution was magnetically stirred at room temperature overnight. The resulting solution was purified by membrane dialysis (molecular weight cutoff 3.5 kDa) in distilled water for 48 hours, and then lyophilized to obtain PEG8k-PEI25k. PEG-PEI was characterized by 1H-NMR (Varian Mercury 300-mHz NMR spectrometer, Mountain View, CA) in deuterium oxide. Fourier transform infrared measurement was carried out using a Fourier transform infrared analyzer (Nicolet/Nexus 670, Woodland, CA) at a resolution of 4 cm⁻¹ using the KBr method.

Preparation and properties of plasmid-encapsulated PEG-PEI nanoparticles

Preparation

The plasmid-enhanced green fluorescent protein (pEGFP-C1, 4.7 kb) was expanded in Escherichia coli (E. coli strain DH5α) and purified with the Plasmid Midi Prep System following the manufacturer’s protocol. The quantity and quality of the purified plasmid were assessed by optical density at 260 nm and 280 nm, and by electrophoresis in 0.8% agarose gel. The purified plasmid was resuspended in ultrapure water and kept in aliquots at a concentration of 0.7 mg/mL. The plasmid solution, and the desired amount of PEG-PEI solution in accord with the required N/P ratio (molar ratio of the positive amino groups of polyethylenimine to the phosphoric anions of plasmid) were separately diluted with 0.9% NaCl to 100 μL. The copolymer solution was then
added to the plasmid solution and mixed by incubating at 200 rpm at room temperature for 30 minutes. The complexes obtained were kept standing at room temperature for 30 minutes before use.

Agarose gel retardation assay
To condense 1 μg of plasmid, the plasmid-encapsulated PEG-PEI complexes were prepared at designated N/P ratios of 0, 0.5, 1, 1.5, 2, 2.5, 3, and 4 without dilution. Loading buffer was added to the samples. The samples were loaded onto 0.8% agarose gel stained with 3 μL ethidium bromide solution (10 μg/μL), and electrophoresed at 90 V for 45 minutes. An ultraviolet image station (Olympus, Japan) was used to record the gel images.

Dynamic light scattering analysis
To condense 1 μg of plasmid, plasmid-encapsulated PEG-PEI was prepared at designated N/P ratios of 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 7, 10, 15, 20, 25, and 30. The plasmid solution and the desired amount of PEG-PEI solution in accord with the required N/P ratio were separately diluted with ultrapure water to 100 μL. The copolymer solution was then added to the plasmid solution and mixed by incubation at 200 rpm and room temperature for 30 minutes in order to form complexes. The size of the nanoparticles was determined by a Zetasizer 3000HS (Malvern, UK) at specified time intervals at room temperature. Samples were run in triplicate.

Cytobiological assessment of rat mesenchymal stem cells
Cell culture
The rat mesenchymal stem cells were harvested from the femurs and tibias of four-week-old (about 60 g) female Sprague-Dawley rats (Center of Experimental Animal of Sun Yat-sen University, Guangzhou). The rats were sacrificed by cervical dislocation. Briefly, the bones were excised aseptically from the hind limbs of rats and placed in high-glucose DMEM after removing the parenchyma. After the knee joint end of each bone was removed using sterile scissors to create a hole, the bone marrow was flushed from the shaft with DMEM using a 26 gauge needle. The marrow solution was harvested by 5000 rpm centrifugation and aspiration of the supernatant, then the precipitate was resuspended in DMEM (supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 g/mL streptomycin), and seeded into two 25 cm tissue culture flasks. After 36 hours, the flasks were rinsed three times with phosphate-buffered saline to remove the nonadherent cells. The medium was exchanged every three days throughout the studies. Twenty-four hours before the transfection experiments, adherent cells were rinsed with phosphate-buffered saline, trypsinized, and seeded in 24-well plates with complete DMEM without antibiotics. Cells were incubated at 37°C in a fully humidified atmosphere of 5% CO2.

MTT assay
The rat mesenchymal stem cells were seeded in 96-well plates at an initial density of 5000 cells/well in 200 μL of complete DMEM and cultured for 24 hours prior to adding PEG-PEI. The growth medium was replaced by complete DMEM containing different amounts of copolymers corresponding to delivery of 1 μg of plasmid. Each concentration was replicated in three wells. Cells incubated without treatment were considered as controls, and cell viability values were set at 100%. After incubation for four, eight, 12, and 24 hours, respectively, the medium was changed to normal culture medium. Cells were incubated for another 48 hours. For cell viability assay, 20 μL of sterile, filtered MTT stock solutions in phosphate-buffered saline (5 mg/mL) was added to each well. After four hours, the unreacted dye was removed, and the formazan crystals were dissolved with 150 μL of dimethyl sulfoxide. After gentle agitation for five minutes, the absorbance was read at 570 nm using a DU730 nucleic acid/protein analyzer (Beckman Coulter, Brea, CA).

Wound and healing assay
The rat mesenchymal stem cells were plated on six-well plates until they reached a confluence of 80%-90% with complete DMEM containing PEG-PEI 14 μg/mL. A cell scraper was used to prepare a scratch across the layer of rat mesenchymal stem cells, as described previously by Faber-Elman et al.31 Rat mesenchymal stem cells were incubated at 0, two, four, and eight hours after scratching, and the time-dependent percentages of the scratched area were calculated.

Stem cell differentiation assay
After being incubated in complete DMEM containing PEG-PEI 14 μg/mL, the multilineage differentiation potential of the rat mesenchymal stem cells was assessed by testing their ability to differentiate into adipocytes and osteoblasts. Briefly, adipocyte and osteoblast differentiation was achieved after one week of culture of rat mesenchymal stem cells with adipogenic and osteogenic medium, respectively (Cyagen Biosciences, China). Oil-red-O and silver nitrate were used to identify the adipocytes and osteoblasts, respectively.
Gene transfection experiments
The rat mesenchymal stem cells were seeded in six-well plates at a density of $2 \times 10^5$ cells per well, and cultured about 24 hours before transfection until they reached 70%–80% confluence. Plasmid 4 µg was utilized for making a series of N/P ratios. The plasmid-encapsulated PEG-PEI complexes were prepared at N/P ratios of 0–50, and the cationic liposome (Lipofectamine)/plasmid complexes were used as controls. Cells were incubated for eight hours with each complex. The cells were then washed and replated with complete DMEM and incubated at 37°C in 5% CO₂ atmosphere for 48 hours. The expression of pEGFP-C1 was observed by fluorescence microscopy (Nikon, Tokyo, Japan). Fluorescence was detected using 520 nm and 570 nm band pass filters for pEGFP-C1. The transfection efficiency of cells expressing pEGFP-C1 was determined using a FACS Aria™ System from Becton-Dickinson (San Jose, CA). After being photographed by fluorescence microscopy, the cells were washed with phosphate-buffered saline and collected with a cell scraper and resuspended in phosphate-buffered saline for flow cytometry assay. Fluorescence parameters from approximately 10,000 cells were acquired, and transfection experiments were carried out in triplicate. Data analysis was carried out by using the WinMDI program.

Interaction of plasmid-encapsulated PEG-PEI and rat mesenchymal stem cells
The rat mesenchymal stem cells were seeded on cover slips and incubated with plasmid-encapsulated PEG-PEI for six hours. The rat mesenchymal stem cells were then washed with phosphate-buffered saline, fixed with 2.5% glutaraldehyde for 15 minutes, washed with distilled water, and dried at room temperature. The samples were scanned with a UL20B silicon probe on atomic force microscopy (Autoprobe CP Research, Thermomicroscopes, Goleta, CA) and analyzed by atomic force microscopy original software (IP2.1). The measurements were carried out in contact mode.

Results and discussion
Synthesis and characterization of PEG-PEI copolymer
On Fourier transform infrared spectrum analysis of the PEG-PEI copolymer (shown in Figure 1), strong absorption around 1657 cm⁻¹ appeared and was attributed to amide linkage, and a strong and broad peak around 3400 cm⁻¹ was attributed to the characteristic absorption of amines. These results indicated that polyethylene glycol was grafted onto the polyethylenimine chains through amide linkage.

Toxicity and biocompatibility of PEG-PEI copolymer
The superior transfection efficiency was based on cell viability. The cytotoxicity of PEG-PEI was related to copolymer concentration and incubation time in the culture medium. PEG-PEI showed minimal toxicity below a concentration of 8.75 µg/mL, corresponding to condensed 4 µg DNA at an N/P ratio of 25 in the six-well plate. Figure 3 shows the influence of rat mesenchymal stem cell viability on PEG-PEI concentration and incubation time. The results indicate that rat mesenchymal stem cells still maintained high viability after being incubated with PEG-PEI 14 µg/mL (corresponding to N/P 40) for 4–8 hours. However, cell viability attenuated remarkably when the cells were incubated, PEG-PEI concentration exceeded 14 µg/mL, and incubation time was longer than eight hours. Because superior transfection efficiency depends on cell viability, cytotoxicity was the main cause of the transfection efficiency decrease at an N/P ratio of 50.
We also assessed whether the migration and differentiation ability of stem cells was influenced when they were incubated with PEG-PEI. The wound and healing assay was designed to simulate the ability of stem cells to reconstruct a tissue. Rat mesenchymal stem cells incubated with culture medium containing PEG-PEI 14 µg/mL for eight hours migrated to the scratched area and resettled (Figure 4). The migration velocity of rat mesenchymal stem cells incubated with PEG-PEI for less than four hours did not change significantly compared with those incubated with DMEM. This incubation time-dependent regularity was consistent with that of the cell viability test. Furthermore, after incubation with PEG-PEI 14 µg/mL for eight hours, the rat mesenchymal stem cells were induced to differentiate into adipocytes and osteoblasts. The N/P ratio of 40 was considered to be safe for stem cells of multiple potency for differentiating to adipocytes and osteoblasts (Figure 5). In summary, the PEG-PEI copolymer exhibited low toxicity and good biocompatibility at a concentration of 14 µg/mL.

**Properties of plasmid-encapsulated PEG-PEI nanoparticles**

To demonstrate the interaction between the positive PEG-PEI and the negative plasmid, a gel retardation assay was performed using electrophoresis. A typical result obtained during these electrophoresis experiments for the copolymer is presented in Figure 6A. Gel electrophoresis showed that PEG-PEI completely bound with plasmid at an N/P ratio of 3. Moreover, PEG-PEI condensed plasmid to form stable nanoparticles 100–200 nm in diameter at an N/P ratio of 15 (Figure 6B). In order to obtain nanoparticles effective for packing and delivery of plasmid, an N/P ratio >15 was chosen. Plasmid-encapsulated PEG-PEI nanoparticles with
low toxicity and good compatibility for gene delivery are anticipated to achieve a long circulation time in blood.

Gene transfection efficiency of plasmid-encapsulated PEG-PEI nanoparticles

Mesenchymal stem cells are attractive targets in gene therapy for several human diseases. However, their ability to internalize and express exogenous DNA via nonviral gene delivery vehicles has been poorly investigated so far. Although the feasibility of using liposome and other lipid-based gene carriers for transfecting many cell lines has been confirmed, there has been little research reported on cationic copolymer PEG-PEI-mediated transfection in stem cells. Here, we evaluated the transfection efficiency of PEG-PEI for delivering plasmid to rat mesenchymal stem cells. Passage 2–4 rat mesenchymal stem cells were used for the transfection experiments. The cationic liposome is a commercial nonviral gene delivery reagent and has been commonly used in transfecting many cell lines, so the transfection efficacy of cationic liposome was used as a positive control.

Forty-eight hours after transfection, the green fluorescence of the enhanced green fluorescent protein (EGFP) was observed qualitatively using fluorescence microscopy and

Figure 5 The multilineage potential of rat mesenchymal stem cells after incubation with the copolymer.

Note: After incubating with PEG-PEI 14 µg/mL for eight hours, rat mesenchymal stem cells were induced to differentiate into adipocytes (A red cells) and osteoblasts (B black cells). Original magnification × 400.

Abbreviation: PEG-PEI, polyethylene glycol-grafted polyethylenimine.
transfection efficacy(I) and has better transfection efficacy
than does the cationic liposome. These results suggest that PEG-PEI is a
valid gene delivery agent and has better transfection efficacy.

Moreover, the increase in the N/P ratio of PEG-PEI to DNA resulted in increased gene expression in rat mesenchymal stem cells, but the transfection efficacy degraded remarkably at an N/P ratio of 50. Considering the cytotoxicity of PEG-PEI concentrations above 14 μg/mL, cell death and limited cell viability were the main causes of the lower transfection efficiency at an N/P ratio of 50.

Our results for the gene transfection efficiency of plasmid-encapsulated PEG-PEI nanoparticles is in agreement with that previously reported.33–36 The feasibility of using polyethyleneimine for transfection of many cell lines, in addition to liposomal and other lipid-based carriers, has been confirmed,33 but several researchers have demonstrated that it is difficult for polyethyleneimine to obtain higher transfection efficiency than a viral vehicle.34–36 Farrell et al34 reported that the 48-hour transfection efficiency of polyethyleneimine-mediated gene delivery to rat mesenchymal stem cells is below 4% at an N/P ratio of 7. Ahn et al35 reported that introducing DNA/PEI nanoparticles into rat mesenchymal stem cells resulted in 2%–10% transfection efficiency. In human adipose-derived stem cells, polyethyleneimine produced the highest transfection of 19% at an N/P ratio of 8, which was superior to that achieved by Lipofectamine.36

Cell internalization is correlated with the buffer action of the cell membrane reservoir, the functioning of the cytoskeleton, and the location of the nanomicrocapsules within cells. The interaction between nanomicrocapsules and various cell lines may be very different. The mechanical properties of stem cells, including cell membrane tension and adhesion, and cytoskeleton molding and elasticity, are different from those of terminally differentiated cells.37,38 Moreover, Farrell et al34 found that less than 10% of PEI/DNA or poly-L-lysine/DNA intake by mesenchymal stem cells occurred in the nuclei. It is suggested that relatively low gene internalization and expression in rat mesenchymal stem cells during cationic polymer-mediated gene delivery might be caused by their low uptake capacity and the distinct cytoskeleton mechanism involved in cellular trafficking.39

Superior transfection efficiency was obtained at the optimized N/P ratio of 40, which was much more than the N/P ratio of 15 needed for stable nanoparticle formation, implying that there was much dissociative PEG-PEI in the culture medium. Dissociative PEG-PEI did not pack and deliver plasmid, but probably enhanced the permeability of the cell membrane, activated cell membrane components, and indirectly increased endocytosis.40

Figure 6 Electrophoretic migration and dynamic diameter distribution of PEG-PEI/EGFP-C1 nanoparticles at various N/P ratios.

Abbreviations: PEG-PEI, polyethylene glycol-grafted polyethylenimine; EGFP, enhanced green fluorescent protein.
Interaction of nanoparticles and rat mesenchymal stem cells

To highlight the interactions between plasmid-encapsulated PEG-PEI microcapsules and the cell membrane, atomic force microscopy were used to scan the ultrastructure of rat mesenchymal stem cells in the 1–70 µm range. Figures 8A and 8B showed a typical rat mesenchymal stem cell which is fusiform-shaped with a well-arranged cytoskeleton. Although some small introcessions can be detected, the surface of the nuclear region and the boundary of the cell are relatively smooth (Figures 8C and 8D).

After six hours’ incubation of plasmid-encapsulated PEG-PEI, nanoparticles 200–400 nm in diameter adsorb onto the cell membrane (Figures 8E–8H) by concentrating negatively charged plasmid onto the positively charged nanoparticles. Some samples were washed with phosphate-buffered saline and cultured for another six hours. Some biggish hollows were observed (Figures 8I–8L). Compared with the pits on the control cells, the hollows are fewer, deeper, and presumed to reflect cytophagocytosis of the plasmid-encapsulated PEG-PEI nanoparticles (Figures 8M and 8N). The interactions between the plasmid-encapsulated PEG-PEI and the cytoskeleton of rat mesenchymal stem cells will be investigated further in the future. New insights into the behavior of nanoparticles might guide improvements in vector design for clinical application, such as improved interaction with the cytoskeleton by promoting binding of motor proteins (see Figure 8).
Figure 8  Cellular uptake of plasmid-encapsulated PEG-PEI microcapsules observed by atomic force microscopy.

Note: The samples were scanned in the range of 1–70 µm with a UL20B silicon probe on atomic force microscopy and analyzed by original software (IP2.1). The measurements were carried out in contact mode. Typical passage 4 rat mesenchymal stem cells comprised the controls (A, B), and the surface of the nuclear region and the boundary of the cell are relatively smooth (C, D). After six hours of plasmid-encapsulated PEG-PEI incubation, nanoparticles of 200–400 nm diameter adsorb onto the cell membrane (E, F). Some samples were washed with phosphate-buffered saline and cultured for another six hours. Some biggish hollows can be observed (G, H, I, and J). Simulation at three dimensions of (I and J) are showed in (K and L) separately.

Abbreviation: PEG-PEI, polyethylene glycol-grafted polyethylenimine.

Conclusion
Our results demonstrate that polyethylenimine modified with polyethylene glycol sufficiently condensed plasmid into nanoparticles. After optimizing the N/P ratio for plasmid delivery, PEG-PEI/pEGFP-C1 nanoparticles displayed low cytotoxicity, good solubility, and compatibility with serum. The migration velocity and multiple differentiation potency of rat mesenchymal stem cells were not affected after exposure to the copolymers during 48 hours of transfection. PEG-PEI/DNA at an N/P ratio of 40 obtained the highest efficiency (21%) in rat mesenchymal stem cells, which was better than for cationic liposomes.

Our data indicate that PEG-PEI is a valid gene vehicle and an even better choice for transfecting rat mesenchymal stem cells than conventional liposomes. PEG-PEI is a representative synthetic gene vector and displays superiority in many situations. Biocompatibility was guaranteed by dense polyethylene glycol shells, which endowed the nanoparticles with water solubility and prevented their interaction with serum protein in the culture medium. At an optimum N/P ratio, PEG-PEI also may have the advantage of lower toxicity and better adsorption properties than liposomes. This makes PEG-PEI a promising gene vector in vivo, with PEGylation preventing aggregation of PEI/DNA complexes under physiological conditions.

Acknowledgments
This work was supported by the Sun Yat-sen University Postgraduates’ Training Project, the Yi-xian Innovators’
Training Project by Outstanding Mentor(s) at Sun Yat-sen University (NO:50000-3126203), the Graduates’ internationally cooperated research project of Sun Yat-sen University (2008–2009), the National Natural Science Foundation of China, and the Science and Technology Planning Project of Guangdong Province, China.

Disclosure
The authors report no conflicts of interest in this work.

References


