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Stimulation of Gene Transfection by Silicon Nanowire Arrays Modified with Polyethylenimine

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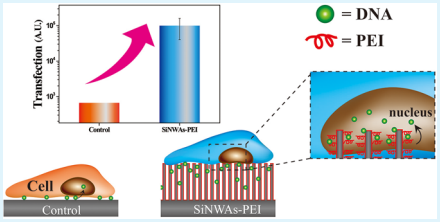
Supporting Information

ABSTRACT: In this work, a novel gene delivery strategy was proposed based on silicon nanowire arrays modified with high-molecular-weight 25 kDa branched polyethylenimine (SN-PEI). Both the plasmid DNA (pDNA) binding capacity and the in vitro gene transfection efficiency of silicon nanowire arrays (SiNWAs) were significantly enhanced after modification with high-molecular-weight bPEI. Moreover, the transfection efficiency was substantially further increased by the introduction of free pDNA/PEI complexes formed by low-molecular-weight branched PEI (bPEI, 2 kDa). Additionally, factors affecting the in vitro transfection efficiency of the novel gene delivery system were investigated in detail, and the transfection efficiency was optimized on SN-PEI with a bPEI grafting time of 3 h, an incubation time of 10 min for tethered pDNA/PEI complexes consisting of high-molecular-weight bPEI grafted onto SiNWAs, and with an N/P ratio of 80 for free pDNA/PEI complexes made of low-molecular-weight bPEI. Together, our results indicate that high-molecular-weight bPEI modified SiNWAs can serve as an efficient platform for gene delivery.



KEYWORDS: *SiNWAs, SN-PEI, polyethylenimine, surface modification, gene transfection, cytotoxicity*

1. INTRODUCTION

Gene therapy, the introduction of exogenous DNA into somatic cells of a patient that results in a therapeutic effect, is one of the most important milestones in biomedical development. Currently, the clinical study of gene therapy has expanded from genetic diseases to cancer, cardiovascular diseases, and many other incurable diseases.[1−3] A key issue for successful gene therapy is the selection of a suitable gene vector, which should have good biocompatibility, a high DNA binding capacity, and targeting activity toward a specific tissue or cell type to ensure safe, efficient, and targeted expression of the exogenous DNA.[4−6]

Although most gene delivery therapeutics in clinical trials are based on viral vectors, safety issues remain a major concern. Over the past few decades, nonviral vectors have attracted great attention in research worldwide for their facile synthesis, flexibility in construction, easy modification for targeted gene delivery, and a carrying capacity with no limitations for DNA size, among other advantages.[7,8] Polycationic polymers, such as polyethylenimine (PEI),[9,10] poly(2-(dimethylamino) ethyl methacrylate) (PDMAEMA),[11,12] and chitosan,[13,14] are the most widely used nonviral vectors and have been studied extensively. However, they are still much less efficient than their viral counterparts.[15] Moreover, they have poor biodegradability, a short time window for efficient expression, and tend to aggregate in vivo, which can cause high cytotoxicity.[16,17] These disadvantages of polycationic polymers strongly limit their application in clinical trials.

Substantial effort has been made to improve the transfection efficiency and decrease the cytotoxicity of polycationic polymers. Currently, surface-mediated gene delivery has been approved as an effective method.[18−20] Compared to conventional gene delivery, in which the vectors are dissolved in aqueous media, the transfection efficiency of surface-mediated gene delivery can be enhanced by modifying the surface in various ways. For example, in a study by Li et al.,[21] polycaprolactone (PCL) surfaces functionalized by (PDMAEMA)/gelatin complexes exhibit substantially enhanced gene transfection efficiency after the adsorption of pDNA. Moreover, Holmes et al. discovered that polyelectrolyte multilayers composed of glycol-chitosan and hyaluronic acid can embed lipoplexes containing plasmid DNA and enable surface-mediated gene delivery.[22] For surface-mediated gene delivery, the key hurdle is whether the exogenous DNA can effectively spread in the cytoplasm and cross the nuclear membrane into the nucleus.[23] Thus, it is of vital importance to select suitable materials with surface properties that not only support cell adhesion and proliferation but also assist the entrance of exogenous DNA into cells or even deliver DNA directly into the cell nucleus.

Vertically aligned silicon nanowire arrays (SiNWAs) have been reported to be efficient in penetrating cells attached to the surface, in particular the cell nuclei.[24−26] Moreover, SiNWAs

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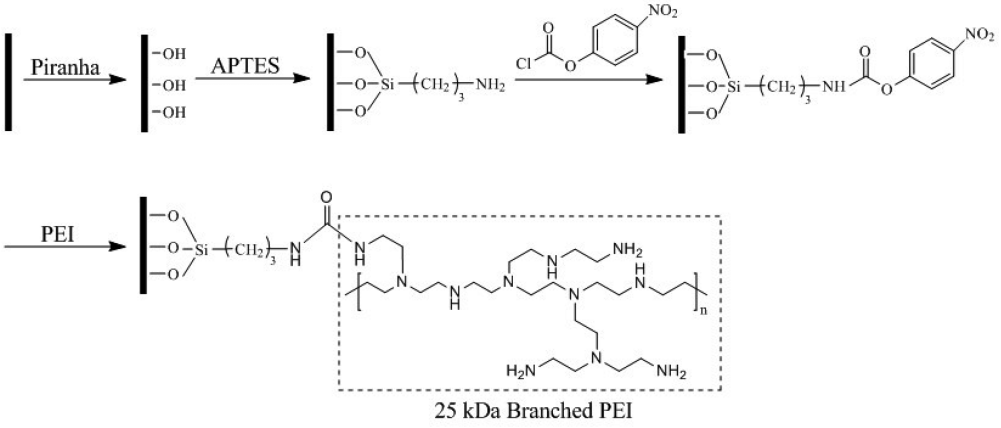
Scheme 1. Surface Modification of SiNWAs



enable the direct delivery of a broad range of loaded biological effectors (drugs, DNAs, and RNAs, among others) into cells and cell nuclei and achieve beneficial therapeutic effects.[26,27] Furthermore, SiNWAs show good biocompatibility and can be used in the culture of various kinds of cells.[25,26] However, the gene transfection efficiency of SiNWAs alone is quite low, less than 1%,[26] which might be caused by the layer of silicon oxide on unmodified SiNWAs leading to restricted gene loading capacity. This disadvantage limits its direct use for gene delivery.

It has been shown that the surface properties of SiNWAs can be improved by various polymer surface modification technologies. Through surface modification, we can not only enhance gene transfection efficiency but also promote targeting ability toward specific proteins, nucleic acids, and cells, as well as improving biocompatibility.[28−30] Building on the studies mentioned above, in the present work, we report a novel gene delivery strategy based on SiNWAs using surface modification with high-molecular-weight branched polyethylenimine (bPEI, 25 kDa), which is considered one of the most efficient candidates for gene delivery and often serves as the "gold standard".[9,10] Our results revealed that the transfection efficiency of SiNWAs modified with 25 kDa bPEI (SN-PEI) was significantly enhanced compared to unmodified SiNWAs. In addition, the transfection efficiency can be further improved by adding free pDNA to a complex with low-molecular-weight bPEI (2 kDa) to the gene delivery system and modulating the bPEI grafting density on SiNWAs, as well as optimizing the incubation time for pDNA/PEI complexes formed by high-molecular-weight bPEI grafted onto SiNWAs. Our strategy combined the high DNA loading capability of polymers and the efficient cell-penetrating ability of SiNWAs. In this way, the efficient delivery of the exogenous DNA directly into the cell nuclei and a substantial reduction in cytotoxicity caused by the aggregation of free polymer vectors was achieved. Moreover, efficient and long-term gene delivery was obtained by continuously supplying free pDNA/PEI complexes formed by low-molecular-weight bPEI.

2. MATERIALS AND METHODS

Silicon wafers [n-doped, (100)-oriented, 0.56 mm thick, 100 mm diameter] were purchased from Guangzhou Semiconductor Materials (Guangzhou, China). The silicon wafers polished on one side were cut into square chips of approximately 0.5 cm × 0.5 cm. 3-Aminopropyltriethoxysilane (APTES) and 4-nitrophenyl chloroformate (NPC, 95%) were purchased from Sigma-Aldrich. Polyethylenimine (PEI, branched, MW 2 kDa and 25 kDa) was purchased from Sigma-Aldrich. Triethylamine (TEA) and all of the other chemical and biological reagents were purchased from Shanghai Chemical Reagent Co. and purified according to standard methods before use. Deionized water purified with a Millipore water purification system to give a minimum resistivity of 18.2 MΩ cm was used for all experiments. Nitrogen gas was of high purity grade.

Preparation of SiNWAs Modified with 25 kDa bPEI (SN-PEI). SiNWAs were prepared as described previously using the chemical etching method.[31] Briefly, cleaned silicon wafers were immersed in an etching solution containing HF (5 M) and $AgNO_3$ (0.015 M) at 50 °C for 10 min. The resulting materials were immersed in 20% nitric acid for 1 min and then rinsed extensively with deionized water.

For the surface modification of SiNWAs with 25 kDa bPEI, SiNWAs were washed with "Piranha" solution [H_2SO_4:H_2O_2 = 7:3 (v/v)] at 90 °C for 2 h to obtain a clean surface with hydroxyl groups. After rinsing with an excess amount of deionized water and drying in a nitrogen stream, the freshly prepared hydroxyl-terminated silicon wafers were immersed in 20 mL of anhydrous toluene solution containing 0.4 mL of APTES at 80 °C for 18 h to obtain chemically bonded $-NH_2$ groups, followed by washing with toluene, dichloromethane, methanol, deionized water, and acetone and then drying under a nitrogen stream. The amino-functionalized surfaces were further incubated in 10 mL of anhydrous acetonitrile solution containing NPC (0.51 g) and 0.36 mL of TEA. The reaction was performed at 30 °C under nitrogen protection for an additional 18 h. The NPC-activated SiNWAs were cleaned with acetonitrile, deionized water, and acetone and then dried under a nitrogen stream. Finally, bPEI was grafted to the SiNWAs-NPC surfaces by immersing the samples into an aqueous solution containing bPEI (25 kDa, 5 mg/mL) at 30 °C for a specific reaction time. The bPEI-modified surfaces were washed with abundant deionized water and acetone to remove the unreacted PEI and dried under a nitrogen stream. The general process for SN-PEI is illustrated in Scheme 1.

SEM and TEM. The topology of the unmodified SiNWAs and SN-PEI was characterized using scanning electron microscopy (SEM, S-4700, Hitachi). Transmission electron microscopy (TEM) characterization of SiNWAs and SN-PEI was performed using a JEOL JEM-2100 TEM operating at 200 kV.

Water Contact Angle. The surface wettability of the unmodified and modified SiNWAs at each modification step was determined with an SL200C optical contact angle meter (USA Kino Industry Co., Ltd.) using the sessile drop method at room temperature.

Density of 25 kDa bPEI Grafted to SiNWAs. The density of bPEI grafted to SiNWAs was determined by reacting the amino groups on the surface with 4-nitrobenzaldehyde to form imines, followed by hydrolysis to yield 4-nitrobenzaldehyde.[32] Typically, four discs of SN-PEI with different bPEI grafting times were immersed in 20 mL of anhydrous ethanol containing 20 mg of 4-nitrobenzaldehyde and 0.016 mL of acetic acid at 50 °C for 3 h. After washing in absolute ethanol

for 2 min and drying under a nitrogen stream, the samples were immersed in 3.5 mL of water containing 0.007 mL of acetic acid, and the solution was incubated at 40 °C for 3 h. The amount of 4-nitrobenzaldehyde liberated, which is equivalent to the surface amine content, was determined by measuring the absorbance at 268.5 nm.

Cell Culture. HeLa and L929 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, Hyclone) and RPMI-1640 medium (Hyclone), respectively. All media were supplemented with 10% fetal bovine serum (FBS, Gibco), 100 U/mL penicillin (Genview) and 100 μg/mL streptomycin (Solarbio). The cells were incubated at 37 °C with 5% CO_2 in the humidified chamber and the medium was changed every other day.

Amplification and Purification of Plasmid DNA. The plasmid used was pRL-CMV (Promega, USA), which encodes the *Renilla* luciferase reporter gene originally cloned from the marine organism *Renilla reniformis*. The plasmid DNA was amplified in *Escherichia coli* and purified using the TIANprep Midi Plasmid Kit (TIANGE, China) according to the manufacturer's protocol. The purity and concentration of the purified plasmid DNA was determined by agarose gel electrophoresis and by measuring the absorption at 260 and 280 nm.

Measurement of Sample DNA Binding Capacity. The DNA-free SiNWAs and SN-PEI samples were immersed in 200 μL NaCl solution (150 mM) containing a specific amount of plasmid pRL-CMV for 30 min to ensure maximum loading of the pDNA. After removal of the sample, the solution was transferred into a 96 well UV microplate to read the absorbance at 260 nm. The pDNA concentration after sample adsorption was determined by comparison to a standard curve calibrated with pDNA samples of known concentrations, which was previously acquired.

In Vitro Gene Transfection and Luciferase Assay. Transfection studies were performed in HeLa cells using the plasmid pRL-CMV as a reporter. In brief, sterile SiNWAs and SN-PEI were placed into a 48 well plate. pDNA (1.5 μg) was added to all sample surfaces and incubated for a specific amount of time. Subsequently, cells were seeded at a density of 5×10^4 cells/well and cultured for 3 h under serum-free condition. bPEI (2 kDa, 50 mM)/DNA complexes (20 μL/well containing 1.5 μg of pDNA) at various N/P ratios were prepared by the dropwise addition of the PEI solution into the DNA solution, followed by thorough mixing and incubation for 10 min at room temperature. At the time of transfection, the complexes were added into each well and further incubated in media containing 10% FBS for 24 h at 37 °C with 5% CO_2 in the humidified chamber. Before the luciferase assay, the cells were washed twice with PBS and lysed with 65 μL of cell culture lysis reagent (Promega, USA) per well. Luciferase expression was quantified using a commercial kit (Promega, USA) according to the manufacturer's protocol and a microplate reader (Thermo Fisher Scientific, Inc.). Protein concentrations in the samples were analyzed using the Coomassie brilliant blue method.[33] Adsorption was measured on a microplate reader (Thermo Fisher Scientific Inc.) at 570 nm and compared to a standard curve calibrated with bovine serum albumin (BSA) samples of known concentration. The results were expressed as relative light units per milligram of cell protein lysate (RLU/mg protein).

Cell Viability Assay. For cell viability assays, L929 cells were seeded at a density of 2×10^4 cells/well on various samples placed in a 48-well plate (Nunc). After incubation for 24 h, the cells attached to the sample surfaces were washed twice with sterilized phosphate buffered saline (PBS, pH 7.4) and stained with live dye 5-chloromethylfluororescein diacetate (CM-FDA, Invitrogen).Images were captured using an Olympus IX71 fluorescence microscope, and the density of adherent cells was determined by Image-Pro Plus 6.0 software.

HeLa cells were adjusted to a density of 2×10^4 cells/mL and seeded on different samples plated in a 48 well plate (Nunc). After 48 h of incubation, the cells were washed twice with sterilized PBS and stained using the live/dead viability/cytotoxicity kit (Invitrogen) according to the manufacturer's protocol. Briefly, 20 μL of EthD-1 stock solution (2 mM) was added to 10 mL of sterile, tissue-culture-grade D-PBS and vortexed to ensure thorough mixing. After adding 5 μL of calceinAM stock solution (4 mM) and mixing thoroughly, we added 200 μL of the diluted solution to each well to stain the adherent cells for all samples. After incubation for 20 min, images were captured using an Olympus IX71 fluorescence microscope, and the viability of adherent cells was determined by Image-Pro Plus 6.0 software.

Statistical Methods. Each independent experiment was performed at least three times, and the results were presented as the mean ± standard error (SE). P-values less than 0.05 were considered to be significant.

3. RESULTS AND DISCUSSION

Surface Characterization of SiNWAs and SN-PEI. *SEM and TEM.* SEM image showed the topography of SiNWAs (Figure 1A), with a length approximate 10 μm of and a diameter of approximate 52 nm. TEM was used to observe the change to single silicon nanowires, after surface modification with 25 kDa bPEI. As shown in Figure 1B, the single silicon nanowire after bPEI modification was covered by a layer with a thickness of approximately 6 nm. Moreover, we studied the change in the thickness of the silicon wafer after surface modification with 25 kDa bPEI using an ellipsometer (M-88, J. A. Woollam Co., Inc.), and the results revealed that after surface modification, the thickness of Si-PEI increased by approximate 3 nm. The increase in the grafting thickness was not altered by increasing the bPEI grafting time (data not shown). On the basis of these results, we concluded that the layer present after surface modification of SiNWAs with 25 kDa bPEI was a layer of PEI.

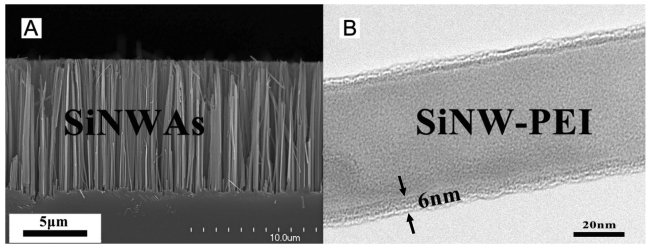


Figure 1. (A) SEM image of SiNWAs and (B) TEM image of single SiNW modified with 25 kDa bPEI.

Water Contact Angle. A static water contact angle test was performed on SiNWAs at different modification stages to study the conditions of the surface modification process as well as the wettability of different samples. As shown in Figure 2, the water contact angle for SN−OH was approximately 0°, but after surface modification with NPC, which is hydrophobic, it increased to approximately 35°. However, after further surface modification with 25 kDa bPEI, which strongly interacts with water through hydrogen bonding, the water contact angle of SN-PEI with various bPEI grafting times all obviously decreased to less than 10°. The water contact angle results demonstrated the success of the surface modification process.

Density of bPEI Grafted on SiNWAs. To investigate the influence of bPEI grafting time on the final bPEI density grafted onto SiNWAs, we studied the amine density of SN-PEI with bPEI grafting times of 1, 3, and 8 h, respectively, by reacting the exposed ε-NH_2 groups on the surfaces with 4-nitrobenzaldehyde to form a reversible Schiff base.[32]

For the silicon wafers, after 8 h of bPEI grafting, the surface amine density can reach approximately 10 nmol/cm^2 (data not shown). For SiNWAs, the surface amine density reached 53 nmol/cm^2 after only 1 h of bPEI grafting. Moreover, after 3 h of bPEI grafting, the surface amine density reached 149 nmol/

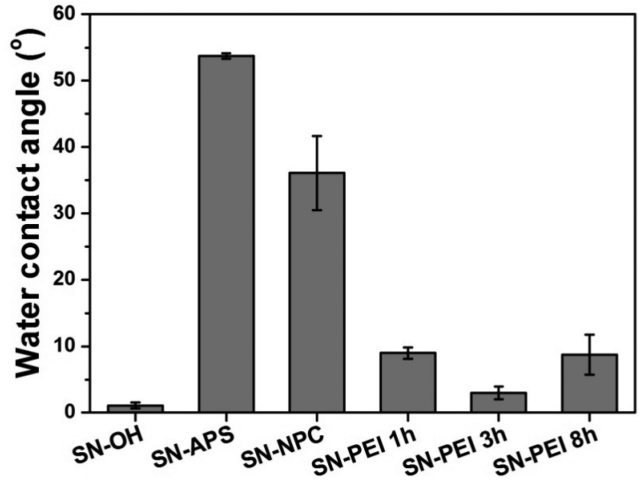


Figure 2. Water contact angles for SiNWAs at different modification stages. OH, APS, NPC, bPEI 1 h, 3 h, and 8 h represent SiNWAs subjected to "Piranha" solution treatment, APTES treatment, NPC activation, and bPEI (25 kDa) grafting for 1 h, 3 h, and 8 h, respectively. Data are the mean ± SE (n = 3).

cm^2, which is much higher than that of silicon wafers (Figure 3). We think there are two possible reasons why the surface

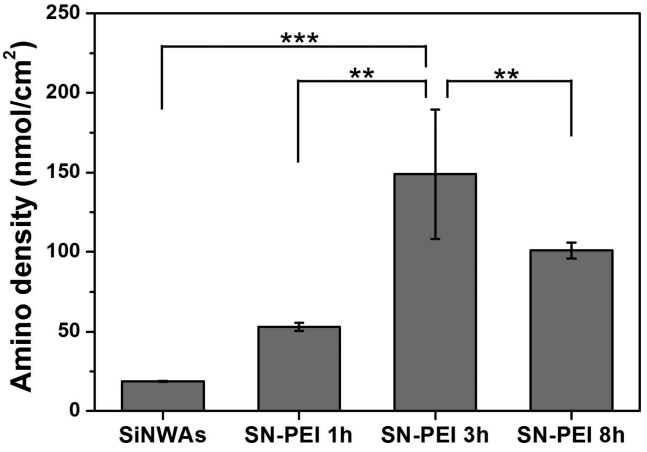


Figure 3. Amine density of SiNWAs after surface modification with 25 kDa bPEI. Data are presented as the mean ± SE (n = 3), **p < 0.01, ***p < 0.001.

amine density of SN-PEI reduced at grafting time of 8 h compared with that of 3 h. First, with the increase in PEI grafting time, more amine groups of the PEI that were already grafted onto SiNWAs might continue to react with SiNWAs, leading to the decrease in the amine density of SN-PEI; Beside, at PEI grafting time of 8 h, a larger amount of 25 kDa-bPEI might graft onto SiNWAs, which can result in stronger entanglement among the grafted PEI chains. However, the tighter entanglement among the PEI chains might result in less effective contact between the amine groups on grafted PEI and the 4-nitrobenzaldehyde in the reaction solution and cause the decrease in the surface amine density of SN-PEI at grafting time of 8 h.

Together, these results revealed that bPEI can be successfully grafted onto SiNWAs using our method. Furthermore, a much larger amount of bPEI can be grafted onto SiNWAs than onto silicon wafers because of the larger specific surface area.

Transfection Efficiency of SiNWAs and SN-PEI. The in vitro transfection efficiency of SiNWAs and SN-PEI was assessed in HeLa cells using pRL-CMV encoding *Renilla* luciferase as a reporter gene. After adsorbing pDNA, silicon wafers poorly deliver pDNA into cells (data not shown), whereas the transfection efficiency of SiNWAs after adsorbing pDNA was increased to approximately 933 RLU/mg protein (Figure 4), which was similar to the results reported by other

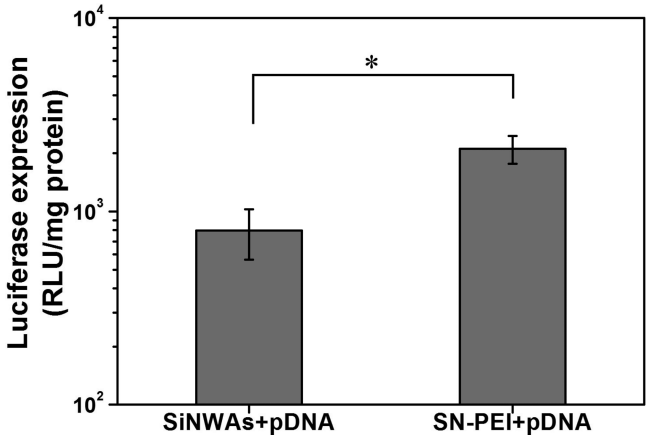


Figure 4. In vitro transfection efficiency of SiNWAs and SN-PEI after adsorbing pDNA. Data are presented as the mean ± SE (n = 3), *p < 0.05.

researchers.[26] This might be due to the vertical alignment of SiNWAs, which have been demonstrated to be efficient in penetrating cell membranes and able to deliver exogenous DNA into cell nuclei.[24] However, compared to other gene delivery materials, the transfection efficiency of SiNWAs is relatively low due to its limited capacity for loading exogenous DNA. It was found that the maximum DNA amount loaded onto SiNWAs was only 1 μg/cm^2 (see the Supporting Information, Figure S1).

To improve the transfection efficiency of SiNWAs, polyethylenimine (PEI, 25 kDa), which is considered to be one of the most efficient candidates to deliver genes and often serves as the "gold standard," was covalently grafted onto SiNWAs. It was discovered that after modifying SiNWAs with 25 kDa bPEI, the transfection efficiency was significantly enhanced, generating a luciferase activity of greater than 1900 RLU/mg protein (Figure 4). Overall, our results indicated that modifying SiNWAs with 25 kDa bPEI substantially improved the gene delivery ability of SiNWAs.

We think there are several possible reasons for this increase. The high specific surface area of SiNWAs allows a high bPEI grafting density, and the 25 kDa bPEI grafted onto the SiNWAs in turn complexes larger amounts of pDNA than SiNWAs, as demonstrated by the result that the DNA bound by SN-PEI was approximately 1.56 μg/cm^2 (see the Supporting Information, Figure S1). Moreover, bPEI grafted onto SiNWAs can condense pDNA and form positively charged pDNA/PEI complexes. With the help of SiNWAs to penetrate cell membranes, pDNA in a complex with bPEI can be efficiently delivered into the cytoplasm and even the nucleus. Additionally, the amine groups on the bPEI chains enhance the surface properties of SiNWAs by favoring cell attachment and proliferation.[34,35]

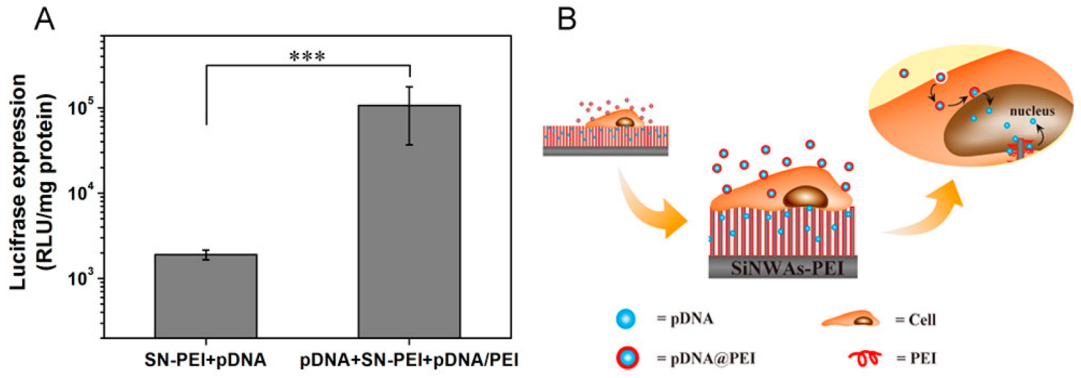


Figure 5. (A) In vitro gene transfection efficiency of SN-PEI with and without exogenous pDNA/PEI (2 kDa) complexes. Data are presented as the mean ± SE (n = 3), ***p < 0.001. (B) Scheme of gene delivery with exogenous pDNA/PEI (2 kDa) complexes on SN-PEI.

Synergistic Effect between SN-PEI and Exogenous pDNA/PEI (2 kDa) Complexes on Transfection Efficiency. One strategy for enhancing transfection efficiency is to improve the concentration of exogenous DNA around targeted cells and increase the amount of exogenous DNA that can cross cell membranes.[36,37] Some problems remained using the system mentioned above, in which exogenous pDNA was absorbed and condensed by 25 kDa bPEI grafted onto SiNWAs and delivered into cells with cell penetration aided by vertically aligned nanowires. First, the amount of pDNA in a complex with bPEI attached to SiNWAs was limited, preventing SN-PEI from further assisting in gene delivery when all of the adsorbed pDNAs are delivered into cells. Furthermore, the area of contact between the cells and the transfection material (SN-PEI) was limited, and the exogenous pDNA can only be transfected into cells at the area of contact between the cell and the material. Thus, to improve the long-term transfection efficiency of this system, the introduction of a larger amount of exogenous pDNA and a method that increases the effective contact time and area between the pDNA and cells would be necessary.

We therefore condensed pDNA with low-molecular-weight bPEI (2 kDa) and added the complexes to the current gene delivery system after the pDNA was adsorbed by 25 kDa bPEI grafted onto SiNWAs (Figure 5B). As shown in Figure 5A, luciferase activity was enhanced to more than 1.1×10^5 RLU/mg protein using this technique, a value that is approximately 55 fold higher than for SN-PEI. Besides, we condensed pDNA with 2-kDa bPEI and added the complexes to the cells cultured in 48-well plate (Costar) as control. After 24 h, the luciferase activity was approximate 7.8×10^4 RLU/mg protein, which is lower than the luciferase activity in cells cultured on SN-PEI and treated with free pDNA/PEI complexes formed by 2 kDa-bPEI.

It has been shown by previous studies that the reason for the poor gene delivery of low-molecular-weight bPEI (such as 2 kDa) is because pDNA in a complex with low-molecular-weight bPEI has difficulty entering cells, leading to a low effective amount of pDNA inside of the cells.[10,38] In our current gene delivery system, SN-PEI penetrated cell membranes, and the entry points for bPEI grafted onto SiNWAs remained in the cell membranes after the complex of bPEI and exogenous pDNA was delivered into the cells, leading to cell membranes with higher permeability for complexes formed by low-molecular-weight bPEI and pDNA. Thus, a high transfection efficiency was achieved by adding pDNA/PEI (2 kDa) complexes that entered cells through the channels provided by SiNWAs penetrating the cell membranes.

Factors Influencing Gene Delivery with the Addition of Exogenous pDNA/PEI (2 kDa) Complexes. Several important factors that might regulate the gene transfection efficiency of the current gene delivery system were investigated. First, SN-PEI prepared with different bPEI grafting times (1, 3, or 8 h) were used for in vitro gene delivery experiments. SN-PEI with a bPEI grafting time of 3 h exhibited the highest amine density (Figure 3), as well as the highest gene transfection efficiency (Figure 6A). This result demonstrated that SN-PEI with small amounts of bPEI grafted onto SiNWAs caused a small amount of pDNA to be adsorbed onto SN-PEI, and the grafted bPEI does not complex the pDNA well, causing the low transfection efficiency. The amount of pDNA adsorbed increased with increasing amounts of bPEI grafted onto SN-PEI, and the increasing amount of bPEI better condensed the pDNA, resulting in much more efficient gene delivery.

Furthermore, the incubation time for the pDNA complex to form with the surface-bound bPEI also directly affects transfection efficiency by influencing the ability of bPEI to condense and release pDNA from the complexes.[39] It was found that for 10 min, the transfection efficiency increased with increasing incubation time and reached its highest efficiency at 10 min; when the incubation time was longer than 10 min, the transfection efficiency decreased (Figure 6B). Our results were consistent with the results reported by Sang et al.[40] and are most likely explained by a longer incubation time resulting in pDNA that was difficult to release from the pDNA/PEI complexes once inside the cells, reducing transfection efficiency.

Moreover, the addition of free pDNA/PEI (2 kDa) complexes played a vital role in significantly enhancing the transfection efficiency of our system. With the assistance of the entry points on cell membrane made by SiNWAs, the cells continually absorb the added exogenous pDNA/PEI complexes. We chose low-molecular-weight bPEI (2 kDa) to condense pDNA for several reasons. First, low-molecular-weight bPEI has lower cytotoxicity compared to high-molecular-weight bPEI, dramatically reducing the cytotoxicity caused by retention of PEI in the cytoplasm.[41] In addition, the strong binding ability of high-molecular-weight bPEI for pDNA makes it difficult for pDNA to be released from the complexes, hindering the delivery of pDNA from the cytoplasm to the nucleus, whereas pDNA in a complex with low-molecular-

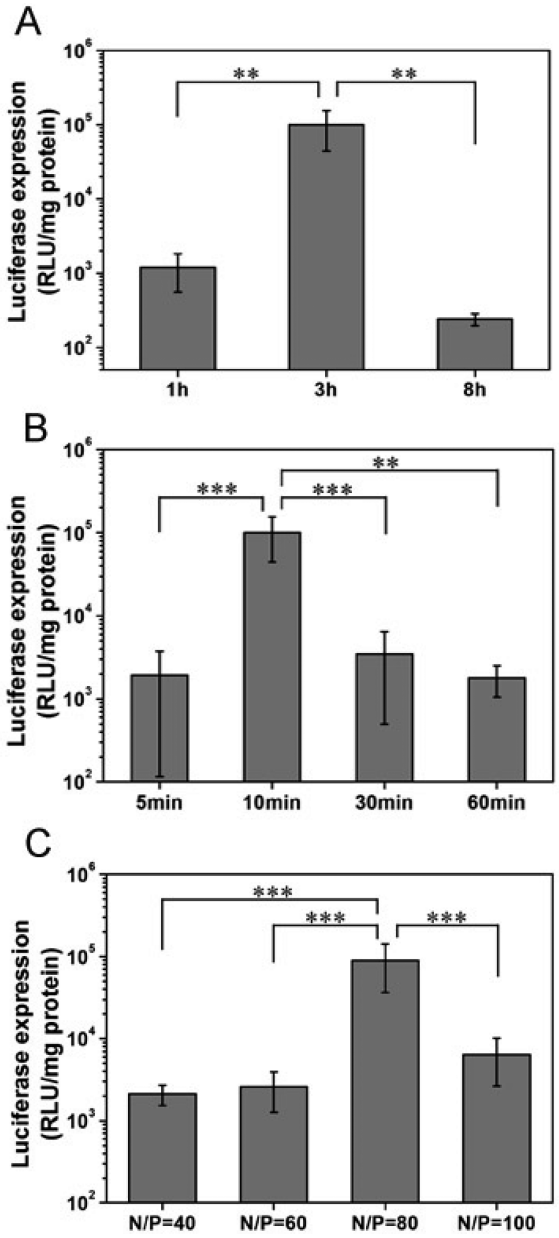


Figure 6. (A) Influence of bPEI grafting time on the in vitro gene transfection efficiency. (B) Influence of incubation time of the tethered DNA/PEI complexes formed with high-molecular-weight bPEI grafted onto SiNWAs on the in vitro gene transfection efficiency. (C) Effect of N/P ratio of the free DNA/PEI complexes formed by low-molecular-weight bPEI on the in vitro gene transfection efficiency. Data are presented as the mean ± SE (n = 3), $^{**}p < 0.01$, $^{***}p < 0.001$.

weight bPEI releases from the complexes more easily and rapidly.[42,43]

The structure and particle size of DNA/PEI complexes formed by low-molecular-weight bPEI by electrostatic interactions directly determines the effective uptake of pDNA by cells and the transfection efficiency. The N/P ratio plays an important role in the formation of the complex made of the gene delivery vector and pDNA, the particle size of the complexes, the transfection efficiency, and the cytotoxicity.[39,44] In this study, the transfection efficiency of pDNA/PEI complexes formed at a relatively low N/P ratio was investigated to optimize the transfection efficiency as well as to reduce the cytotoxicity of PEI. Our results showed that the transfection efficiency increased with an increasing N/P ratio, revealing that pDNA is better protected with a higher N/P ratio (see the Supporitng Information, Figure S2). The transfection efficiency was the highest at an N/P ratio of 80 but decreased at an N/P ratio of 100 (Figure 6C). This might be because at an N/P ratio of 100, the strong binding of the pDNA to PEI makes it difficult for the pDNA to be released in the cytoplasm, reducing the transfection efficiency.[39,44]

Biocompatibility of the Gene Delivery System. In the current gene delivery system, using both SiNWAs modified with 25 kDa bPEI and the introduction of exogenous pDNA in a complex with low-molecular-weight bPEI (2 kDa), as well as the penetration of cell membranes by SiNWAs and the bPEI grafted onto SiNWAs, might be detrimental to cell growth. Therefore, it was important to investigate the biocompatibility of the gene delivery system by assessing the cell density and morphology as well as the damage to cell membrane integrity caused by SiNWAs and SN-PEI.

It was discovered that the number of cells attached to SN-PEI was larger than to SiNWAs after both 24 and 48 h (Figure 7A). Moreover, cells adhering to SN-PEI (Figure 7B b and d) maintained more normal morphology compared to the cells adhering to SiNWAs (Figure 7B a and c). Our results showed that SiNWAs grafted with 25 kDa bPEI supported improved cell attachment and the maintenance of normal morphology.

In addition, live/dead staining was used to study the viability of HeLa cellsgrown on SiNWAs and SN-PEI after 48 h of culture. The results showed that SiNWAs and SN-PEI were both conducive to the maintenance of cell viability and cell proliferation, especially SN-PEI (Figure 8).

Furthermore, the cell membrane integrity of the current gene delivery system was studied by SDS-PAGE, and it was found that the cell membranes were effectively maintained, demonstrated by the fact that no cell content was observed (see the Supporting Information, Figure S3).

Overall, we conclude that SiNWAs are biocompatible and conducive to cell adhesion and proliferation. Our results were consistent with those reported by other researchers, indicating that SiNWAs with small diameters that are dozens of nanometers long do not damage or destroy cell membrane structures.[26] SiNWAs grafted with bPEI supported improved cell adhesion and proliferation. Moreover, cell morphology is better maintained on SN-PEI than on SiNWAs, likely because compared to SiNWAs, the positive charge on SN-PEI enhances cell attachment and growth. In addition, the high-molecular-weight bPEI was grafted onto SiNWAs and therefore was unable to cause cytotoxicity. Furthermore, the exogenous pDNA was in a complex with low-molecular-weight bPEI at a low concentration and generated very low toxicity.

4. CONCLUSIONS

In summary, high-molecular-weight bPEI (25 kDa)-modified SiNWAs were successfully prepared in this study. Using this tool for gene delivery was investigated in detail, leading to the following conclusions: (a) The bPEI grafting density can be adjusted by controlling the bPEI grafting time. (b) The DNA loading capacity and the in vitro transfection efficiency of SiNWAs can be significantly enhanced by modification with 25 kDa bPEI, revealing that grafted bPEI can form a complex with larger amounts of DNA and deliver the exogenous DNA into the cytoplasm and nucleus by penetrating cells with the assistance of vertically aligned silicon nanowires. The high cytotoxicity of high-molecular-weight bPEI was avoided in this

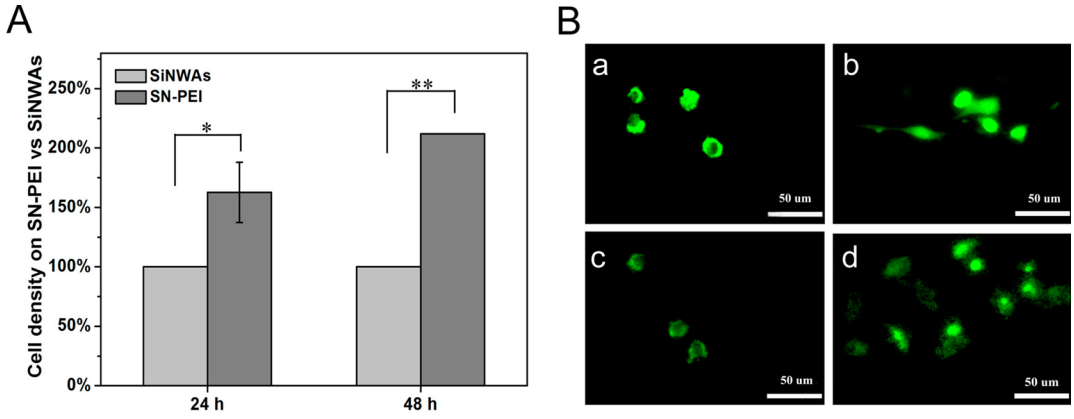


Figure 7. (A) Percentage of L929 cells cultured on SN-PEI compared to the cells cultured on SiNWAs for 24 or 48 h. Data are represented as the mean ± SE (n = 3), *p < 0.05, **p < 0.01. (B) Immunofluorescence images of L929 cells after culture for (a, b) 24 h or (c, d) 48 h on (a, c) SiNWAs or (b, d) SN-PEI. Cells were stained for cytoskeleton (Phalloidin; green). Bar, 50 μm.

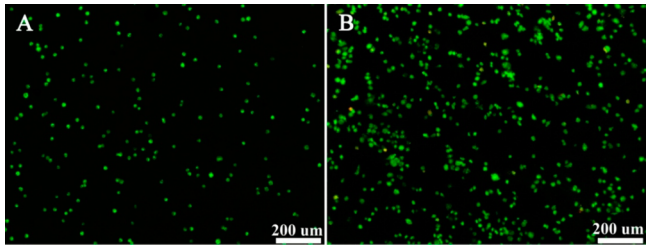


Figure 8. Immunofluorescence images by live/dead staining of HeLa cells cultured on (A) SiNWAs or (B) SN-PEI after 48 h. Bar, 200 μm.

gene delivery system by grafting it to the SiNWAs. (c) The introduction of exogenous DNA condensed by low-molecular-weight bPEI (2 kDa) to this system greatly increased the transfection efficiency and achieved long-term and efficient gene delivery. In addition, low-molecular-weight bPEI has the benefit of very low cytotoxicity. (d) The variables influencing the gene delivery system with the addition of exogenous pDNA/PEI (2 kDa) complexes were studied, and it was found that SN-PEI with a bPEI grafting time of 3 h exhibited an optimal transfection efficiency with a 10 min incubation time for pDNA condensed by bPEI grafted onto SiNWAs and an N/P ratio of 80 for exogenous DNA in a complex with low-molecular-weight bPEI. (e) The biocompatibility of this gene delivery system was assessed, and the results from cell density measurements, live/dead staining, and cell morphology tests showed that our system is biocompatible and conducive to cell adhesion and proliferation. Altogether, these conclusions demonstrate that a gene delivery strategy based on SiNWAs modified with high-molecular-weight bPEI is a novel, safe, and highly efficient gene delivery system.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

DNA binding capacity of SiNWAs and SN-PEI; Agarose gel electrophoreses assay of pDNA/bPEI (2 kDa) complexes formed at different N/P ratio; Analysis of cell content in the culture media by SDS-PAGE after cells were cultured on SiNWAs and SN-PEI, respectively for 24 h. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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