Rare-earth-incorporated polymeric vector for enhanced gene delivery

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Keywords: PEI-CyD
Neodymium
Energetic ions
Plasma treatment
Cellular pathways

ARTICLE INFO

Article history:
Received 16 July 2013
Accepted 20 September 2013
Available online 5 October 2013

ABSTRACT

Cationic polymer PEI-CyD is doped with Nd by plasma technology to produce the gene vector: Nd@PEI-CyD. Luciferase expression and EGFP transfection experiments performed in vitro reveal that Nd@PEI-CyD has significantly higher transfection efficiency than lipofectamine 2000 and PEI-CyD and the mechanism is studied and proposed. The rare-earth element, Nd, stimulates the energy metabolism of cells, enhances cell uptake of complexes/pDNA, and regulates the cellular pathways. These special features suggest a new strategy involving metal-incorporated non-viral gene vectors.

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1. Introduction

In recent years, two kinds of gene delivery systems for gene therapies: viral and non-viral [1] have been widely reported [2,3]. Viral gene vectors have high efficiency, but serious safety risks such as immunogenicity, carcinogenicity and inflammation limit their clinical implementation [4]. In comparison, non-viral gene vectors with several advantages over viral ones such as low immunogenicity and toxicity, large DNA loading capacity, and tissue-specific targeting are typically less effective [2]. Therefore, it is critical to develop more efficient non-viral vectors for clinical applications [2,5].

Among the various types of non-viral vectors, polyethylenimine (PEI), especially high-molecular weight (HMW) PEI (~25 kDa), is a widely used cationic polymer known as the gold standard [6]. Although HMW PEI (~25 kDa) has high gene transfection efficiency, the high toxicity remains a drawback for application in vivo [7]. Our previous studies have shown that β-cyclodextrin-polyethylenimine(PEI-CyD), in which β-CyD is crosslinked by low molecular weight PEI (600Da), has lower cytotoxicity than PEI 25 kDa and similar transfection efficiency as PEI 25 kDa [8]. Our preliminary studies suggest that the gene transfection efficiency of PEI-CyD can be improved by surface modification of the polymer [9,10].

Metallic elements play vital roles in biological systems and activities, for example, bones and teeth [11], functional components of proteins [12], activators of enzymes [13], essential parts in the electron transport of the respiratory chain [14], and maintenance of normal functions in cell membranes [15]. In particular, rare earth elements (REEs) can change the mitochondrial metabolic activity [16,17], promote DNA synthesis [18], regulate the activity of calmodulin [19,20], and participate in a variety of physiological and biochemical processes [21]. Although REEs have been used to promote the growth and development of plants and animals [22,23], their use in gene vectors have seldom been explored.

In this work, the benefits of metallic elements in biological processes such as DNA transcription, mRNA translation [24–26] and surface modification of polymeric materials by ion beams and plasmas [27–29] are combined. The cationic polymer PEI-CyD is doped with a rare earth element, neodymium (Nd), to produce Nd@PEI-CyD complexes. To determine the effects of the plasma treatments, the chemical, physical, and biological characteristics of the Nd@PEI-CyD are investigated. The transfection efficiency of Nd@PEI-CyD is compared to that of PEI-CyD and the commercial transfection agent Lipofectamine 2000. To illustrate the underlying mechanism (Fig. 1), the ATP assay is performed to evaluate the
energy metabolism of cells and the reverse transcription-polymerase chain reaction (RT-PCR) and western blot analysis are conducted to assess several mRNA and proteins.

2. Materials and methods

2.1. Materials

Branched polyethylenimine (PEI; average 25 000 Da), linear polyethylenimine (average MW 600), β-cyclodextrin (β-CyD, MW 1135), 1,10-carbonyldiimidazole (CDI, MW 162.15), dimethyl sulfoxide (DMSO, ≥99.5%), N,N-dimethylformamide (DMF, ≥99%), [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT, MW 218.1), phosphate buffered saline (PBS), and triethylamine (Et3N, 99%) were obtained from Sigma (St. Louis, MO, USA). DMF and DMSO were dried by refluxing over CaH2 and distilled before use. Calcium hydride (CaH2) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The plasmid DNA (pDNA) encoding firefly luciferase (pGLO3-Luc) or green fluorescent protein (pEGFP) were purchased from Promega Corporation (Madison, WI, USA) and Guangzhou Jetway Biotech CO., Ltd. (Guangzhou, Guangdong, China). The BCATM protein assay kit and luciferase activity assay kit were purchased from Pierce Biotechnology, Inc. (Rockford, IL) and Promega Corporation (Madison, WI, USA), respectively. The ATP assay kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China).

2.2. Production of the Nd@PEI-CyD

The PEI-CyD was synthesized as described previously [8]. The PEI-CyD films were prepared by dropping the solution containing 10 mg of PEI-CyD dissolved in 200 μL of deionized water onto a 1 cm × 1 cm silicon chip and dried in air overnight.

Table 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequences</th>
<th>Annealing temperature (°C)</th>
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</thead>
<tbody>
<tr>
<td>calmodulin</td>
<td>Sense primer: 5'-TGAGATAGGGTTCCTGGTTG-3'</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Antisense primer: 5'-AGAGGGTGTAGGGTTTCTGGTT-3'</td>
<td></td>
</tr>
<tr>
<td>caveolin</td>
<td>Sense primer: 5'-TAGAGATAGGGTTGCTGCG-3'</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Antisense primer: 5'-TTCTCTTCCTGCTACGTGTC-3'</td>
<td></td>
</tr>
<tr>
<td>metallothionein</td>
<td>Sense primer: 5'-CTCAACTTTCTTGGGATGAC-3'</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Antisense primer: 5'-ATGCAGGCTCTGCTAG-3'</td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>Sense primer: 5'-AGCTGGGACTCAACGATTT-3'</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Antisense primer: 5'-AGATGATAGCCTTTCGCT-3'</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 2. (A) Tomoscan of Nd@PEI-CyD films; (B) XPS spectra acquired from Nd@PEI-CyD at different depths; (C) XPS spectrum of O 1s at different depths; (D) Theoretical fitting of O 1s at 50 nm; (E) XPS spectrum of Nd 3d at different depths; (F) Theoretical fitting of Nd 3d at 50 nm.
Nd plasma immersion ion implantation (Nd-PIII) was conducted on a metal plasma ion implanter equipped with a neodymium cathodic arc source for 10 min at a pulsed voltage of -20 kV.

2.3. Polymer characterization

The structure of the PEI-CyD was characterized by 1H nuclear magnetic resonance (1H NMR) spectra. The 1H NMR spectra were recorded on a Bruker AV-400NMR spectrometer at 400 MHz and room temperature.

The Nd@PEI-CyD samples were analyzed by attenuated total-reflection Fourier transformed infrared spectroscopy (ATR-FTIR, Varian, ExcaliburTM, USA). Each sample was scanned 16 times in the spectral region of 4000–600 cm⁻¹ with a resolution of 4 cm⁻¹ and averaged to produce each spectrum. The surface chemical structure of Nd@PEI-CyD was determined by X-ray photoelectron spectroscopy (XPS, Physical electronics PHI 5802). The overlapping peaks were resolved into individual components by the peak analysis software, XPSPEAK version 4.1 adopting the Lorentzian–Gaussian sum function [30]. The tomoscan of Nd@PEI-CyD was examined by field-emission scanning electron microscopy (FE-SEM, JEOL JSM-7400F, Japan).

2.4. Preparation and characterization of Nd@PEI-CyD/pDNA

The Nd@PEI-CyD/pDNA complexes were prepared by mixing Nd@PEI-CyD with pDNA at different N/P ratios. Briefly, the Nd@PEI-CyD solutions were added to the pDNA solutions with the same volume, mixed, and incubated for 30 min at room temperature to form the Nd@PEI-CyD/pDNA complexes. The images of the Nd@PEI-
CyD/pDNA complexes in the 293T cell lines were acquired by field-emission scanning electron microscopy (FE-SEM, JEOL JSM-7400F, Japan). The energy-dispersive X-ray spectroscopy (EDS) spectra were taken on the SEM to determine the chemical composition of the complexes in the 293T cell lines.

The particle size and zeta potentials of the complexes were measured at 25 °C by dynamic light scattering (DLS) on the Zetasizer 3000 (Malvern Instruments, Worcestershire, UK). Complex solutions (20 μL) containing 1 μg of pDNA were prepared at various N/P ratios. The average size (nm) and zeta potential (mV) were used in the data analysis. The morphology of the complexes was examined by transmission electron microscopy (TEM, Phillips CM20, Holland).

2.5. Cell culture

The 293T cells, HEK-293 cells, and 3T3 cells were cultured in Dulbecco’s modified eagle medium (DMEM) containing 10% FCS. The MCF-7 cells were cultured in DMEM containing 10% FCS and the SGC-7901 cells were cultured in RPMI-1640 containing 10% FCS. The cells were grown under humidified air containing 5% CO2 at 37 °C.

2.6. In vitro transfection efficiency assay

The 293T cells were seeded at a density of 2 × 104 cells/well on a 48-well plate in 400 μL of DMEM medium containing 10% FCS and incubated for 18 h before transfection. The medium was replaced by 400 μL of fresh serum-free DMEM containing complexes (of Nd@PEI-CyD with pEGFP or Nd@PEI-CyD with pGL3-Luc) with 1 μg pDNA at different N/P ratios. After incubation for 6 h at 37 °C, the transfection medium was replaced with a fresh growth medium and the cells were incubated for additional 36 h.

In the green fluorescent protein assay, the cells expressing the green fluorescent protein were observed and the pictures were captured by fluorescence microscopy (Nikon Eclipse TE2000, Nikon Corporation, Tokyo, Japan) at 36 h transfection. The EGFP fluorescence was excited at 488 nm and emission was collected using a 515 nm filter. The EGFP transfection efficiency was also determined by flow cytometry. After transfection, the cells were trypsinized, washed, and re-suspended in PBS. The FACS analysis of the EGFP-expressing cells was performed by flow cytometry (BD LSRII, USA). The untransfected cells were used to establish the background. The Cell Quest 3.3 software (BD Biosciences, San Jose, CA) was used for data acquisition and data analysis was performed with the FCS Express V3 (De Novo Software, Thornhill, Canada).

The luciferase activity was measured according to standard protocols of the luciferase assay system (Promega). The measurements were conducted in a single-tube luminometer (Berthold luminat LB9507, Germany) for 10 s. The relative light units (RLU) were normalized against protein concentration in the cell extracts, which was measured using a BCA protein assay kit (Pierce, Rockford, USA). The luciferase expression efficiency was reported in terms of RLU/mg cellular protein.

2.7. MTT cytotoxicity assay

The cytotoxicity of the complexes was evaluated using the MTT assay using 293T, 3T3, and MCF-7 cells. Generally, the cells were seeded on 96-well plates at 1 × 103 cells/well in 200 μL of DMEM medium containing 10% FCS and allowed to grow for 18 h. The medium was replaced by 200 μL of serum-free culture media containing serial dilutions of Nd@PEI-CyD/pDNA solutions at a series of N/P ratios for 4 h. After replacing the medium with 90 μL of fresh medium and 10 μL of MTT solution (5 mg/mL in PBS buffer), the cells were incubated for another 4 h. Finally, each well was replaced with 100 μL of DMSO and measured spectrophotometrically on an ELISA plate reader (Model 550, Bio-Rad) at a wavelength 570 nm. The relative cell growth (%) related to control cells cultured in the media without the polymer was calculated by the following relationship: (\(\frac{[A]_{\text{control}} - [A]_{\text{blank}}}{[A]_{\text{control}} - [A]_{\text{blank}}}\)) × 100.

2.8. ATP assay

In the ATP assay, the cells were transfected as described in section 2.6. After cell disruption, each well was treated with the ATP Assay Kit. The samples were incubated for 5 min at room temperature. Each sample was analyzed by Vis-Vis spectrophotometry at a wavelength 636 nm. The concentration of ATP was calculated by (\(\frac{[A]_{\text{control}} - [A]_{\text{blank}}}{\text{dilution factor}}\)) × 1 mmol/L. The concentration of ATP was normalized against the protein concentration in the cell extracts. It was measured using a BCA protein assay kit (Pierce, Rockford, USA). The concentration of ATP was reported in terms of mmol/g cellular protein.

2.9. In vitro analysis of calmodulin, caveolin, and metallothionein expressions

The HEK-293 cells and SGC-7901 cells were seeded on 6-well culture plates at a density of 5.0 × 103 cells per well and incubated at 37 °C in 5% CO2 for 18 h to reach 70% confluency. The medium was replaced by 3 mL of fresh serum-free DMEM or RPMI-1640 containing different complexes (PEI-CyD/pDNA or Nd@PEI-CyD/pDNA) with 4 μg of pDNA with different N/P ratios, PBS, or Nd@PEI-CyD, respectively. After incubation for 6 h at 37 °C, the medium was replaced by a fresh growth medium and the cells were incubated for additional 48 h. The cellular levels of calmodulin, caveolin, and metallothionein mRNA as well as protein were assessed using the RT-PCR and Western blot analysis.

In the Western blot analysis, the cells were transfected as described above and the cell proteins were extracted after transfection for 48 h. The total protein was quantified by the BCA protein assay kit (Pierce, Rockford, USA). An equal amount of protein was separated on the SDS-PAGE, transferred onto the nitro-cellulose membrane, and blocked and incubated overnight with monoclonal antibodies against calmodulin (1:400), caveolin (1:400), and metallothionein (1:400). After washing, the membrane was incubated with the HRP-conjugated secondary antibody (1;5000) for 2 h at room temperature. The bands were visualized using the WestZol enhanced chemiluminescence kit (Intron, Sungnam, Korea) and the expression was normalized to the housekeeping gene (β-actin) expression.

In the RT-PCR analysis, mRNA was extracted from a total of 1 × 106 cells 48 h post-transfection and then reverse-transcribed into cDNA using the Qiagen RNeasy Mini Kit (Cat. No.74104). Quantification of the PCR product was performed in the 1× TE buffer using absorption values at 260 nm and 280 nm (cRNA) – OD260 – OD280 × dilution factor × 0.04 μg/μL. The RNA was reverse-transcribed with Oligo(dT) (Fermentas) according to the protocol. The relative gene expression values were determined using the BandScan4.3 software and the data representing the calmodulin, caveolin, and metallothionein expression were normalized to the housekeeping genes: glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the endogenous reference. Three selected genes and housekeeping gene (GAPDH) used in this study are listed in Table 1. The PCR parameters consisted of 5 min of Taq activation at 95 °C, followed by 32 cycles of 95 °C 30 s, 56 °C 45 s, and 72 °C 45 s.

2.10. Statistical analysis

All the experiments were repeated at least three times and the data are presented as means ± standard deviation. The statistical significance (p < 0.01) was evaluated by the Student t-test when only two groups were compared. If more than two groups were compared, evaluation of significance was performed using the one-way analysis of variance (ANOVA) followed by Bonferroni’s post hoc test. In all the tests, the statistical significance was set at p < 0.01.

3. Results and discussion

Fig. S1 depicts 1H NMR spectrum of PEI-CyD in D2O. Fig. 2A shows the tomoscan of the Nd@PEI-CyD samples. The modified surface darkens after the plasma treatment. The X-ray SEM micrographs of 293T cells incubated with Nd@PEI-CyD/pDNA complexes and corresponding EDS spectra.
Fig. 6. Fluorescence microscopy images of 293T cells incubated with (A) PEI-CyD/pEGFP complexes, (B) Lipofectamine/pEGFP complexes 2000, (C) Nd@PEI-CyD/pEGFP complexes, and (D) Nd-PEI-CyD/pEGFP complexes; Quantified expressions of EGFP by flow cytometry: (E) PEI-CyD/pEGFP, (F) Lipofectamine/pEGFP, and (G) Nd@PEI-CyD/pEGFP, and (H) Nd-PEI-CyD/pEGFP; (I) In vitro gene transfection - Luciferase expression levels in 293T cells transfected with PEI-CyD/pDNA, Lipofectamine/pDNA, Nd@PEI-CyD/pDNA, and Nd-PEI-CyD/pDNA.
photoelectron spectroscopy (XPS) spectra of the Nd@PEI-CyD in Fig. 2B with different colors representing various depths show the Nd\(^{3+}\) and O\(^{2-}\) peaks. The O\(^{2-}\) peak remains at about 532 eV at a depth of 100 nm (Fig. 2C), indicating the existence of C–O–H [31] and that the O–H bonds of PEI-CyD are unreacted. The O\(^{2-}\) peak in the near surface shifts to 530 eV as a result of the Nd–O bond [32] disclosing the formation of Nd@PEI-CyD and complete reaction of C–O–H. The O\(^{2-}\) peak at 50 nm in Fig. 2D is Gaussian fitted to locate the C–O–H and Nd–O peaks. By integrating the peaks, 86% of O–H is found to be converted into Nd–O. The Nd\(^{3+}\) peaks at a depth of 50 nm (Fig. 2E and F) consist of two subpeaks at 1003 eV (3d\(_{5/2}\)) and 979 eV (3d\(_{3/2}\)) indicating the formation of Nd–O bond (Nd\(^{3+}\)) [33,34]. According to Gaussian fitting, the satellite peaks on the lower binding energy side of the 3d\(_{5/2}\) and 3d\(_{3/2}\) peaks are separated by about 5 eV. The broad peaks may not arise from different chemical states but be attributed to the special nature of rare earth atoms [35].

The ATR-FTIR spectra in Fig. 3 further confirm the formation of the Nd–O bond. Compared to PEI-CyD, Nd@PEI-CyD exhibits a sharp peak at 654 cm\(^{-1}\) which is the characteristic peak of Nd–O. In addition, the characteristic peak of O–H at 3291 cm\(^{-1}\) diminishes substantially and the other peaks remain at about the same position as in PEI-CyD, confirming that the plasma treatment does not damage the polymer skeleton. However, after the Nd@PEI-CyD is dissolved and freeze-dried, the characteristic peak of Nd–O at 654 cm\(^{-1}\) almost shift back to 629 cm\(^{-1}\), which is part of the original PEI-CyD, as shown in Fig. 3. The red (in web version) profile and characteristic O–H peak at 3291 cm\(^{-1}\) reappear showing aging which is common to polymeric materials. The aged materials are termed Nd@PEI-CyD to distinguish them from the fresh sample designated as Nd@PEI-CyD.

Cationic polymers bind with DNA and form nanoparticles via electrostatic interactions. The particle size and zeta potential of the Nd@PEI-CyD/pDNA complexes with various N/P ratios are determined by dynamic light scattering and the results are displayed in Fig. 4A. The PEI-CyD/pDNA and Nd@PEI-CyD/pDNA complexes with the same N/P ratios have similar particle size and zeta potentials. The particle size and zeta potential of the Nd@PEI-CyD/pDNA complexes with an N/P ratio of 30 are about 150 nm and 28 mV, respectively, which are suitable for cell uptake [36]. The transmission electron microscope (TEM) picture of the Nd@PEI-CyD/pDNA complexes with an N/P ratio of 30 in Fig. 4B shows that the nanoparticles are spherical with a size of about 100 nm.

In order to visualize the transfection, the gene expression of the complexes decrease when the N/P ratio is larger than 35 possibly due to the increased cytotoxicity or strong interaction between polycation and DNA preventing the release of pDNA [37]. By using the optimal N/P ratio in the 293T cells, Nd@PEI-CyD shows 27-folds and 15-folds higher luciferase release of pDNA [37]. By using the optimal N/P ratio in the 293T, 3T3, and MCF-7 cells. The N/P ratio has an obvious impact on the cytotoxicity of Nd@PEI-CyD/pDNA complexes in the three types of cells. The cell viability decreases with increasing N/P ratios. At the optimal N/P ratio of 35 for the in vitro transfection assay, the cell viability of the Nd@PEI-CyD/pDNA

quantify the amounts of transfected cells, the EGFP expression in the 293T cells is analyzed by flow cytometry (FCM) (Fig. 6D–F). At the respective optimal N/P ratios, the percentage of the EGFP-positive 293T cells mediated by Nd@PEI-CyD is up to 58.55 ± 4.55% which is dramatically higher than 12.04 ± 2.51% for PEI-CyD without plasma treatment and 24.25 ± 3.61% for Lipofectamine 2000.

The transfection efficiency of Nd@PEI-CyD, which is the polymer formed after Nd@PEI-CyD is dissolved and freeze-dried, is about the same as that of PEI-CyD, as shown in Fig. 6D and I. The results reveal that freeze drying degrades the Nd–O bond (as seen in FTIR) and this affects the transfection efficiency. Ideal non-viral gene vectors should have both high transfection efficiency and low cytotoxicity [7]. Fig. 7 shows the in vitro MT assay results of the cytotoxicity of the Nd@PEI-CyD/pDNA complexes with various N/P ratios in the 293T, 3T3, and MCF-7 cells. The N/P ratio has an obvious impact on the cytotoxicity of Nd@PEI-CyD/pDNA complexes in the three types of cells. The cell viability decreases with increasing N/P ratios. At the optimal N/P ratio of 35 for the in vitro transfection assay, the cell viability of the Nd@PEI-CyD/pDNA

- **Fig. 7.** Cell viability in 293T, 3T3, and MCF-7 cells incubated with Nd@PEI-CyD/pDNA complexes at various N/P ratios (mean ± SD, n = 3).

- **Fig. 8.** ATP assay in HEK293 and SGC-7901 cells incubated with PEI-CyD/pDNA, Nd@PEI-CyD/pDNA, respectively (mean ± SD, n = 3, *P < 0.01).
complexes is over 75%, suggesting that the toxicity of the plasma-treated cationic polymers remains low, in fact much lower than PEI (non-viral gold standard for gene transfection) [38]. The results also indicate that the plasma treatment affects the gene transfection ability of the PEI-CyD significantly. Nd@PEI-CyD exhibits a very high transfection efficiency of about 60% which is close to that of some viral vectors [37] and at the same time, the in vitro cytotoxicity of Nd@PEI-CyD is quite low.

Fig. 9. (A) Representative CaM, Caveolin, and Metallothionein protein expressions determined by Western blot analysis in HEK-293 cells; (B) Analysis of light intensities of CaM, Caveolin, and Metallothionein protein expressions from Western blot results; (C) Expression of CaM, Caveolin and Metallothionein mRNA determined by RT-PCR in HEK-293 cells; (D) Analysis of light intensities of CaM, Caveolin, and Metallothionein protein expressions determined by Western blot analysis in SGC-7901 cells; (E) Representative CaM, Caveolin, and Metallothionein protein expressions determined by Western blot results; (G) Expressions of CaM, Caveolin, and Metallothionein mRNA determined by RT-PCR in SGC-7901 cells; (H) Analysis of light intensities of CaM, Caveolin, and Metallothionein mRNA from RT-PCR results (mean ± SD, n = 3, *P < 0.01).
To investigate the underlying mechanism of the high transfection efficiency exhibited by the Nd@PEI-CyD, the ATP assay is performed to evaluate the energy metabolism of cells and RT-PCR and western blot analysis are conducted to assess several mRNA and proteins. The results of the ATP assay in HEK293 and SGC-7901 cell incubated with PEI-CyD/pDNA and Nd@PEI-CyD/pDNA are shown in Fig. 8. Both cells produce more ATP, suggesting that Nd@PEI-CyD can stimulate the energy metabolism of cells. Rare-earth elements have been reported to change the mitochondrial metabolic activity [16,17]. The synthesis of proteins requires a lot of ATP for energy. Nd@PEI-CyD changes the mitochondrial metabolic activity of the cells to produce more ATP to enhance the transfection efficiency. Calmodulin (CaM) is a ubiquitous multifunctional calcium-binding protein participating in many physiological and biochemical processes and thus essential in cellular regulation [21,39,40]. Some rare-earth elements can decrease the CaM levels and regulate the cellular pathways [41]. Fig. 9A and B for HEK293 cells and Fig. 9E and F for SGC-7901 show lower levels of CaM mediated by Nd@PEI-CyD. Nd affects the cell activity by down regulating the expression of CaM. PEI-CyD is internalized by the caveola pathway as one possible means and the inhibition of the caveola pathway can decrease the PEI-CyD mediated gene transfection [42]. Caveolins play a vital role in the formation and stability of caveolae [43]. There is a remarkable increase in the expression of caveolin induced by Nd@PEI-CyD in the HEK293 cells (Fig. 9A and B) and SGC-7901 cells (Fig. 9E and F) according to the western blot analysis. Moreover, the RT-PCR analysis shown in Fig. 9C and D for the HEK293 cells and Fig. 9G and H for the SGC-7901 cells confirm the same results for caveolin mRNA. These results furnish strong evidence that Nd@PEI-CyD enhances cell uptakes by up-regulating the expressions of caveolins. Our data show an up-regulation of caveolin and down-regulation of CaM, which are the reasons for the high transfection.

Metallothioneins (MTs) which have a low molecular weight are cysteine-rich metal-binding proteins existing widely in the biosphere. MTs have a wide range of biological functions in the storage, transport, and metabolism of metallic elements [44,45], and REEs can induce the expression of MTs [46]. Expression of MT induced by Nd@PEI-CyD is assessed by the western blot analysis (Fig. 9). Obvious increases in MTs are observed from both the HEK293 cells (Fig. 9A and B) and SGC-7901 cells (Fig. 9E and F). Additionally, the increased MT mRNA expression is verified by the RT-PCR analysis illustrated in Fig. 9C and D for HEK293 cells and Fig. 9G and H for SGC-7901 cells. The results indicate that Nd@PEI-CyD up-regulates the expression of MTs which participate in the metabolism of Nd.

4. Conclusion

A new type of gene vector, Nd@PEI-CyD which combines the rare-earth element Nd and non-viral polymeric gene vector, is obtained by plasma treatment and efficient delivery of DNA into cells is demonstrated. Nd@PEI-CyD exhibits a transfection efficiency of 58.6% which is dramatically higher than that of PEI-CyD without the plasma treatment and commercial transfection agent Lipofectamine 2000. The luciferase expression level mediated by Nd@PEI-CyD is 15 times and 27 times higher than that induced by Lipofectamine 2000 and PEI-CyD. Because of the special biochemical characteristics of Nd, Nd@PEI-CyD enhances the energy metabolism of cells to produce more ATP, strengthens cell uptakes by increasing the caveolin expression, and regulates the cellular pathways by adjusting the CaM. In addition, metallothioneins are involved in the discharge of Nd. Our study provides a new strategy for highly effective metal-incorporated non-viral gene vectors.

Acknowledgments

This work was jointly supported by the National High Technology Development Program of China (863 Program 2007AA03Z255, 2009AA02Z416), National Natural Science Foundation of China (grant # 30970711 and 21074111), Major Scientific and Technological Innovation Project of Hangzhou (20122511A43), Hong Kong Research Grants Council (RGC) General Research Funds (GRF) Nos. 112510 and 112212, and City University of Hong Kong Applied Research Grants Nos. 9667066 and 9667069.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biomaterials.2013.09.069.

References


