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The PEI-introduced CS shell/PMMA core nanoparticle for silencing the expression of E6/E7 oncogenes in human cervical cells

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ABSTRACT

In this study, we examined the potential of cationic nanoparticle – polyethyleneimine-introduced chitosan shell/poly (methyl methacrylate) core nanoparticles (CS-PEI) for siRNA delivery. Initially, DNA delivery was performed to validate the capability of CS-PEI for gene delivery in the human cervical cancer cell line, SiHa. siRNA delivery were subsequently carried out to evaluate the silencing effect on targeted E6 and E7 oncogenes. Physicochemical properties including size, zeta potential and morphology of CS-PEI/DNA and CS-PEI/siRNA complexes, were analyzed. The surface charges and sizes of the complexes were observed at different N/P ratios. The hydrodynamic sizes of the CS-PEI/DNA and CS-PEI/siRNA were approximately 300–400 and 400–500 nm, respectively. Complexes were positively charged depending on the amount of added CS-PEI. AFM images revealed the mono-dispersed and spherical shapes of the complexes. Gel retardation assay confirmed that CS-PEI nanoparticles completely formed complexes with DNA and siRNA at a N/P ratio of 1.6. For DNA transfection, CS-PEI provided the highest transfection result. Localization of siRNA delivered through CS-PEI was confirmed by differential interference contrast (DIC) confocal imaging. The silencing effect of siRNA specific to HPV 16 E6/E7 oncogene was examined at 18 and 24 h post-transfection. The results demonstrated the capacity of CS-PEI to suppress the expression of HVP oncogenes.

1. Introduction

Effective gene delivery is needed for effective DNA and RNA-based therapeutics, since both DNA and RNA are easily degraded by the digestive enzymes in the body. This results in a decrease in the quantity of DNA or small interference RNA (RNAi) entering the cells after intracellular delivery. To make DNA and RNA delivery more efficient, both viral and non-viral carriers have been developed (Mastrobattista, van der Aa, Hennink, & Crommelin, 2006; Walther & Stein, 2000). Among the non-viral gene delivery approaches, polymeric-based nanoparticles have been intensively studied because of their safety and biocompatibility (Anderson, 1998; Schaffert & Wagner, 2008).

Adsorption of nucleic acid onto cationic nanoparticles is one of the approaches used for DNA or RNA delivery (Wong, Pelet, & Putnam, 2007). This technique facilitates the immediate release of DNA or RNA at target site. Furthermore, the preparations of polymer and DNA/RNA complexes by adsorption can avoid the chemical effects used in other approaches such as encapsulation. Polyethyleneimine (PEI) is a cationic polymer widely used for nucleic acid delivery. PEI possesses very high positive charges from amines in molecules which can form complexes with phosphate groups of nucleic acids through electrostatic interaction (Bivas-Benita, Romeijn, Junginger, & Borchard, 2004). The complexes can later be delivered into the cell through endocytosis (Urban-Klein, Werth, Abuharbeid, Czubayko, & Aigner, 2005). While PEI is considered to be the most effective cationic polymer due to its buffering capacity via the proton sponge effect, it is a highly cytotoxic polymer which presents a major obstacle to applying PEI in human gene therapy (Boussif et al., 1995; Kunath et al., 2003). Another polymer that is often used for gene therapy is chitosan which is well-known as a biocompatible polymer (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). Chitosan is a cationic polysaccharide, produced by deacetylation of chitin from crustacean shells. Previously, the gene delivery effectiveness of PEI-introduced chitosan shell/poly(methyl methacrylate) core nanoparticles (CS-PEI

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nanoparticles) was demonstrated in rat mesenchymal stem cells (Pimpha, Sunintaboon, Inphonlek, & Tabata, 2010). The study concluded that the CS-PEI nanoparticle was a promising carrier for gene delivery. In the current study, we focused on the application of CS-PEI nanoparticles for both DNA and siRNA delivery in cervical cancer cells.

Cervical cancer is a malignant tumor which occurs when abnormal cells in the cervical area divide rapidly and grow out of control. The main cause of cervical cancer is infection by the sexually transmitted human papillomavirus (HPV) (Walboomers et al., 1999). HPV is a double-stranded DNA virus, which is divided into low risk and high risk categories based on the ability to transform normal cells into cancer (Bosch et al., 1995; Govan, 2008). Viral DNA replication, regulatory functions, and activation of the lytic life cycle are controlled by six early expressed genes (E1, E2, E4, E5, E6 and E7). Meanwhile, the viral capsid protein is regulated by two late expressed genes (L1, L2). Malignancy occurs through integration of the HPV DNA with the host genome, resulting in over-expression of the viral genes E6 and E7 (Grassmann, Rapp, Maschek, Petry, & Iftner, 1996; Jeon & Lambert, 1995). The HPV oncoproteins E6 and E7 interfere with the cell cycle by inhibiting the two tumor suppressor proteins, p53 and pRb, which control cell division (Dyson, Howley, Munger, & Harlow, 1989; Werness, Levine, & Howley, 1990).

In general, the treatments for cervical cancer are surgery, radiation and/or chemotherapy. However, there is no satisfactory approach which completely eliminates cancer. RNA interference (RNAi) is a promising, novel mechanism for gene silencing which is being considered for use in cancer therapy. Introduction of exogenous short interfering RNA (siRNA) causes of RNA silencing at the posttranscriptional level, providing the novel approach to manipulate gene of interest in sequence-specific manner (Hannon, 2002). The over-expression of the HPV E6/E7 oncogenes results in cellular transformation into HPV-positive cervical cancer cells (von Knebel Doeberitz, Rittmüller, Hausen, & dürst, 1992). The loss of E6/E7 gene over-expression results in suppression of cervical cancer development. Therefore, the use of RNAi to inhibit the expression of E6/E7 genes has been studied intensively (Alvarez-Salas, Benitez-Hess, & DiPaolo, 2003; Butz et al., 2003). Furthermore, several studies revealed that treatment of cancer with siRNA was able to enhance the effect of anti-tumor drugs such as cisplatin (Tan et al., 2012), doxorubicin, and gemcitabine (Koivusalo, Krausz, Helenius, & Hietanen, 2005).

The aim of this study was to develop the nanoparticle for delivery of siRNA into cervical cancer cells. The feasibility study of the role of CS-PEI nanoparticles in siRNA delivery was validated initially by DNA delivery. The cervical cell line SiHa was selected as a cell model for transfection because SiHa is the cervical cancer cell infected with high-risk strains of human papillomavirus type 16 which is one of the prevalent genotypes of HPV (Howley, Munger, Romanczuk, Scheffner, & Huibregtse, 1991; Khan et al., 2005). After evaluation, we then subsequently performed siRNA delivery to investigate the potential of CS-PEI as a siRNA carrier.

2. Materials and methods

2.1. Chemicals

Chitosan (Mw 45 kDa) was purchased from Seafresh Chitosan Lab, Thailand. Branched PEI (Mw 750 kDa) was purchased from Aldrich, Missouri, USA. The PEI-introduced CS shell/PMMA core nanoparticles (w/w, 0.5/0.5) was synthesized as previously described (Inphonlek, Pimpha, & Sunintaboon, 2010). Lipofectamine 2000™ was purchased from Invitrogen, New York, USA. Plasmid pGL3-basic containing CMV promoter/enhancer was used to monitor transfection efficiency (Tencomnao, Rakkhitawatthana, & Sukhontasing, 2008). The plasmids were propagated in *Escherichia coli* which were grown in LB broth (10 g/l tryptone, 5 g/l yeast extract and 10 g/l NaCl) supplemented with ampicillin under shaking conditions, 250 rounds per minute at 37 °C. The plasmid was extracted using the PureLink™ Hipure Plasmid DNA Purification Kit (Invitrogen, New York, USA). The extracted plasmid was observed by electrophoresis on a 1.0% agarose gel. Plasmid purity and concentration were determined by measuring light absorbance at 260 and 280 nm using a SpectraMax M2 microplate reader (MDS Inc., Canada). The siRNA duplex was synthesized by Stealth technology (Invitrogen, New York, USA). The sense strand and antisense strand sequences for siRNA 16E6 sequence 10 were: 5′-GCAACAGUUACUGCGACGUUU-3′ and: 3′-UUCGUUGUCAAUGACGCUGCA-5′, respectively (Putral et al., 2005).

2.2. Preparation of CS-PEI/DNA and CS-PEI/siRNA 16E6 complexes

CS-PEI/DNA or CS-PEI/siRNA complexes were prepared by adding CS-PEI particles into DNA or siRNA solutions. Briefly, CS-PEI was suspended in doubled-deionized water to prepare a stock solution with final concentration 1 μg/μl. Different amount of CS-PEI were then mixed with 1 μl of doubled-deionized containing 1 μg of plasmid. The mixtures of complexes were incubated for 30 min at room temperature. The complexes were prepared at various nitrogen/phosphate molar ratios (N/P) depending on the amine group (N) in the nanoparticle and the phosphate group (P) in the DNA or siRNA. The amount of amine groups on CS-PEI surface was determined by conventional 2,4,6-trinitrobenzene sulfonic acid (TNBS) assay as previously described in Pimpha et al. (2010).

2.3. Characterization of complexes by AFM analysis, size and zeta potential measurement

The AFM images of CS-PEI/DNA and CS-PEI/siRNA 16E6 complexes were obtained using a dynamic force microscope (DFM) mode (Seiko SPA4000, Japan). Samples were prepared by dropping the complex solutions on a mica surface and leaving them for air drying. All images were obtained with a scanning speed of 1.0 Hz over 5 μm × 5 μm areas. The *Z*-average hydrodynamic diameter, polydispersity index (PDI), and surface charge of methylated Ch derivatives/DNA complexes were determined by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK) at room temperature. The complexes were prepared and made up to 1 ml with DI water. All samples were measured in triplicate.

2.4. Gel retardation assay

The ability of CS-PEI to bind DNA or siRNA was confirmed using gel retardation. The CS-PEI/DNA and CS-PEI/siRNA complexes were mixed and incubated at room temperature for 30 min at different N/P ratios. Then, their DNA binding affinity was analyzed using 1% agarose gel containing 0.1 mg/ml of ethidium bromide (EtBr). The gel electrophoresis was performed at 100 V for 30 min in a TAE buffer solution (40 mM Tris–HCl, 1%, v/v, acetic acid and 1 mM EDTA). The gel were visualized under a UV transilluminator using a GelDoc imaging system (G:box, Syngene, Cambridge, UK).

2.5. In vitro gene transfection and luciferase assay

SiHa (HPV type 16 positive) was grown in MEM alpha supplemented with 10% FBS containing 0.1 mM non-essential amino acids (100 μg/ml L-glutamine, 100 μg/ml streptomycin and 100 U/ml penicillin). Cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2. The medium was changed every other day.

For cell study, SiHa were plated in T-75 ml culture flasks. For *in vitro* transfection, SiHa cells were seeded in a 96-well plate at a density of 1×10^5 cells/well. Prior to transfection, the media were removed and the cells were rinsed twice with PBS then plated and incubated with serum-free media. Cells were incubated with complexes at 37 °C for 4 h then replaced with growth media. Twenty-four hours post transfection, the luciferase activity in each well was determined according to the manufacturer's recommendations (Promega, Wisconsin, USA). Luciferase expression was quantified as relative light units (RLU) using a luciferase assay system (Promega, Wisconsin, USA). Luciferase activity was normalized for protein concentration using the Bradford assay. Lipofectamine 2000™ (Invitrogen, New York, USA) was used as a control carrier for gene transfection.

2.6. Evaluation of cytotoxicity

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed to evaluate cell viability after treating with the complexes. Cells were seeded at the same density as during transfection and were incubated overnight at 37 °C in humidified air with a 5% CO_2 atmosphere. The assay was performed 24 h after transfection according to the manufacturer's recommendations.

2.7. Determination of siRNA 16E6 loading efficiency

The CS-PEI/siRNA complexes at N/P ratios of 0.4, 0.6, 0.8, 1.6, 4.0 and 8.0 were centrifuged at 13,000 × *g* for 15 min. The siRNA loaded on nanoparticles was detected by Quant-iT™ RiboGreen® RNA reagent (Invitrogen, New York, USA) which is an ultra-sensitive fluorescent nucleic acid stain for quantization of RNA in solution. The siRNA loading efficiency was calculated from the percentage difference between the total amount of siRNA added and the amount of non-loaded siRNA. Three replicate experiments were conducted to obtain data for statistical analysis.

2.8. Internalization of CS-PEI/siRNA complexes

siRNA was labeled with CX-Rhodamine using a *Label* IT® siRNA Tracker Intracellular Localization Kit (Mirus Bio Corporation, Madison, USA). The labeled-siRNA was utilized to form complexes with CS-PEI resulting in CS-PEI/Rho-siRNA complexes. SiHa cells were seeded on a cover slip in a 35 mm^2 petri dish at a cell density of 5×10^5 cells. The CS-PEI/Rho-siRNA complex with a N/P ratio of 1.6 was incubated with the cells for 4 h at 37 °C. 18 h after transfection, cells were fixed using 70% ethanol for 10 min and were stained with Hoechst 33258 solution. Confocal imaging was observed using an Olympus FluoView 1000.

2.9. siRNA delivery, RNA isolation and gene silencing determination

For siRNA delivery, CS-PEI was diluted to a concentration of 1 μg/μl and mixed with 1 μg of siRNA to form complexes with N/P ratios of 0.8 and 1.6. The complexes were incubated at room temperature for 30 min. A day before transfection, plated cervical cancer cell lines in a 96-well plate at a density of 3×10^5 cells/well and incubated the cells at 37 °C in a CO_2 incubator for 24 h. The CS-PEI/siRNA complexes were incubated with cells for 4 h and then replaced with growth media for detecting gene silencing using RT-PCR. Cells transfected with naked siRNA and normal cells were used as controls.

Total RNA from SiHa and SiHa transfected with CS-PEI/siRNA complexes was extracted after transfection for siRNA delivery and incubation for 18 and 24 h. One hundred milliliters of Trizol reagent

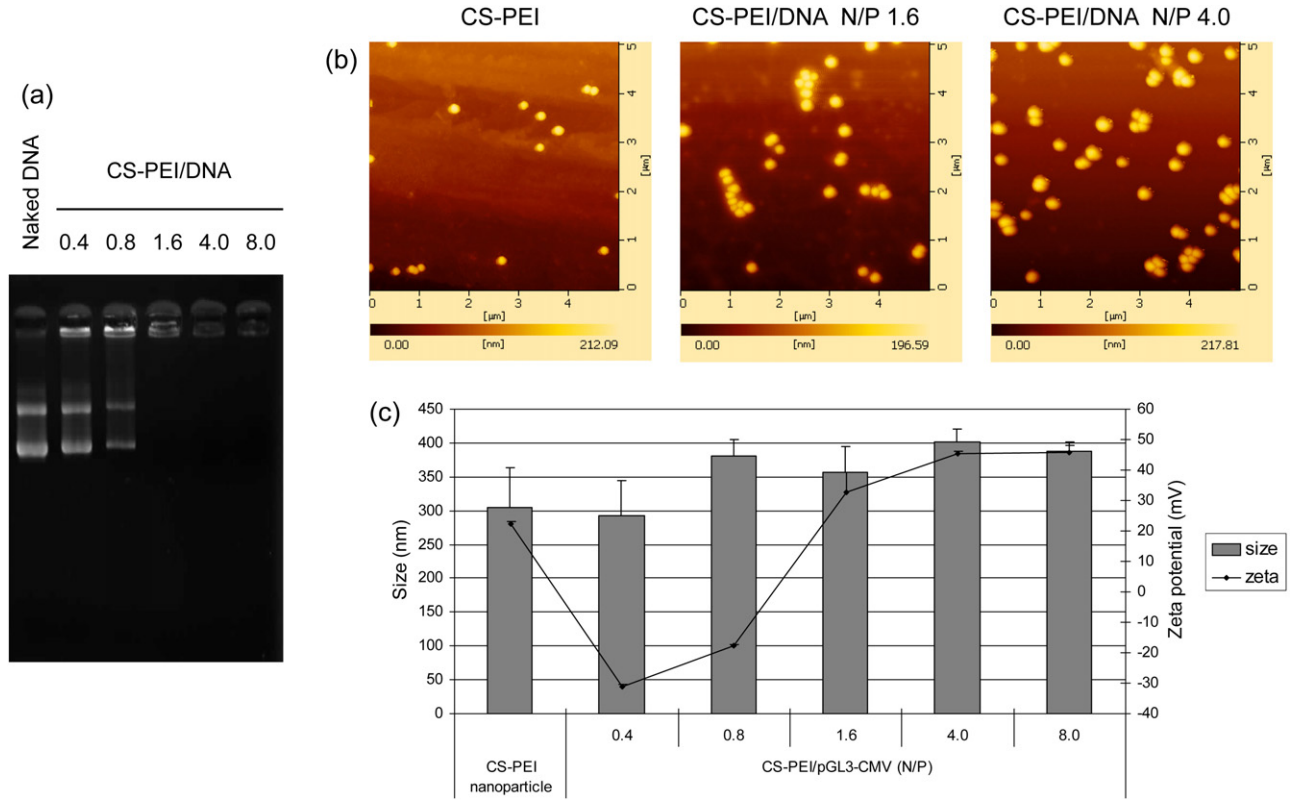

Fig. 1. Gel retardation analysis of CS-PEI/DNA complexes at different N/P ratios. One microgram of DNA was applied to form a complex (a). Atomic force microscope (AFM) images of the topology of CS-PEI/DNA complexes (b). Size and zeta-potential of CS-PEI/DNA at different N/P ratios (c).

(Invitrogen, New York, USA) was added to the cells and incubated at room temperature for 10 min. Then 200 μl of chloroform was added to extract the RNA in the aqueous layer after centrifugation at 4 °C 12,000 × *g* for 15 min. The RNA was precipitated using 500 μl of isopropanol, washed with 75% ethanol, dried, and then dissolved in DEPC-treated water. For the RT-PCR, the isolated RNA samples were treated with RNase-Free DNase (Promega, Wisconsin, USA) to removed double-stranded and single-stranded DNA from the RNA sample. The resulting RNA was finally diluted to a concentration of 5 ng/μl for RT-PCR. Gene silencing was monitored by a one-step reverse-transcription-polymerase chain reaction (RT-PCR). A transcriptor one-step RT-PCR kit (Roche, Indiana, USA) was used to synthesize cDNA and to carry out the PCR reaction. The primer sequences for HPV type 16 E6, E7, and GAPDH are shown in Table 2. GAPDH gene expression was also measured to be a factor for normalization of E6 and E7 gene expression. RT-PCR products were separated on a 3% agarose gel at 100 volts for 30 min. The RT-PCR products were analyzed by the GeneTools program in a G:Box (Syngene, Cambridge, UK).

2.10. Statistical analysis

Luciferase assay, siRNA loading efficiency, cell viability, and gene silencing assay were performed in triplicate. One-way ANOVA with Tukey's post hoc test was applied in the statistical analysis with $p < 0.05$ considered as a statistically significant difference.

3. Results and discussion

3.1. CS-PEI/DNA complex formation and physicochemical characterization

The CS-PEI/DNA complexes were prepared simply by physically mixing CS-PEI nanoparticles and DNA via a micropipette. The mixtures were then incubation for 30 min at room temperature to promote electrostatic interaction. Gel retardation was employed to monitor the binding affinity of DNA on the CS-PEI surface. The complexes were prepared by fixing 1 μg of plasmid DNA to form a complex with CS-PEI at N/P ratios of 0.4, 0.8, 1.6, 4.0 and 8.0. The results revealed that DNA binding affinity increased in parallel with CS-PEI concentration (Fig. 1a). The complexes were completely formed since no DNA migration was found on the gel with a N/P ratio of 1.6. The AFM result for N/P ratios of 1.6 and 4.0 revealed

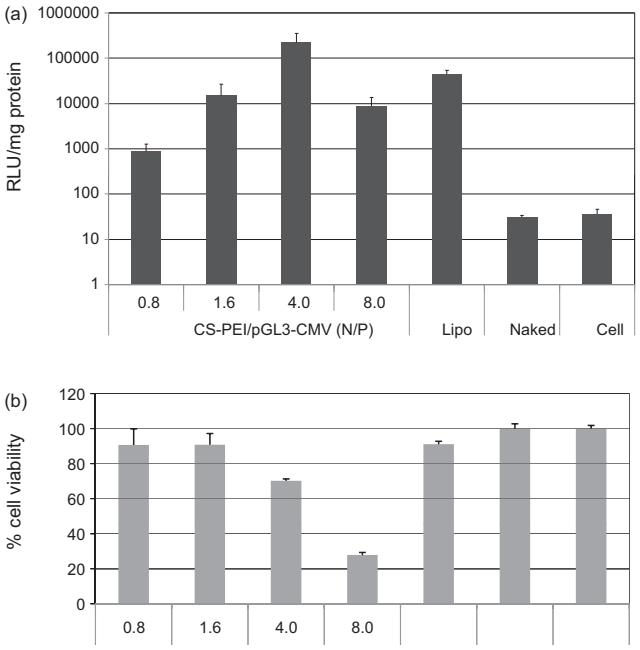

Fig. 2. Transfection efficiency (a) and cell viability (b) of CS-PEI/DNA complexes in SiHa. The results are compared to positive control Lipofectamine 2000™ (Invitrogen, USA) (Lipo), naked plasmid pGL-3-basic containing CMV promoter/enhancer (Naked) and control free cells (Cell).

the adsorption of DNA onto nanoparticles as shown in Fig. 1b. The adsorption of the DNA strands was visible especially at N/P ratios of 1.6 but the complex was difficult to see at a N/P ratio of 4.0. This may have been because of the high number of particles forming complexes with DNA in the mixture. The particle size and zeta-potential were determined at a pH of 7.4. As shown in Fig. 1c, there were no size differences among CS-PEI/DNA-formed complexes at different N/P ratios. Generally, the average size of the complexes ranged from approximately 300–400 nm which is acceptable for transfection (Rakkhithawatthana et al., 2010; Tencomnao et al., 2011). Zeta potential measurements exhibited a dynamic change in total surface charge in proportion to the CS-PEI concentration. The CS-PEI nanoparticles exhibited a zeta potential of 22.23 mV in accordance with their cationic properties. Similar to the gel retardation results, at N/P ratios of 0.4 and 0.8, the total surface charge

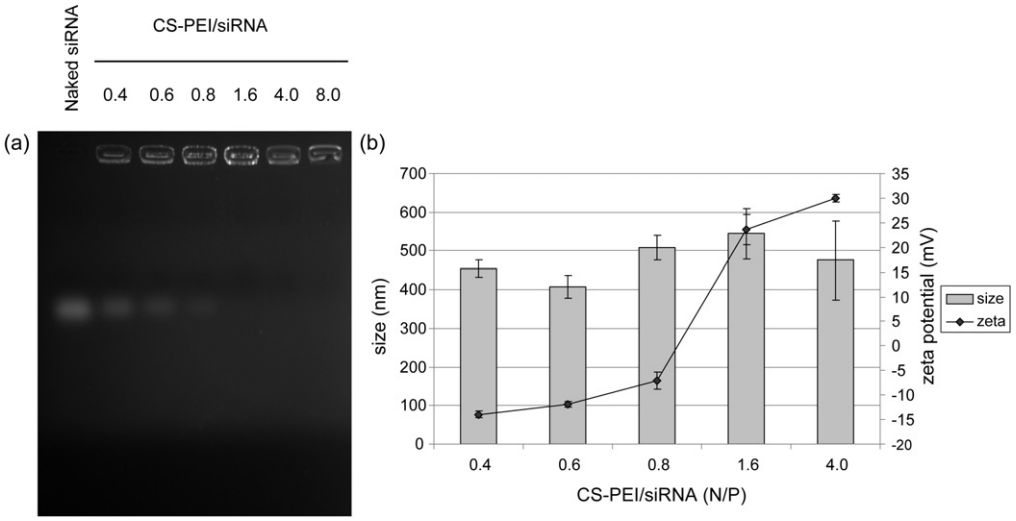

Fig. 3. Gel retardation analysis of CS-PEI/siRNA at different N/P ratios. One microgram of siRNA was applied to form a complex (a). Size and zeta-potential of CS-PEI/siRNA complexes (b).

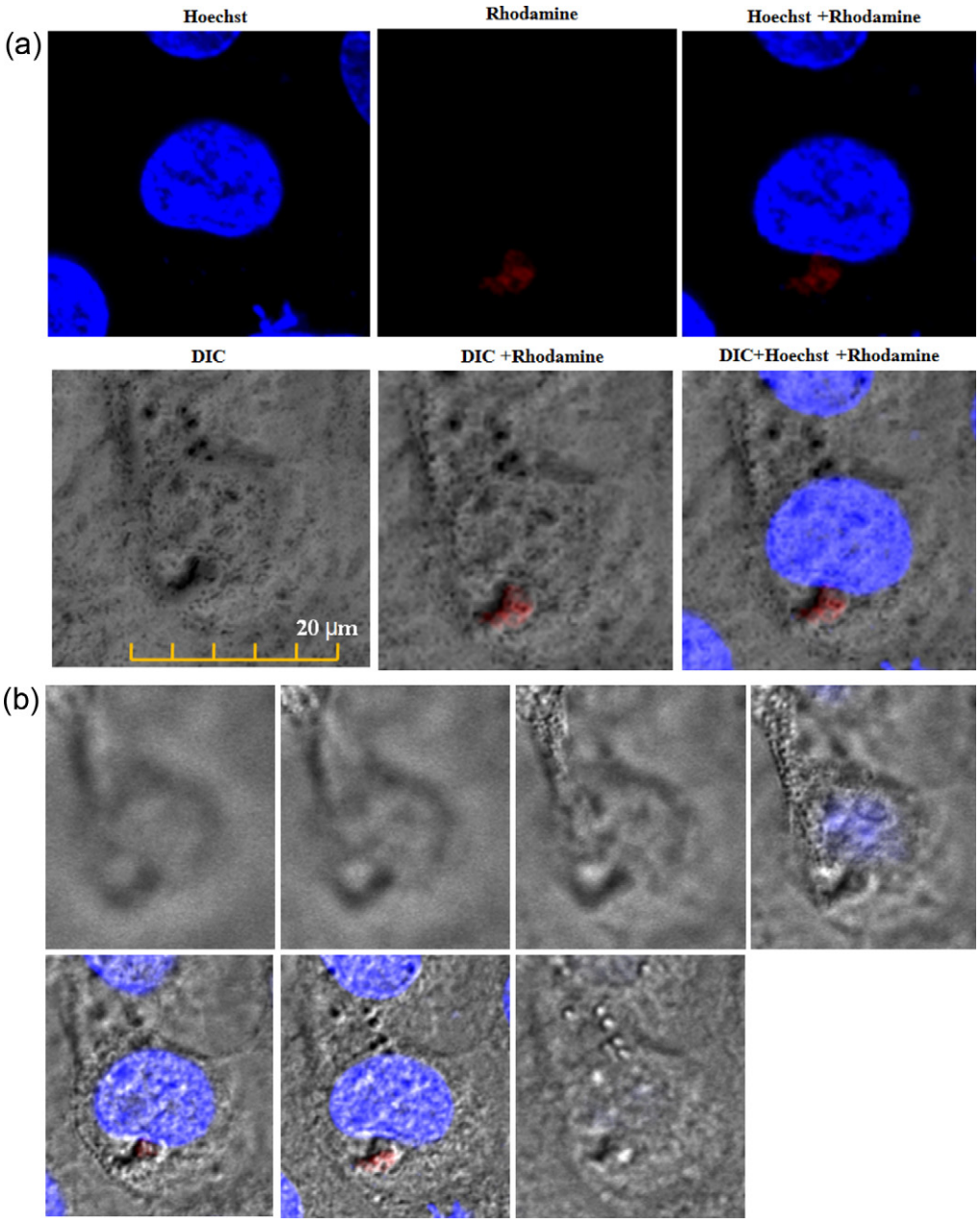

Fig. 4. Confocal images of CS-PEI/siRNA complexes in a SiHa cell. The blue fluorescence is the nucleus of a SiHa cell and the red fluorescence is the signal of rhodamine-labeled siRNA (a). Confocal Z-stack series of images of a SiHa cell 18 h post-transfection by CS-PEI/Rho-siRNA (b). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

of all nanoparticles was negative while the complexes were not completely formed. Supplementation with more CS-PEI in the formulation induced an increase in zeta potentials to 32.80, 45.36 and 45.80 mV at N/P 1.6, 4.0 and 8.0, respectively. These zeta values were strongly positively charged and facilitated cellular uptake. This result, taken together with the results from gel retardation and AFM, confirmed that the adsorption of DNA onto the surfaces of CS-PEI nanoparticles occurred through charge neutralization. Therefore, the CS-PEI nanoparticle has promise for use as a carrier for gene delivery. This result is corresponded with the previous study of Pimpha et al. (2010).

3.2. CS-PEI/DNA delivery into human cervical cells

In this study, we aimed to silence the expression of oncogenes in cervical cancer cell lines. Therefore, the human cervical cancer cell, SiHa (HPV type 16 positive) was selected to investigate the transfection efficiency of CS-PEI. To evaluate the ability of CS-PEI, *in vitro* DNA transfection was performed with 1 μg of plasmid pGL3-CMV promoter/enhancer containing a luciferase gene marker for quantitative gene expression signals (Tencomnao et al., 2008). Control-free cells, cells incubated with naked DNA, and cells transfected with DNA-loaded Lipofectamine 2000™ were used as controls. As shown in Fig. 2a, the results demonstrated the potency of CS-PEI as a gene carrier since the DNA cargo was successfully introduced and expressed. The transfection efficiency depended on the N/P value. Compared with the control-free cells and cells transfected with naked DNA, the optimal transfection efficiency was obtained from CS-PEI/DNA complexes at N/P ratios in the range of 1.6–4.0. At an N/P ratio lower than 1.6 or higher than 4.0, the transfection efficiencies were low indicating that transfection efficiency depended on CS-PEI concentration. Complex formation at

Table 1
Binding capacity of siRNA 16E6 sequence 10 (Putral et al., 2005) to the CS-PEI at N/P ratios of 0.4, 0.6, 0.8, 1.6, 4.0 and 8.0.

CS-PEI/siRNA (N/P)	Binding capacity (%)
0.4	35.9 ± 12.8
0.6	59.7 ± 14.7
0.8	72.5 ± 13.3
1.6	99.5 ± 0.50
4.0	99.3 ± 0.30
8.0	97.9 ± 0.50

low N/P ratios is not effective due to remnant unbound DNA while the excess complexes can cause toxicity to the cells. To evaluate the cytotoxicity of CS-PEI, we employed the MTT assay on SiHa transfected with the CS-PEI/DNA complex at different N/P ratios. While the viability of control cells and cells transfected with naked DNA remained constant, cytotoxicity of the CS-PEI/DNA complex which was dependent on the N/P ratio was induced (Fig. 2b). Excess CS-PEI can induce cell membrane leakage due to the aggressive penetration of highly positively charged complexes (Godbey, Wu, & Mikos, 1999). Therefore, the use of cationic nanoparticles needs to be considered carefully because excess amounts of particles will ultimately induce cellular leakage.

3.3. CS-PEI/siRNA complex formation

CS-PEI/siRNA complexes were prepared and characterized for siRNA delivery. Similar to CS-PEI/DNA complexation, the complex formation of CS-PEI/siRNA was confirmed by gel retardation using 1 μg of siRNA. The results indicated that siRNA strands were adsorbed onto CS-PEI as a result of retardation of siRNA migration by gel electrophoresis (Fig. 3a). Complete binding occurred at a N/P ratio of 1.6. The bind affinity corresponded with the CS-PEI concentration. The complexes were approximately 400–500 nm in size (Fig. 3b) and the zeta potential increased with increasing N/P ratio. Unlike the CS-PEI/DNA complex, there was no distinction observed between CS-PEI and CS-PEI/siRNA using AFM analysis due to the limitation of AFM in detecting the siRNA strand (data not shown).

3.4. Binding capacity of siRNA on CS-PEI

The binding capacity of siRNA adsorbed on the surfaces of CS-PEI at N/P ratios of 0.4, 0.6, 0.8, 1.6, 4.0 and 8.0 was measured quantitatively using the Quant-iT™ RiboGreen® RNA reagent (Invitrogen, New York, USA). As shown in Table 1, almost 100% siRNA-binding capacities were detected in CS-PEI/siRNA complexes at N/P ratios of 1.6, 4.0, and 8.0. The results suggest that the adsorption of siRNA is dependent on CS-PEI. This result corresponds well with gel retardation in which no migrated bands of siRNA were observed for the complexes with N/P ratios ranging from 1.6 to 8.0.

3.5. Cellular internalization of the CS-PEI/siRNA complex

Cellular internalization of the siRNA complex was confirmed by differential interference contrast (DIC) confocal imaging (Fig. 4a). At 18 h after transfection, a siRNA signal (red fluorescence) was detected in the cellular compartment. A Z-stack confocal image confirmed the localization of siRNA inside the SiHa cell (Fig. 4b). These results suggest that siRNA can be delivered into the cell by CS-PEI.

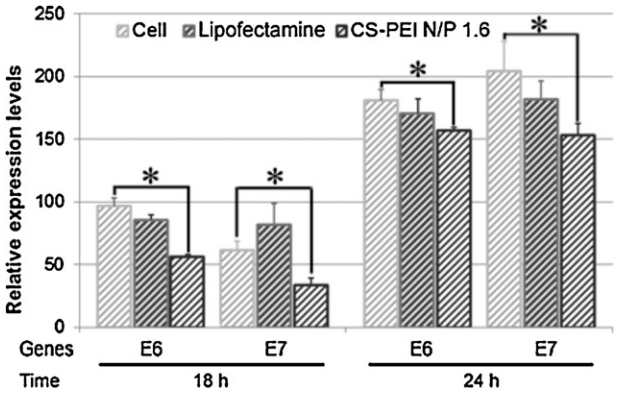

Fig. 5. Determination of E6 and E7 mRNA expression of a SiHa-transfected cell at 18 and 24 h post-transfection. The siRNA strands were delivered in the form of naked siRNA (1 μg), Lipofectamine 2000™/siRNA lipoplex and CS-PEI/siRNA complex. The expression profiles were investigated by semi-quantitative RT-PCR. Samples were prepared in triplicate and statistically analyzed by one-way ANOVA ($p < 0.05$).

3.6. Gene silencing efficiency

The role of CS-PEI as a DNA carrier in HPV-transformed cells was verified. Based on the same strategy, CS-PEI was expected to be a carrier of siRNA as well. *In vitro* transfection was performed in SiHa cells. We inhibited the activity of two oncogenes encoded by HPV, namely E6 and E7, through the activity of siRNA. These two genes are best known for their ability to inactivate the tumor suppressors, p53 and pRb, respectively (Schwock, Pham, Cao, & Hedley, 2008). After transfection, the cells were continually incubated for 18 and 24 h. The RNA was then isolated for RT-PCR as described in Section 2. The RT-PCR was performed to examine E6 and E7 expression using the primer (Table 2) (Divya & Pillai, 2006). The expression signals of both E6 and E7 were normalized by GAPDH, a house-keeping gene. The differences in the expression levels of E6 and E7 between free cells and transfected cells were detected using gel electrophoresis.

The gene silencing effects were evaluated as presented in Fig. 5. It is noted that amount of Lipofectemine 2000™ used in this study was not optimized the time period for transfection with our siRNA and cell. Lipofectemine 2000™ was used to be the control for our experimental procedure. Therefore, the gene knockdown efficiency obtained from Lipofectamine 2000™ may not represent its best result. For CS-PEI-mediated siRNA, expression of E6 and E7 genes was statistically significant different compared with free cell. The achievement of siRNA mediated CS-PEI delivery to silence of E6 and E7 expressions was verified at 18 and 24-h post transfection. However, siRNA suppression of gene expression usually is time dependent (Liao et al., 2010). Therefore, the silencing effect of siRNA may vary over time. The use of CS-PEI is proposed here as the

Table 2
Primers for RT-PCR (Divya & Pillai, 2006).

Gene	Sequence	Size (bp)
HPV type 16 E6	Sense strand: 5′-TGAGGTATATGACTTTGCTTTTC-3′ Antisense strand: 5′-CAAGACATACATCGACCGGTCC-3′	297
HPV type 16 E7	Sense strand: 5′-AAATGACAGCTCAGAGGAGGAG-3′ Antisense strand: 5′-GTTTCTGAGAACAGATGGGGCAC-3′	209
GAPDH	Sense strand: 5′-GACCACAGTCCATGCCATCACT-3 Antisense strand: 5′-TCCACCACCCTGTTGCTGTAG-3′	452

carrier of choice for both DNA and siRNA delivery using cationic polymeric-based nanoparticles.

4. Conclusions

The capacity of CS-PEI to deliver nucleic acids to cervical cancer cell lines was reported here. CS-PEI can form a complex with either DNA or siRNA via electrostatic interaction between the amine groups of cationic polymers and the phosphate groups of nucleic acids. The binding affinities of both DNA and siRNA to the surfaces of CS-PEI nanoparticles were confirmed by gel retardation assay. The results indicated that the complexes were completely formed at a N/P ratio of 1.6. The particle sizes were 300–400 nm on average while the zeta potentials became positively charged when the N/P ratio of the complex was 1.6. The optimal transfection efficiency of CS-PEI/DNA complexes delivered into SiHa was detected at a N/P ratio of 1.6. A confocal laser scanning microscope confirmed the penetration of siRNA-bound CS-PEI complexes. *In vitro* siRNA delivery revealed gene silencing efficiency at 18 and 24 h post-transfection. As a result, CS-PEI nanoparticles show potential as carriers for both gene and siRNA into cervical cancer cell lines.

Acknowledgments

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