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Research Article

Targeted Gene Delivery to MCF-7 Cells Using Peptide-Conjugated Polyethylenimine

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Abstract. Specific and effective delivery of drugs and genes to cancer cells are the major issues in successful cancer treatment. Recently, targeted cancer gene therapy has been emerged as a main technology for the treatment of different types of cancers. Among various synthetic carriers, polyethylenimine is one of the most well-known polymers for gene delivery. In this study, we conjugated phage-derived peptide (DMPGTVLP) to polyethylenimine (10 kDa) *via* disulfide bonds for targeted gene delivery into breast adenocarcinoma cells (MCF-7). As negative-control cells, we used non-related hepatocellular carcinoma cells (HepG2). Peptide-conjugated polyplex exhibited low cytotoxicity and significantly increased the transfection efficiency in comparison with unmodified polyethylenimine. Therefore, the peptide-modified vector can be used as a good targeting agent for gene or drug delivery into breast adenocarcinoma cells.

KEY WORDS: breast cancer; gene therapy; phage-derived peptide; polyethylenimine.

INTRODUCTION

Cancer gene therapy is considered as a promising treatment for various forms of cancer. To improve the stability and efficiency of gene delivery, research efforts are currently focused on designing both viral and non-viral vectors. Viral vectors are efficient in delivery of nucleic acids. However, fundamental issues of viral vectors such as immunogenicity, toxicity, mutagenesis, limited capacity, and high cost of production have encouraged researchers to use non-viral gene delivery systems (1,2).

During recent years, non-viral vectors such as liposomes (3), cationic polymers (4), cationic lipids (5), peptides (6), and dendrimers (7) have received a large deal of attention for gene delivery. Many different types of cationic polymers have been evaluated as gene delivery vectors. Polyethylenimine (PEI) is one of the widely used examples of cationic polymers capable of gene transfection due to its native endosome-buffering property (1,8). It has been shown that PEIs with medium to low MW (5–25 kDa) are more efficient and less toxic compared to PEIs with molecular weights greater than 25 kDa (9–11).

The success of gene therapy is basically dependent on the safety and efficiency of gene delivery systems for delivering a gene to specific target cells. Recently, distinctive markers of tumor cells have been identified and several targeting ligands such as antibodies, nanobodies, peptides, and aptamers have been evaluated for gene or drug delivery to specific cancer cells (12,13). Different peptides such as RGD, Tet-1, CNGRC, TGN, and CAP have been conjugated to the polymeric carriers such as PEI in order to specifically target the cells and enhance cellular uptake (14–18).

Phage display technology allows expression of exogenous (poly)peptides as fusions to capsid proteins on the outside of phage virions and was first described in 1985 by George P. Smith (19). Surface display is achieved by inserting protein or peptide encoding gene into the pIII or pVIII gene, encoding either the minor or main surface protein, respectively. This technology offers a new way to identify peptides that bind to molecular targets with good affinity and specificity, including tumor cells (20, 21). For example, it has been shown that small interfering RNA (siRNA) delivery to breast cancer cells was efficiently increased via DMPGTVLP-targeted liposomes (22). In the mentioned study, Bedi *et al.* selected a novel targeting peptide (DMPGTVLP) against MCF-7 cells using phage display technique. They used HepG2, MCF-10A, and ZR-75-1 cell lines as negative-control cells and VPEGAFSS sequence as negative-control peptide. The peptide VPEGAFSS was designed against streptavidin and did not have any specificity for MCF-7 cells (22).

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In the present study, we evaluated the potential use of phage display-derived targeting peptide DMPGTVLP for modifying the PEI 10-kDa structure to enhance its transfection specificity for breast adenocarcinoma cells MCF-7. To reach this objective, branched PEI 10 kDa which has low cytotoxicity was conjugated to DMPGTVLP sequence via disulfide bonds, and its gene delivery activity was investigated in MCF-7 cell line. Non-related hepatocellular carcinoma cell HepG2 was used as negative cell line.

MATERIALS AND METHODS

Materials

Branched polyethylenimines (bPEI 10 kDa) was purchased from Polysciences, Inc. (Warrington, PA, USA). HEPES and N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) were obtained from Sigma-Aldrich (Munich, Germany), and ethidium bromide (EtBr) was purchased from Cinnagen (Tehran, Iran). Peptides were obtained from China Peptide Company, China (purity>98%). Plasmid pCMVLuc and a luciferase assay kit were purchased from Promega (Madison, WI, USA). Tissue culture reagents were obtained from Gibco (Gaithersburg, MD, USA). Amicon centrifugal filter devices with 5000 Da molecular weight cutoff were supplied by Millipore (Billerica, MA, USA).

Cell Culture

HepG2 cells (ATCC, HB-8065) and MCF-7 cells (ATCC, HTB-22) were grown in Eagle's Minimum Essential *Medium* (EMEM) and RPMI medium supplemented with 10% fetal bovine serum (FBS), penicillin at 100 U mL^{-1} and streptomycin at 100 μg mL^{-1}. All cells were incubated at 37°C in a humidified 5% CO_2 atmosphere.

Preparation of Plasmid DNA

Plasmid DNA (pDNA) encoding Renilla luciferase (pRLCMV) (Promega, Madison, Wisconsin) was transformed into *Escherichia coli* bacterial strain DH5-α, incubated in selective Luria-Bertani medium, and extracted from the culture pellets using a QIAGEN endotoxin-free Mega Plasmid kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The concentration and purity of the plasmid was measured as the A260/A280 ratio using an ultraviolet spectrophotometer (Pharmaspec, UV-1700; Specto, Shimadzu, Japan). Samples with A260/A280 ratios higher than 1.8 were used in transfection experiments (10,23).

Synthesis of 3-(2-Pyridyldithiol) Propionate-Modified PEI

The synthesis procedure was performed based on previous reports (10,23). In brief, PEI 10 kDa was treated with SPDP (cross-linking reagent) in HEPES-buffered solution (2 mL, 20 mM, pH 8.0 containing 0.35 M NaCl) to prepare thiol-functionalized PEI for peptide conjugation. After stirring for 2 h at room temperature under argon, unreacted SPDP was removed using centrifugal membrane dialysis filter devices with 5-kDa molecular weight cutoff membranes that were conditioned before use with a solution of PEI 10 kDa (23). The preparations were diluted with buffer and centrifuged in the filter devices for 30 min at room temperature, the retentate diluted with buffer (20 mM HEPES, 250 mM NaCl, pH 7.4), and the process repeated twice. The reaction yield was determined by the formation of 2-thiopyridone (DTP) detected at 343 nm after reduction of an aliquot of each product with excess dithiothreitol. The degree of modification with SPDP was determined in an aliquot of each preparation by releasing DTP by reduction for 30 min at room temperature with excess dithiothreitol (DTT). The DTP released was determined spectrophotometrically (extinction coefficient at 343 nm: 8.08×10^3 $M^{-1}cm^{-1}$). The primary amine contents of PEI-DTP were determined using trinitrobenzenesulfonic acid (TNBS) assay at 405 nm as described previously (10,23). Aliquots of each preparation were flash frozen and stored at –80°C until use.

Synthesis of Peptide-Conjugated PEI

For conjugation of peptides to PEI 10 kDa, we used the desired synthetic sequences with a three-amino-acid linker as Cys-Gly-Gly (CGG) attached into the C-terminus of the peptides. The cysteine sulfhydryl group could be used for creating the disulfide bonds, while the two glycine residues made the conjugates more flexible at the conjugation site.

Aliquots of peptides were added to the activated PEI-DTP from the previous step in amounts calculated to achieve coupling of 10% of primary amines with peptides and kept under argon. The degree of grafting was adjusted to 10%, as calculated according to the primary amine contents, with 2.2-M excess. The reaction mixtures were diluted to 2 mL with buffer (20 mM HEPES, 500 mM NaCl, pH 7.1). After 4-h incubation at room temperature, the released thiopyridone was measured at 343 nm to determine the extent of peptide conjugation. Unreacted peptides were removed by dialysis against HEPES-buffered solution (20 mM, pH 7.4 containing 50 mM NaCl) using an ultrafiltration cell equipped with a 5-kDa molecular weight cutoff membrane (Amicon). The amine content of each product was determined by TNBS assay at 405 nm. Solutions of the peptide conjugates in the retentate were flash-frozen in aliquots and stored at –80°C until used (10,23).

Reverse-Phase High-Pressure Liquid Chromatography

The final products were further analyzed by HPLC on a C18 reverse-phase column (5 μm, 250×4.6 mm, I.D.). First, the retention time of peptide was confirmed based on the HPLC certificate and then the final conjugates were injected into the column. The programmed gradient elution was performed at a flow rate of 1 mL min^{-1} with the mobile phases of 0.1% TFA in acetonitrile (solution A) and 0.1% TFA in H_2O (solution B) based on the chromatography program of peptide sheet. To examine the conjugation of peptide to PEI-DTP, excess DTT-treated products were then injected into the HPLC column. The presence of the peptide peak after incubation with DTT while it did not exist prior to DTT treatment could confirm the peptide coupling (10).

Fourier Transform Infrared Spectroscopy

Appropriate amount of final products were used to make KBr disks. Fourier transform infrared spectroscopy (FTIR) was conducted using a FTIR spectrometer (Spectrum 2000; Perkin-Elmer, Waltham, MA, USA) in the spectral region 400 to 4000 cm^{-1}.

EtBr Exclusion Assay

DNA condensation ability of PEI and peptide-conjugated PEI was evaluated by ethidium bromide (EtBr) exclusion assay. Fluorescence intensity was determined (excitation λ_{ex} 510 nm and emission λ_{em} 590 nm) in a Jasco FP-6200 fluorometer (Tokyo, Japan). Plasmid DNA (5 μg) was complexed with EtBr (400 ng mL^{-1}) in 1-mL HBG buffer and the fluorescence intensity recorded was taken as the 100% value. The fluorescence intensity of 400 ng mL^{-1} EtBr solution was used as the background value. A series of concentrations of PEI or PEI–peptide were added to the fluorometer cuvettes containing DNA and EtBr. Plasmid condensation by PEI or its derivatives was measured in triplicate as decreased fluorescence intensity and results are reported as mean±SD (10,23).

Agarose Gel Retardation Assay

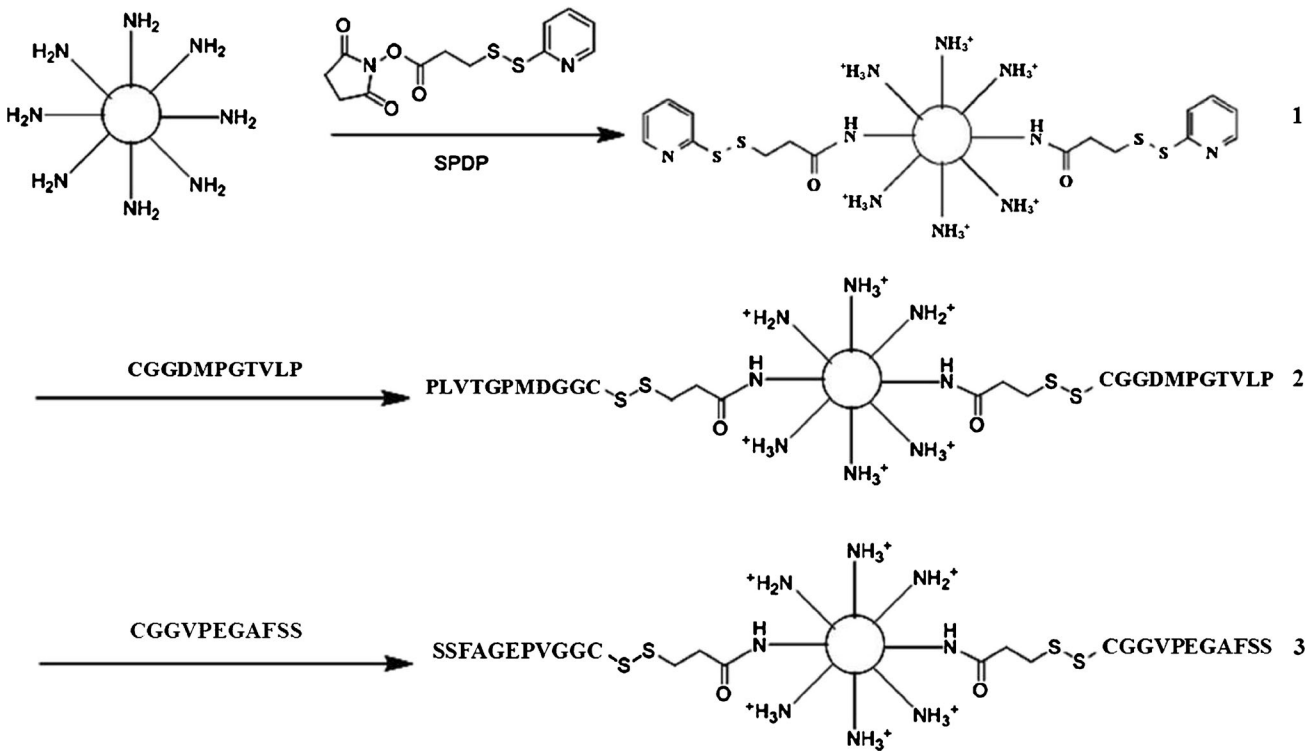
PEI 10 kDa and peptide-conjugated polyplexes were prepared in different C/P ratios by mixing the polymers with 0.5 μg of pDNA in a total volume of 25 μL in deionized water. After incubating at RT for 20 min, 8 μL of polyplexes and pDNA alone (as control) were mixed with 2 μL of loading dye and transferred to the wells of a 1% agarose gel containing 1 μg mL^{-1} EtBr. The gel was run for 45 min at 80 mV with 0.5% TBE as running buffer, and finally, the bonds were visualized by UV light.

Particle Size and Zeta Potential Measurements

Particle size and zeta potential of the polyplexes (vector/pDNA complexes) containing PEI 10 kDa and peptide-conjugated PEIs were measured using dynamic light scattering (DLS) and laser Doppler velocimetry (LDV), respectively, by Malvern Nano ZS instrument (Malvern Instruments, UK).

Cytotoxicity Assay

MCF-7 and HepG2 cells were seeded in 96-well plates at an initial density of 1×10^4 cells per well in 100 μL EMEM and RPMI complete medium, respectively, and incubated for 24 h. Different polyplexes at different carrier/plasmid *w/w* (C/P) ratios ranging from 2:1, 4:1, and 6:1 were used for cytotoxicity experiments. Twenty microliters of complexes (equivalent of 200 ng pRL-CMV) were added into each well. After 4 h, the media was replaced by fresh complete media containing 10% FBS and the cells were incubated for 18 h, followed by addition of 20 μL MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, 5 mg mL^{-1} in phosphate-buffered saline] solution per well and additional 3 h incubation at 37°C. The medium was then removed and the formazan crystals were dissolved in 100 μL DMSO. Absorbance was measured at 570/630 nm on a STAT FAX-2100 microplate reader (Awareness Technology, Palm City, FL, USA) and cell viability (%) relative to control wells containing cell



Scheme 1. The chemical synthetic process used to make peptide-PEI conjugates. (*1*) PEI-PDP (activated PEI), (*2*) PEI-PP, and (*3*) PEI-NP. *PP* positive peptide, *NP* negative peptide, *PEI* polyethylenimine

Table I. Size and Zeta Potential of Polymer/DNA Complexes Prepared with Either Modified or Unmodified PEI

Polymer	Size of DNA/ polymer complex (nm) (±SD)	Zeta potential of DNA/ polymer Complex (mV) (±SD)
PEI 10 C/P 2	104.95±3.32	13.62±1.05
PEI 10 C/P 4	198.68±6.52	12.5±2.68
PEI 10 C/P 6	214.43±24.035	12.29±3.78
PEI 10-PP C/P 2	58.185±2.94*	7.86±0.88*
PEI 10-PP C/P 4	84.33±19.21*	5.26±073*
PEI 10-PP C/P 6	141.33±8.73*	5.135±3.35*
PEI 10-NP C/P 2	72.93±12*	2.28±0.10*
PEI 10-NP C/P 4	77.28±0.86*	2.56±1.31*
PEI 10-PP C/P 6	136.66±7.63*	2.4±2.07*

Statistically significant differences in size and zeta potential of modified PEI compared to PEI 10 are indicated as $*P \leq 0.05$

culture medium without polymer was calculated by [A] test/[A] control×100.

Cell Transfection with pRL-CMV

For cell transfection, MCF-7 and HepG2 cells were seeded at a density of 1×10^4 cells/well in 96-well plates and incubated for 24 h. Cells were then treated for 4 h with 20 μL of polyplex preparations in serum-free EMEM containing 200 ng pDNA at the same C/P ratios [(*w/w*)] used for the toxicity experiments. After 4 h, the medium was replaced with fresh complete medium containing 10% FBS and gene expression was assayed 18 h later. Then the medium was removed and cells were lysed by adding 50 μL of cell lysis buffer. The luciferase activity in cell lysate was measured using Promega Luciferase Assay Kit on Luminometer (Brthlod Detection System, Pforzheim, Germany). The results were reported as relative luminescence units (RLU) per 4000 seeded cells as mean±SD, $n=3$.

Flow Cytometry Analysis of GFP Reporter Gene Expression

MCF-7 cells were seeded (8×10^4 cells per well) in 12-well plates. Modified and unmodified polymers in different C/P ratio were complexed with 3 μg of pDNA (pEGFP) and added to the cells. After 4 h, the medium was removed and replaced with fresh complete medium. Cells were harvested 48 h post transfection and kept in an ice bath until analysis. The percentage of GFP positive cells was determined using a Partec flow cytometer (Partec, Münster, Germany).

RESULTS

Synthesis of Peptide-Conjugated PEI

About 10% of primary amine content of PEI was modified with (*N*-succinimidyl 3-(2-pyridyldithio)-propionate) (SPDP). The number of DTP groups per average chain of PEI was calculated using excess dithiothreitol (DTT) to release 2-thiopyridone at 343 nm (molar absorptivity=8.08×10^3 M^{-1} cm^{-1}), which was quantified spectrophotometrically.

The release of 2-thiopyridone in the presence of the peptide was indicative of the peptide coupling to PEI–DTPs (Scheme 1) and the reaction was followed up by monitoring the release of 2-thiopyridones. The quantity of peptide grafted to the primary amines was estimated to be approximately 8%. Moreover, reverse-phase high-pressure liquid chromatography (RP-HPLC) method was used to confirm the conjugation of peptides onto PEI. For this, the peptide-conjugated PEI samples were incubated with excess amount of DTT and the release of free peptides, if any, was detected by RP-HPLC (Supplementary Figure 1).

Furthermore, in FTIR spectrum, the amide I bond was shown as a peak at 1659 cm^{-1}. This peak is due to C=O stretching vibration of the peptide bond (Supplementary Figure 2).

The resulting vectors were abbreviated as PEI10–PP and PEI10-NP, in which PP represents the positive peptide with the sequence of "CGGDMPGTVLP" and NP shows the

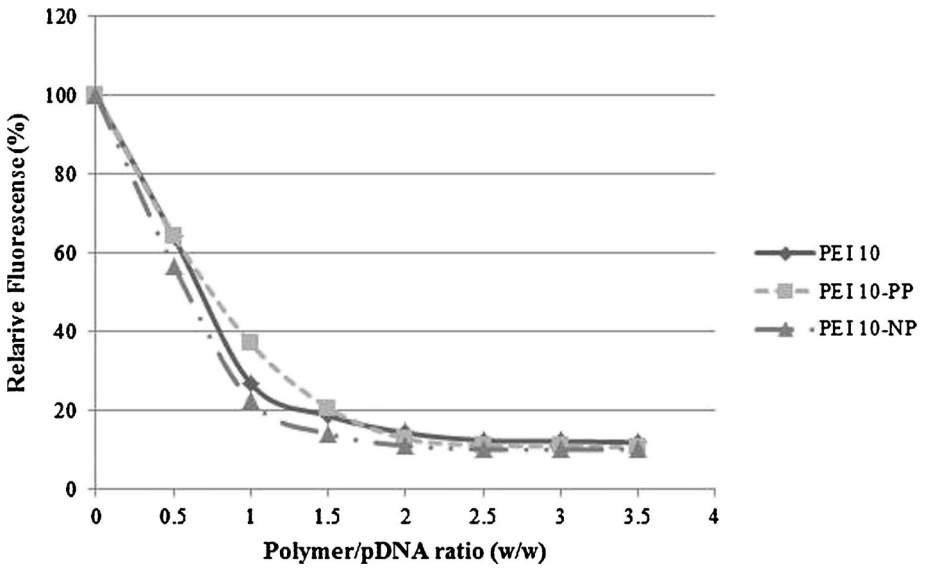


Fig. 1. DNA condensation ability of polycationic vectors measured by EtBr assay

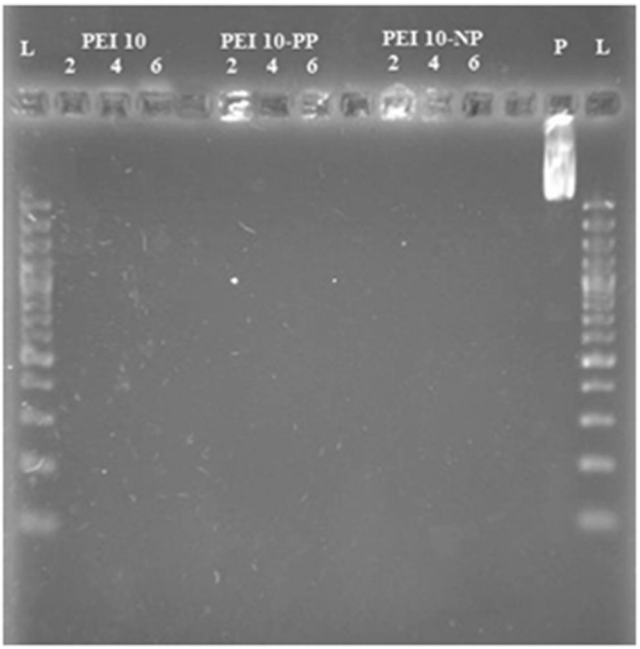


Fig. 2. Gel retardation assay of PEI 10 kDa and peptide-conjugated PEI polyplexes in different C/P ratios. *Lane P* plasmid. *Lane L* ladder

negative peptide with the sequence of "CGGVPEGAFSS" (Scheme 1).

Particle Size and Surface Charge Analysis

The ability of the PEI 10 kDa and peptide-modified PEIs to condense pDNA into nano-sized particles was studied by a zeta sizer. Particle size and ζ-potential measurement results are displayed in Table I. Unmodified PEI 10 kDa was used as control. Peptide modifications resulted in the complexes with the size range of 58.185±2.94 nm to 141.33±8.73 nm and zeta potentials of 2.28±0.10 mV to 7.86±0.88 mV. Therefore, peptide conjugation notably decreased polyplex size and zeta potential when compared to PEI polyplexes at the same C/P ratio.

DNA Condensing Ability

Two methods were employed to determine the binding strength of PEI 10 kDa and peptide-modified PEIs for pDNA, namely their ability to condense pDNA and their ability to retard the migration of nucleic acids in agarose gel electrophoresis.

PEI 10 kDa and peptide-conjugated PEI vectors were prepared in filtered HEPES-buffered glucose (HBG) solution (1 mL, 20 mM, pH 7.4 containing 5% glucose). EtBr, when intercalated in double-stranded pDNA, emits fluorescence. Condensation of pDNA by polymer decreases fluorescence intensity by interfering with the EtBr intercalation process. By adding 2.5 μL aliquots of vectors to the solution containing EtBr bound to plasmid DNA, the fluorescence intensity decreases by releasing EtBr free in solution. Results showed that PEI 10 kDa and peptide-conjugated PEIs reduced the fluorescence intensity of EtBr solution by more than 80% at C/P ratio of 2 (Fig. 1). For higher C/P ratios, no more reduction was observed. As shown in Fig. 1, there is no significant difference between binding affinity for pDNA of PEI 10 kDa and that of peptides-conjugated PEI derivatives.

PEI 10 kDa or peptide-modified PEIs/DNA polyplexes were also loaded on agarose gel in different C/P ratios. The results indicated that only free, unbound pDNA moved through the gel in the electric field (Fig. 2). The decrease in electrophoretic mobility of PEI 10 and peptide-modified PEIs/DNA polyplexes indicated that pDNA binding was irreversible at different C/P ratios.

Cytotoxicity Assay

The cytotoxicity of peptide-conjugated PEIs was evaluated by MTT assay in MCF-7 and HepG2 cell lines at C/P ratios of 2, 4, and 6 and compared with the control PEI 10 kDa. As shown in Fig. 3, both peptide-conjugated PEIs and PEI did not exhibit any significant toxicity at C/P ratios tested on both cells ($P \leq 0.05$).

Gene Transfection Studies

By grafting the positive peptide CGGDMPGTVLP (PP) onto PEI 10-kDa backbone, transfection efficiency of PEI 10 kDa on MCF-7 cells was enhanced by about 2.6- to 4-fold at different C/P ratios. On the other hand, the negative peptide CGGVPEGAFSS (NP) could not improve the transfection efficiency of PEI 10 kDa in

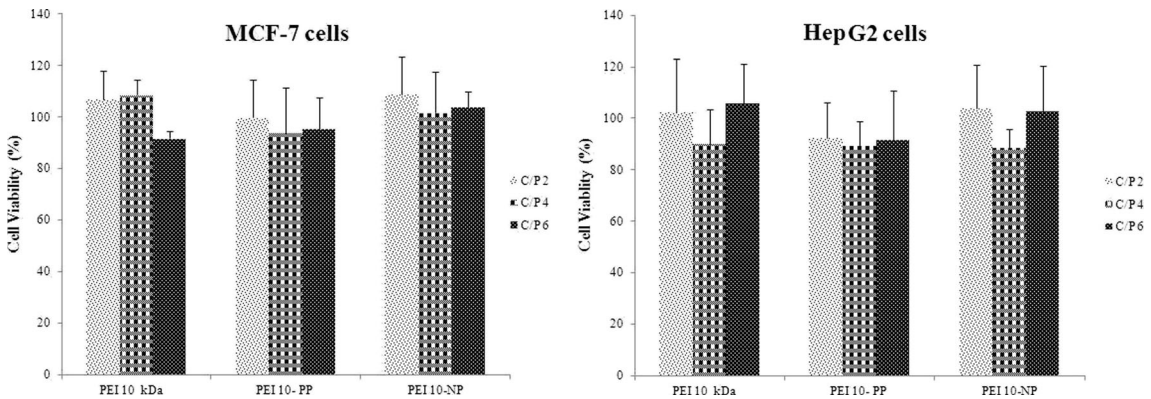


Fig. 3. Viability of MCF-7 and HepG2 cells exposed for 4 h to polyplexes prepared from plasmid DNA and PEI 10 kDa or peptide-conjugated PEI 10 at the indicated C/P ratios

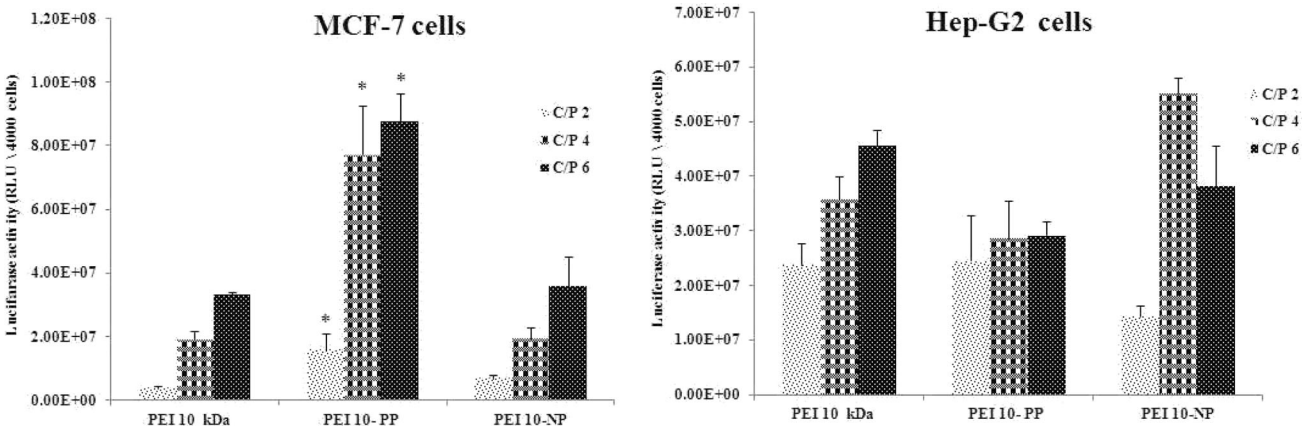


Fig. 4. Transfection efficiency of polyplexes prepared from plasmid DNA encoding the luciferase gene and PEI 10 kDa or the peptide-conjugated PEI 10. Luciferase activity expressed in MCF-7 and HepG2 cells as positive and negative cells, respectively. Statistically significant differences in transfection efficiency of modified PEI compared to PEI 10 kDa are indicated as $*P \leq 0.05$

MCF-7 cells (positive cells). No significant increase in transfection efficiency was observed for any of the peptide-modified PEIs in HepG2 cells as the negative cell line having no receptor for the peptide (Fig. 4). Similar results were obtained using GFP transfection analyzed by fluorescence microscopy (Supplementary Figure 3). Furthermore, flow cytometry data revealed that compared to PEI 10 kDa, peptide-modified PEIs increased the GFP expression about 1.6- to 4-fold at different C/P ratios (Fig. 5). Transfection experiment without carriers (plasmid DNA only), which is considered as negative control, showed an undetectable gene expression.

DISCUSSION

Effective cancer treatment needs efficient drug delivery systems. Reducing the side effects on normal cells as well as specific and efficient delivery of drugs and genes to cancer cells is the main concerns of an effective cancer treatment (24). Targeting strategies can be used for the selective delivery of nucleic acids to the desired cells (22). Up to now, several targeting elements such as antibodies, aptamers, peptides, *etc.* have been employed in carriers for such purpose (12,15,24,25). Phage display technique is one of the best methods that have been used for identification of new cell-specific receptors on the surface of cancer cells. In this approach, a random peptide library expressing on the surface of genetically engineered bacteriophages is screened for the identification of new antigens (receptors) on cells. The selected targeting peptides can be conjugated to the different gene delivery vectors for delivering nucleic acids to the specific cells (17,18,26). Phage display-designed peptides have many advantages such as variety and abundance, easy and cost-effective production, and the rapid process for identification of specific peptides for the cell receptors (21). Inspired by the study of Bedi *et al.* (22), we investigated the specificity and efficiency of

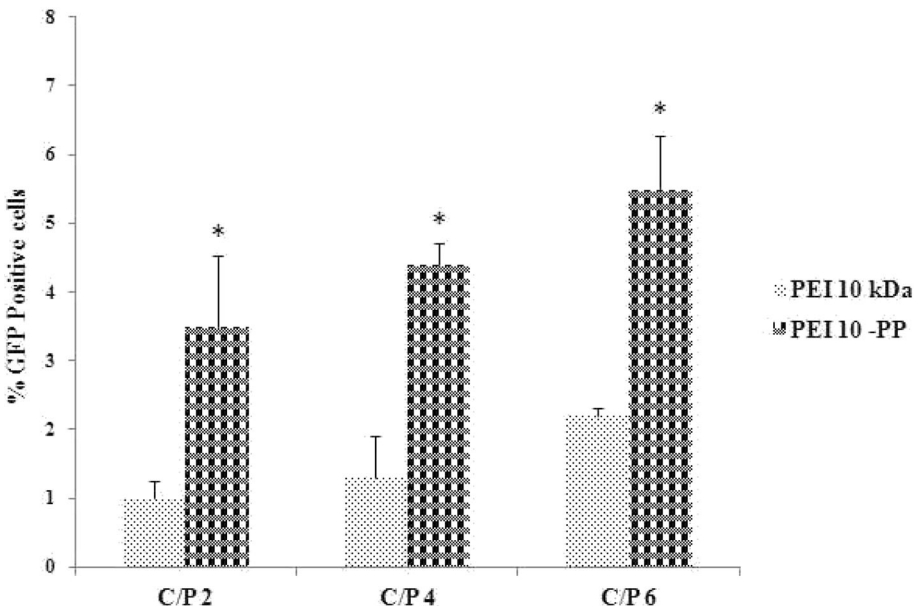


Fig. 5. Transfection efficiencies of PEI 10 kDa and PEI 10-PP in MCF-7 cells evaluated by flow cytometry

CGGDMPGTVLP-targeting peptide in the context of polymeric gene carrier for delivering genes into breast adenocarcinoma cells. We therefore conjugated CGGDMPGTVLP peptide to branched PEI 10 kDa and used such construct for targeted gene delivery to MCF-7 breast cancer cells. Moreover, CGGVPEGAFSS peptide was used for comparison as negative control.

Due to the lower toxicity of PEI 10 kDa compared to higher molecular weight PEIs, PEI 10 kDa could be used as less toxic transfection vector for gene delivery. However, considering its lower transfection efficiency, the structure is still needed to improve via optimization strategies such as surface modification or conjugation with different molecules. For instance, it has been shown in our laboratory that conjugation of different arginine-rich peptides (10) or lysine-histidine peptides (23) to PEI 10 kDa could significantly enhance the transfection efficiency.

In this study, peptides were coupled to the PEI 10-kDa core by disulfide bonds. The disulfide linkages are believed to be cleaved in reducing intracellular environment which exists in both cytosol and nucleus (27). Arginine-modified PAMAM with biodegradable ester bonds has shown better biodegradability compared to the analogous structures with the amide bonds (28).

Based on our results, conjugation of the CGGDMPGTVLP peptide (phage-displayed targeting peptide) to polyethylenimine increased the transfection efficiency about 2.6- to 4-fold at different C/P ratios in MCF-7 cells compared to HepG2 as negative-control cells. On the other hand, results showed that grafting the CGGVPEGAFSS peptide (negative-control peptide) onto the PEI backbone did not increase transfection efficiency at different C/P ratios in both cell lines. These observations suggest that incorporation of the sequence DMPGTVLP in the polymeric PEI carrier could specifically target the breast cancer cells used in this study and therefore increase the transfection efficacy. Our results were also in agreement with Bedi *et al.* (22) findings which have shown enhanced siRNA delivery by incorporation of the mentioned targeting peptide into a liposomal formulation.

The cytotoxicity of polyplexes prepared with peptide-conjugated PEI was also determined in MCF-7 and HepG2 cells. Results showed low toxicity for all vectors with no significant difference between the toxicity of peptide-conjugated PEI 10 and that of PEI 10 kDa in any of the cell lines tested. The observed lack of cytotoxicity of polyplexes containing the modified polymers might be partially related to the hydrophobic nature of the peptide sequences. It can be also related to the biodegradability of peptide sequences and disulfide bonds linking the sequences to the core structure. On the other hand, conjugation of both peptides with PEI 10 kDa significantly affected the physicochemical features such as polyplex size and zeta potential. Both the mean size and zeta potential decreased upon peptide modifications. The reduction in zeta potential is probably due to the covering of the positive charge sources, *i.e.*, amine moieties on the surface of polyethylenimine by the hydrophobic sequences. Moreover, it seems that the presence of hydrophobic amino acid residues in the peptides might be the probable reason for the observed favorable condensing ability. The existence of hydrophobic residues in peptides could improve DNA binding and transfection efficiency in other gene delivery systems as well (29). The suitable interactions between hydrophobic residues may also compact the vectors and decrease the size of resulted polyplexes.

CONCLUSION

Conjugation of DMPGTVLP peptide to PEI 10 kDa could significantly increase transfection efficiency of PEI in MCF-7 cell line with minimal cytotoxicity. Therefore, targeting of PEI with this peptide may be useful for further *in vivo* application.

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