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Preparation and Characterization of Celecoxib-Conjugated Polyethylenimine as a Potential Nanocarrier for Gene Delivery

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Abstract

The objective of the present investigation was to conjugate celecoxib as a cyclooxygenase-2 (COX-2) inhibitor onto polyethylenimine (PEI) in order to prepare nanoparticles for tissue targeting. Since celecoxib binds to COX-2 and this enzyme is over expressed in several pathological conditions including cancer, the final goal of the study was to direct the nanoparticles into specific tissues. Celecoxib was conjugated on PEI structure at two substitution degrees of 5 and 10 % and the new conjugates were characterized in terms of size, zeta potential, buffering capacity, plasmid DNA binding affinity and protection against enzymatic degradation as well as cytotoxicity. The results demonstrated the ability of the PEI conjugates in the formation of nanoparticles with the size of around 200 nm with buffering capacity comparable with unmodified PEI. The celecoxib conjugated PEI derivatives demonstrated high binding affinity to pDNA and protection effect against degradation by DNase I. The conjugation of celecoxib onto PEI structure slightly reduced the toxic effects of unmodified PEI especially at the conjugation degree of 10%. However, this result showed that the significant decrease of PEI cytotoxic effects could not be achieved by the shielding of surface amines even at the conjugation degree of 10%. Therefore, it is suggested to investigate the effects of higher degrees of amine substitution to produce less toxic PEI -based nanocarriers.

Keywords: Celecoxib, Gene Delivery, Nanopartcle, Polyethylenimine.

1. Introduction

Several reports indicate the potential anticancer and anti proliferative properties for non-steroidal anti-inflammatory drugs (NSAIDs) (1-4). Such attribute might be correlated with the inhibition of cyclooxygenase (COX) enzymes in the cancer cells which is supported by experimental and pathological studies (5-8). COX enzymes are responsible for biosynthesis of crucial bioactive prostanoids including prostaglandin E2 (PGE2), prostaglandin D2 (PGD2) as well as prostacyclin, and thromboxane A2 (9). At least three isoforms of the enzyme has been reported (10-12). COX-1 is responsible for the production of physiological protective prostaglandins (PGs) while COX-2 is an inducible enzyme and its expression increases following pathological situations. In other words, COX-2 levels are almost undetectable in normal tissues. COX-3 is expressed in the CNS and it is believed that the prostaglandins derived from this isoform are responsible for the regulation of body temperature (13). Induction of COX-2 is stimulated by tumor promoters or inflammatory cytokines such as interleukin 1β (IL-1β) and TNF-α (14, 15). Traditionally, COX-2 inhibitors have been widely used for the reduction of inflammation but nowadays many clinical trials are being carried out to prove anti-cancer properties of COX-2 inhibitors

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(16-19). PGE2 is synthesized from arachidonic acid by COX-2 enzyme and responsible for several various pathological consequences including fever induction, inflammation-related vasodilation and hyperalgesia (20, 21). Also, it has been shown that PGE2 may stimulate cell proliferation. The association of the elevation of PGE2 levels and cancer initiation is well documented (20). Since PGE2 is produced by the catalytic activity of COX-2, new strategies have been considered based on the importance of the enzyme in the initiation of cancer. The COX-2 over-expression occurs in various types of cancers including human colorectal adenoma and adenocarcinoma (22), human lung cancer (23, 24), head and neck carcinoma (25), as well as pancreatic carcinoma (26) and human cervical cancer (27), (28). Furthermore, the over expression of COX-2 could be considered as a diagnostic tool for cancer in the early stages. (29, 30). There are some investigations reporting the relationship between the overexpression of p53 and COX-2 (30). Also, the role of COX-2 in angiogenesis through the induction of VEGF pathway has been reported in several investigations. (31, 32). On the other hand, COX-2 expression has an established effect on tumor cell resistance against apoptosis and decreasing cell-mediated immunity (33, 34). At the same time, COX-2 peroxidase activity can transform procarcingoenous cells into cancerous ones by activating sevaral oncogenes in the cells (35-37) (38). There are several various studies showing the elevation of COX-2 mRNA level or intact enzyme before the observation of any clinical signs (39-41). These findings highlight the significant importance of COX-2 in diagnosis and treatment of cancer cells. Beside cancer and inflammatory conditions, COX-2 over expression is associated with some neurodegenerative conditions such as Alzheimer's and Parkinson's disease in which the COX-2 level increases before any symptoms appear (42-48). Due to the important role of COX-2 in many pathological conditions, a lot of selective COX-2 inhibitors were designed and enrolled to clinical trials. Celecoxib is the most famous COX-2 inhibitor in the clinic. Since an increase in COX-2 levels occur before the observation of clinical findings, labeled COX-2 inhibitors have been widely used for *in vivo* detection of COX-2 distribution in different organs and tissues. That is why the various radionuclides were used for labeling COX-2 inhibitors for the detection of specific pathological conditions using detection techniques such as SPECT and PET (42, 49-52). Therefore, COX-2 inhibitors have shown the potential to target molecules including drugs and carriers into a specific organ and may act as a tissue/organ targeting ligand.

The delivery of genetic materials into different cells, tissues or organs has been limited due to the lack of targeting moieties to transfer these macromolecules into the specific site of action. The genetic materials including plasmid encoding therapeutic genes need a carrier to cross various borders hampering their efficient transfection. Among different carriers used for gene delivery, polyethylenimine (PEI) has been extensively investigated and showed great ability to transfer various oligonucleotides into cells and tissues. This polycationic compound interacts electrostatically with the negative charge backbone of the nucleic acid materials and compacts them into nanostructures called polyplexes. These properties make PEI an efficient gene delivery carrier. However, lack of targeting quality as well as its relatively high toxicity, limit its wide clinical application. Although the high amine content of the molecule plays a crucial role in the formation of polyplexes, the high positive charge density results in the induction of cytotoxic effects. In other words, the modulation of high amine charge density on the surface of the polymer could be considered as an efficient approach for the reduction of toxic effects of the polymer. Since the conjugation of targeting ligands on the PEI structure occurs via its surface amines, these substitutions may also reduce its toxicity. In other word, conjugation of targeting moieties on the PEI surface not only directs the polyplex into a specific cell or tissue, but also decreases its toxic effects.

Considering over expression of COX-2 genes in pathological conditions and elevation of COX-2 levels in cancer tissues, conjugation of a COX-2 inhibitor, such as celecoxib, on the surface of PEI could be an effective strategy to reduce its high positive charge density and consequently its toxic effects. In this study, celecoxib was conjugat-

ed on the PEI structure at two substitution degrees of 5 and 10% followed by the characterization of the PEI derivatives in terms of buffering capacity, size and zeta potential, pDNA binding affinity as well as the protection of plasmid against nuclease digestion and cytotoxicity.

2. Material and methods

2.1. Materials

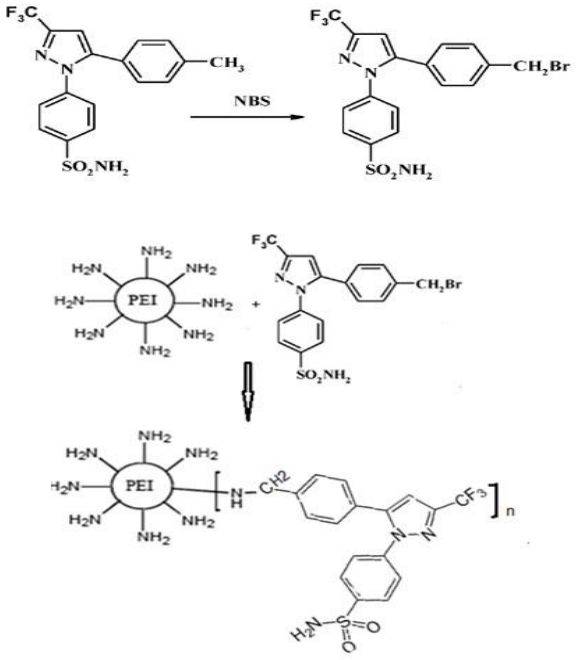
Branched polyethylenimine (b-PEI; average MW 25 kDa) was purchased from Sigma-Aldrich (Munich, Germany). Celecoxib was kindly donated by Darou Pakhsh Company (Tehran, Iran). N-hydroxybenzotriazole, 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC), N-[2-hydroxythyl] piperazine-N0-[2-ethanesulfonic acid] (HEPES) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were obtained from Sigma-Aldrich (Munich, Germany). EndoFree Plasmid Mega Kit was purchased from Qiagen (Valencia, CA, USA). Plasmid pUMVC3-hIL12 (human interleukin-12 under control of the cytomegalovirus enhancer/promoter) was purchased from Aldevron (Madison, WI, USA). Ethidium bromide (EtBr) and DNA ladder 1 kb were purchased from Cinnagen (Tehran, Iran). Fetal bovine serum (FBS) and Dulbecco's modified Eagle's medium (DMEM) were purchased from Gibco (Gaithersburg, MD, USA). Dialyses were performed using Spectra/Por dialysis membranes (Spectrum Laboratories, Houston, TX, USA). All solvents were obtained from Sigma-Aldrich (Munich, Germany) and were of the highest purity available.

2.2. Preparation of brominated derivative of celecoxib

4-[5-(4-bromomethyl-phenyl)-3-trifluromethyl-pyrazol-1-yl]-benzenesulfonamide was prepared according to a previously reported method (53). Briefly, N-Bromosuccinimde (NBS; 0.850 g, 4.77 mmol) and 2-3 crystals of benzoyl peroxide were added to the solution of celecoxib (1 g, 2.26 mmol) in carbon tetrachloride (30 ml). The mixture was reacted under sun lump for 24 hours. Following the confirmation of the reaction by thin layer chromatography (TLC), the solvent was evaporated under vacuum condition. The product was purified by column chromatography using silica gel and a mixture of petroleum ether and ethyl acetate as the mobile phase. The brominated celecoxib was used for the conjugation reaction (Scheme 1).

2.3. Synthesis of celecoxib-conjugated PEI

In order to prepare the celecoxib-conju-



Scheme 1. Synthesis of celecoxib conjugated PEI derivatives at two degrees of substitutions.

gated PEI derivative, the brominated derivative of celecoxib was dissolved in ethanol (6 mg/ml) and added dropwise to the PEI solution at the same solvent. The reaction was monitored by TLC. After around 2 hours the reaction was completed. The reaction mixture was filtered and dialyzed three times against 2 L of 50% ethanol using dialysis membrane (cut off=10000). After removing unreacted materials, ethanol was removed under reduced pressure and the resulting solution was lyophilized and characterized using a Bruker Avance DRX-500 MHz NMR spectrometer (Bruker, Ettlingen, Germany). The reagent/ polymer amine ratio in the feed was adjusted to achieve two conjugation degrees of 5 and 10 % and labeled as PEI-Cel 5% and PEI-Cel 10% in which Cel stands for celecoxib and X% shows the calculated conjugation degree.

2.4. Plasmid preparation

Plasmids pUMVC3-hIL12 were transformed into *Escherichia coli* bacterial strain DH5α and amplified in selective Luria-Bertani (LB) media at 37 ºC overnight. The propagated plasmids were extracted and purified using the Qiagen Endo-Free Plasmid Mega Kit (QIAGEN, Valencia, CA,USA) according to the manufacturer's instructions.

2.5. Nanoparticle preparation

Different concentrations of PEI, celecoxib-conjugated PEI and plasmid DNA were prepared in HBG solution (HEPES buffered glucose; 20 mM HEPES, 5% glucose pH=7.2). In order to prepare the polyplexes, 50 µL of polycation solution were added to 50 µL of plasmid solution in the same medium. Then, the mixture was incubated for 30 min to form stable polymer/plasmid complexes. The composition of nanoparticles was characterized by weight/weight ratio of PEI and its derivatives (C) to the plasmid (P) in the final formulation.

2.6. Determination of the buffering capacity of PEI and its derivatives

The buffering capacity of PEI and its derivatives was measured by a standard procedure (54). In brief, the solution of each polymer (0.4 mg/mL) in double distilled water was prepared. The pH was raised to 12 using NaOH (1M). Then, HCl (1M) was added to each solutions in 5 µl portions and the pH of each solution was recorded. Once the final pH reached about 2, the acid addition was stopped and the pH values were plotted against added volume of acid.

2.7. Measurement of the pDNA binding affinity to the PEI derivatives

The ability of pDNA to bind to the PEI and its modified derivatives was evaluated by ethidium bromide exclusion assay using fluorescent spectroscopy (excitation at 510 nm and emission at 590 nm). According to the previously reported procedure (55), a solution of EtBr (0.4µg/ml) and plasmid (20 µg/ml) were prepared and the fluorescent intensity was set to 100% (C/P=0). Subsequently, 20 µL of PEI or its modified derivatives were added stepwise to the EtBr solution and the decreased fluorescent intensity was measured by fluorescent spectroscopy. Finally, the relative fluorescent intensity was plotted against the PEI/plasmid ratio. Decrease of in the relative fluorescent intensity demonstrated the ability of polymer to condense the pDNA.

Also, the binding affinity of the modified polymers to the plasmid was evaluated by a gel retardation assay. In brief, the polyplexes were formed as described above. Then, the polyplexes were loaded on an EtBr containing agarose gel (1%) and the electrophoresis was carried out at 70 V for 1 h. Finally, the gels were photographed under UV light.

2.8. DNase I protection assay

In order to evaluate the ability of the modified PEI derivatives to protect pDNA against enzymatic degradation, DNase I protection assay was carried out. The polyplexes were prepared in different polymer/plasmid ratios (0.25-8) as described above. One microliter of DNase I or PBS in DNase/Mg^{2+} digestion buffer (50 mM Tris-Cl, pH 7.6 and 10 mM $MgCl_2$) was added to the polyplexes prepared at different C/P ratios and incubated for 30 min at 37 ºC. Instantly following the incubation, DNase I was inactivated by adding 4 µl of EDTA (250 mM) followed by mixing it with

1 % sodium dodecyl sulfate (SDS), dissolved in 1 M NaOH (pH 7.2). Finally, all samples were incubated for 1.5 h at room temperature in order to allow the polyplexes to be dissociated completely. The samples were run electrophoretically using 1 % agarose gel in TAE running buffer, and the location of plasmid bands was visualized by a UV illuminator.

2.9. Particle size and zeta potential of the polyplexes

The particle size and zeta potential of polyplexes were measured using Dynamic Light Scattering (DLS) and Laser Doppler Velocimetry (LDV), respectivelyby Malvern NanoZS (Malvern Instruments, UK) in a sodium chloridefree buffer, HBG (20 mM HEPES, 5 % glucose, pH 7.2). The measurements were performed in an automatic mode, and the results are presented as mean±SD, n=3. Each mean represents the average value of 30 measurements.

2.10. Cell culture and cytotoxicity assay

Human HepG2 hepatocellular carcinoma cells (NCBI C158, Tehran, Iran) were maintained at 37 ºC, 5% CO_2 and 100% humidity in DMEM medium supplemented with 10% fetal bovine serum, streptomycin at 100 µg/mL and penicillin at 100 U/mL. Cells were seeded at a density of 1×104 cells/well in 96-well plates 1 day prior to cytotoxicity measurements, and grown in the appropriate medium with 10% FBS. Different polymer/plasmid DNA ratios (C/P) were used to prepare the polycation/plasmid complexes (i.e. polyplexes). Polyplexes were prepared by adding 50 µl of a solution of polycation at varying concentrations in HBG to 50 µl of a solution of plasmid DNA (40 µg/ml) in HBG. Cytotoxicity assay was performed by adding 10 µl (equivalent of 200 ng pDNA) of polyplex solution to the wells of 96-well plates containing 60%-90% confluent cultures of cells in complete medium containing 10% FBS. In order to assess the cytotoxic effects of the PEI and its derivatives, MTT assay was performed as described elsewhere. Briefly, the polyplexes were added to the medium as described above and after 4 h the medium was replaced by a fresh one and the plates were incubated in 37 ºC for 48h. Subsequently, 10µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/ml) was added to each well and incubated for another 2 h. Then, the supernatant of all wells were aspirated and 100 µl DMSO was added and allowed to dissolve the formazan crystals. The absorbance was measured using a microplate reader at 590 nm with background correction at 630 nm. Data are presented as mean±SD, n=3.

2.11. Statistical analysis

Data are presented as the mean±SD. The statistical significance was determined using Student's t-test and *P* values <0.05 were considered as significant.

3. Results and discussions

3.1. Synthesis of celecoxib conjugated PEI derivatives

In the present study, the most famous COX-II inhibitor, celecoxib, was conjugated to the high molecular weight branched PEI (25kDa). The primary amines on the surface of the polymer were utilized for conjugation. Celecoxib was treated with NBS to form brominated celecoxib (Scheme 1). Then, the brominated celecoxib was reacted with PEI via primary amines. 1H-NMR spectroscopy was used to determine the formation of celecoxib conjugated PEI and its conjugation degree. The aromatic protons were detected at chemical shifts between 7-8 ppm while the chemical shift for PEI protons appeared at 2.5-3.5 ppm. The conjugation degree was expressed as a number of conjugations per PEI monomer unit *100 %. Depending on the reagent/polymer amine ratio in the feed, two conjugation degrees (5 and 10 %) were achieved. The conjugates were labeled as PEI-Cel 5% and PEI-Cel 10% in which Cel stands for celecoxib and X% shows the calculated conjugation degree.

3.2. Buffering capacity

Polycationic polymers such as PEI can escape from endo/lysosomal compartments using their high amine content according to proton sponge hypothesis. Based on this theory, the amines of PEI absorb the protons that have entered the endosomes by V-ATPase pumps. This leads

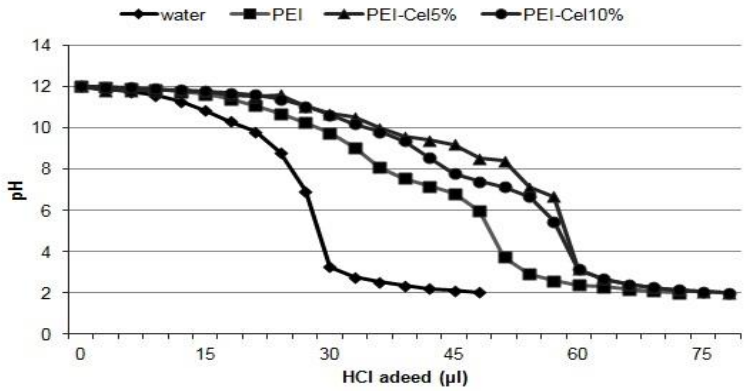


Figure 1. Buffering capacity measurement. Titration curves for aqueous solutions (0.4 mg/ml) of unmodified bPEI and its derivatives from pH 12 to 2.

to the influx of counter ions into endosomes, increased osmotic pressure, swelling, and disruption of endosomal membrane followed by early escape of polyplexes into the cytoplasm (56). Since the conjugation of targeting moieties occurred through the formation of linkages between celecoxib and the amines of PEI, it is necessary to assess whether the buffering capacity of the conjugates has remained in the optimal range. The results of buffering capacity measurement demonstrated that the conjugates were still able to absorb protons comparable with unmodified PEI particularly in the critical pH range of 5-7. This range of pH acts as a driving force for proton sponge effect and induction of early escape from endosomal compartments. As demonstrated in Figure 1, the conjugation of celecoxib at the substitution degrees of 5% and 10% could not decrease the buffering capacity of the whole molecule. In other words, even the substitution of 10% of PEI amines could not affect its buffering capacity. Therefore, these new conjugates may still induce early escape from degrading compartments inside the cells.

3.3. Measurement of the pDNA affinity to the PEI Derivatives

The electrostatic interaction between the positively charged polymers and negatively charged plasmid DNA leads to the formation of complexes which acts as a crucial step in successful gene delivery. Ethidium bromide exclusion assay is able to assess the binding affinity of pDNA to the polymeric compounds such as PEI (57, 58). The addition of PEI into the plasmid containing solutions leads to the exclusion of the intercalated ethidium bromide and a reduction in fluorescent intensity. Polymers with higher binding affinity to DNA result in the significant decrease of fluorescence intensity while the weaker polymers are not able to decrease the fluorescence intensity as so. The results of the binding affinity of PEI and its conjugated forms (Figure 2A) demonstrated that the conjugation of celecoxib on the surface of the polymer did not reduce its binding affinity in comparison with the unmodified parent polymer. In other words, the conjugation of celecoxib at the substitution degrees tested in the present investigation did not substantially reduce its binding properties. As shown in Figure 2B and 2C, all the conjugated were able to retard the migration of pDNA on agarose gel even at the lowest C/P ratio tested in this study (*i.e*, C/P=0.25) The formation of compact and stable nanoparticles outside the cells has been considered as a crucial step for gene delivery. However, these tight complexes will not necessarily lead to higher transfection efficiency. There are some controversial reports suggesting that weaker complexes may lead to the easier dissociation of plasmid from its carrier inside the cells which consequently leads to the more accessibility of the transcription apparatus of the cell (57, 58). Therefore, the binding affinity of polymer to pDNA is not the only factor determining the high transfection efficiency and it must be considered along

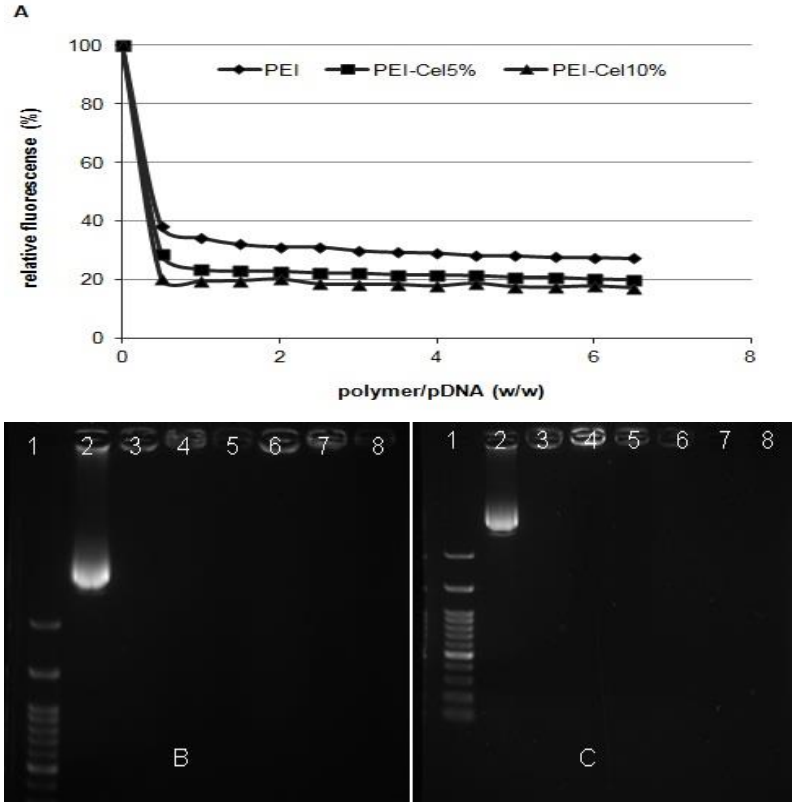


Figure 2. (A) Plasmid DNA binding affinity of 25 kDa PEI and celecoxib-conjugated derivatives determined by the ethidium bromide exclusion assay in HBG buffer. (B) and (C) Plasmid DNA condensation of 25 kDa PEI and celecoxib-conjugated derivatives determined by gel retardation assay at various C/P ratios. (B) lane1, DNA marker; lane 2, naked pDNA; lanes 3-5, 25 kDa PEI/pDNA at C/P ratios of 0.25, 4, and 8, respectively; lanes 6-8, PEI-Cel5%/pDNA at C/P ratios of 0.25, 4, and 8, respectively. (C) lane1, DNA marker; lane 2, naked pDNA; lanes 3-5, 25 kDa PEI/pDNA at C/P ratios of 0.25, 4, and 8, respectively; lanes 6-8, PEI-Cel 10%/pDNA at C/P ratios of 0.25, 4, and 8, respectively.

with the other characteristics of the polyplexes.

3.4. DNase I protection assay

The condensation of pDNA by polycationic polymers such as PEI not only forms nano complexes but also protects the oligonucleic materials from enzymatic degradation. Since the free pDNA is not resistant against enzymatic digestion, the formation of polyplexes is a key factor to the delivery of intact form of nucleic acids into their final site of action inside the cells (55, 59). The results of pDNA protection assay using DNAse I has been demonstrated in Figure 3. Unmodified PEI could not protect pDNA against the nuclease enzyme at the lowest C/P ratio of 0.25. Also, the celecoxib conjugated PEI derivatives were not able to fully protect the plasmid against the degrading enzyme at the same C/P ratio. By increasing the C/P ratio, the protection effect increased and either unmodified PEI or its conjugated forms could optimally show the protection effect at C/P ratios of 4 and 8. In other words, at the lowest C/P ratio of 0.25, there is not enough protection effect. It could be the result of incomplete condensation or the shape of the polyplexes. There are some reports indicating that at the low C/P ratios some parts of pDNA might be exposed on the surface of the polyplexes making it more susceptible for enzymatic degradation compared with the compact parts of the nucleic acids inside the nanoparticles. This assay confirmed that the application of PEI or its conjugated forms at C/P ratio of 0.25 might not be considered for gene delivery purposes while the higher C/P ratios could be tested.

3.5. Particle size and zeta potential measurement

The formation of nano sized complexes with favorable size and zeta potential affects the

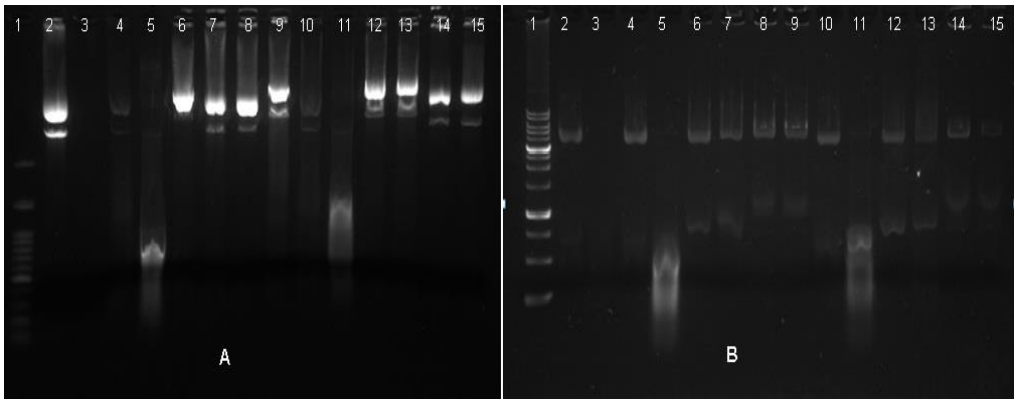


Figure 3. DNase I protection assay. (A) PEI-Cel 5% series; (B) PEI-Cel 10% series. lane 1, DNA marker; lane 2, naked plasmid; lane 3, naked plasmid+DNase I; lanes 4,6,8, unmodified PEI/plasmid polyplexes at C/P ratios of 0,25, 4, 8 respectively. Lanes 5,7,9, DNase I treated unmodified PEI/ plasmid polyplexes in C/P ratios of 0.25, 4, 8 respectively. Lanes 10, 12, 14, celecoxib conjugated PEI/ plasmid polyplexes in C/P ratios of 0.25, 4, 8 respectively; lanes 11, 13, 15, DNase I treated celecoxib conjugated PEI/ plasmid polyplexes in C/P ratios of 0.25, 4, 8 respectively.

ability of polyplexes for gene delivery and has been considered as an important key factor for the evaluation of gene carriers. The size and zeta potential not only determine the route of cell entrance but also have a significant influence on cell-induced toxicity (60). The results of size and zeta potential measurements are shown in Figure 4. The size of the polyplexes formed by unmodified PEI was around 75 nm while the conjugation of celecoxib on the surface of PEI increased the size to around 200 nm in the case of PEI-Cel 10%. The increase of particle size following the conjugation of large molecules on the surface of PEI is consistent with the previous investigations. However, the largest size obtained in this study was still in the size range which allows the nanoparticles to enter the cells through clathrin-mediated endocytosis. The conjugation of celecoxib through the primary surface amines reduced the zeta potential from 35 mV in the case of unmodified PEI polyplexes to 28 mV in the case of PEI-Cel 10%. Since the amines on the polymer surface are responsible for the high positive charge density of the polyplexes and the conjugation of targeting moieties occurred through these amines, it is expected that substitution of these amines may result in the reduced zeta potential. Although the conjugation of celecoxib occurred via the amines of PEI, the remaining positive charge on the surface of the resulted polyplexes was still sufficient to prevent aggregation. The particle size measurement was carried out 1 h after the preparations of the complexes and the results showed no significant increase of size.

3.6. Cell-induced toxicity

The wide clinical application of polymer-based gene carriers has been limited due to their high cytotoxic effects. The high cytotoxicity of the polycationic gene vectors is highly associated with the high positive charge density on the surface of the complexes which leads to the interaction of these nanostructures with the cell membrane and finally the formation of pores in the plasma membrane and cell death. Since this high charge density comes from the amines of PEI, conjugation of targeting moieties through these amines not only directs the complexes into the target site but also modulates the surface charge (60, 61). The modulation of charge density of the polycationic gene carriers has been reported as one of the major strategies to reduce their toxic effects on the cells. As illustrated in Figure 5, the unmodified PEI induced higher toxicity on the HepG2 cells particularly at the highest C/P ratio of 8. By increasing the carrier:plasmid ratio, the toxic effects increased which is consistent with the previous studies in which the higher amounts of unmodified PEI led to the induction of higher toxicity. The conjugation of celecoxib slightly reduced the toxic effects of unmodified PEI especially at the conjugation degree of 10%. However, this reduction is not considerable. This observation is consistent with the results of zeta potential and protection assay as well as the retardation experiment. In these

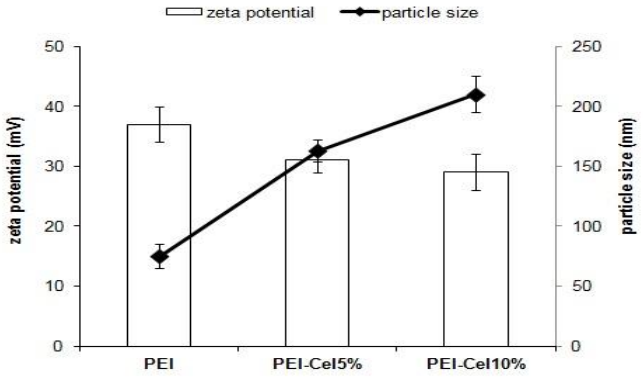


Figure 4. Particle size and zetapotential of the polyplexes. The nanoparticles were formed with bPEI and its conjugate in HBG buffer.

tests, no significant difference between the unmodified and conjugated forms of PEI was observed. In other words, the reduction of toxicity of PEI needs more conjugation degrees to shield the positive charge density on the surface of the polymers. However, the higher conjugation degrees may affect the other characteristics of the PEI polymer such as size, binding affinity and more importantly its buffering capacity. There are some reports suggesting that "wasting" the amines for conjugation of targeting ligands may decrease their ability to induce early escape from endosomes and finally transfection efficiency (62). Therefore, more optimization is suggested for further studies.

4. Conclusion

Using simple conjugation strategies, celecoxib was conjugated onto PEI and characterizaed with respect to pDNA binding affinity, size, zeta potential andprotection against nuclease as well as cytotoxicity. The results demonstrated the ability of new conjugates in pDNA condensation and formation of nanoparticles. However, further studies is necessary to evaluate the transfection efficiency of these new conjugates.

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Conflict of Interest

None declared.

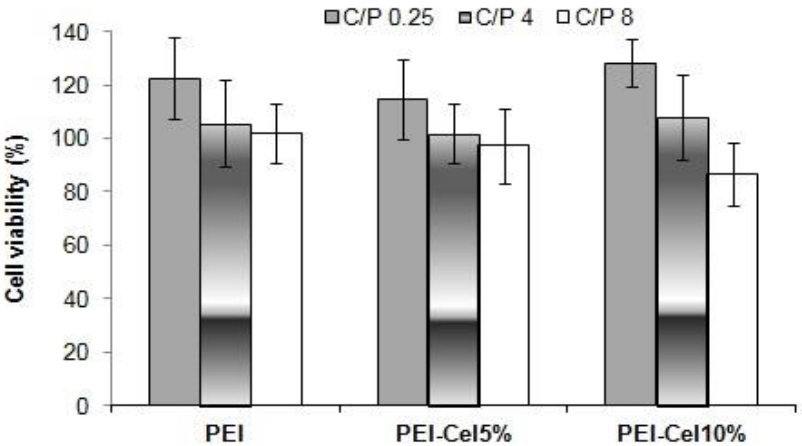

Figure 5. Viability of bPEI and its conjugate complexed with pUMVC3-hIL12 plasmid at C/P ratios of 0.25, 4 and 8 assayed by MTT determined in triplicate HepG2 cell lines.*P< 0.05, conjugated bPEI derivative compared to unmodified parent polymer at the same C/P ratio. (N=3; error bars represent±standard deviation).

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