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Research paper

Polyethylenimine nanoparticles as an efficient *in vitro* siRNA delivery system

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ABSTRACT

Degradation of mRNA by RNA interference is one of the most powerful and specific mechanism for gene silencing. Owing to this property, siRNAs are emerging as promising therapeutic agents for the treatment of inherited and acquired diseases, as well as research tools for the elucidation of gene function in both health and disease. Here we have explored the potential of polyethylenimine (PEI) to deliver siRNA to mammalian cells. Nanoparticles of PEI were prepared by acylating PEI with propionic anhydride followed by cross-linking with polyethylene glycol-bis(phosphate). The nanoparticles size as revealed by DLS studies was found to be ~110 nm and AFM investigations showed spherical and compact complexes with an average size of 100 nm. For electro-neutralization of negative charge of siRNA higher amount of nanoparticles was required as compared to native PEI. The siRNA delivery efficiency of nanoparticles was assessed by using siRNA against gene coding for green fluorescent protein (GFP). The gene silencing efficiency of PEI nanoparticles was found to be comparable to commercially available transfecting agent Lipofectin but with reduced cytotoxicity.

1. Introduction

Recently, RNA interference (RNAi) has emerged as a powerful tool for silencing a target gene in gene therapy. RNAi regulates gene expression in mammalian cells through siRNA, which is a double-stranded RNA molecule having 21–23 bp. In this process the degradation of homologous mRNA by double-stranded RNA (dsRNA) is highly sequence specific. The process of RNAi has been found to be very useful for genetic analysis and is rapidly evolving as a potent therapeutic approach for gene silencing [1,2].The RNAi mechanism involves the cleavage of long dsRNA molecules into 21–23 bp nucleotides called small interfering RNAs (siRNAs) by an endogenous RNase III-like enzyme known as Dicer [3]. The siRNA forms the RNA induced silencing complex (RISC) on complexation with the ribonuclear proteins, which contains the proteins necessary for unwinding the double-stranded siRNA, binding, and cleaving the target messenger RNA [4]. In mammalian cells, the inhibition of mRNA translation takes place by a sequence non-specific interferon response triggered on exposure to dsRNAs with more than 30 bp in length [5,6]. However, the interferon response remains inactive on inserting short siRNAs of 21–23 bp into mammalian cells which results in mRNA degradation with great sequence specificity [1]. Synthetic siRNAs of 21–23 nucleotides have been found to suppress the *in vitro* endogenous and exogenous gene expression in mammalian cells [7].

The successful clinical use of siRNA is limited due to problems such as: (1) rapid enzymatic degradation resulting in a short half-life in the blood; (2) poor cellular uptake; and (3) insufficient tissue bioavailability [8–10]. Because of these limitations, native delivery of siRNA to the cells is not effective. The siRNA can be chemically modified to circumvent these problems but these modifications are also associated with certain disadvantages such as decreased mRNA hybridization, higher cytotoxicity and increased unspecific effects [11]. Therefore, a delivery system is required which can protect and efficiently transport siRNA to the cytoplasm of the target cells.

The majority of approaches tested so far have utilized viral vectors, but recently, the research on non-viral vectors has gained momentum as they offer several advantages, such as easy manipulatibility, stability, safety, low cost and high flexibility regarding the size of transgene delivered [12]. Amongst various delivery systems, polycationic polymers have been preferred over other systems due to their ease of preparation, purification and chemical modification along with the long shelf life [13,14]. Polyethylenimine (PEI), a polycationic polymer has emerged as one of the most promising candidates for the development of efficient gene delivery vectors [15–18]. The high molecular weight PEI (800 kDa) has been found to exhibit high transfection efficiency as compared to low molecular weight PEIs, but is also associated with high cytotoxicity [19–21]. Various mechanisms of gene delivery have been suggested for PEI, amongst which "proton sponge hypothesis" is most accepted [16]. However, this theory has been challenged recently [22].

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PEI has been PEGylated to bear certain advantages such as the introduction of biodegradable linkages, overall reduction in cytotoxicity and decrease in non-specific interactions with the serum proteins, increase in polymer solubility, improvement in blood circulation time, which ultimately leads to enhanced transfection efficiency [23–25]. One of our earlier studies has demonstrated the use of PEG as a cross-linker to prepare nanoparticles with improved transfection efficiency [26]. Nowadays, nanoparticle-mediated delivery of biomolecules has attracted much attention of the researchers in areas related to therapeutics. Nanoparticles being compact in nature are well suited to traverse cellular membranes to mediate drug or gene delivery. It is also expected that due to smaller size, nanoparticles will be less susceptible to reticuloendothelial system clearance and will have better penetration into tissues and cells, when used in *in vivo* therapy.

In the present investigation, we have prepared nanoparticles of PEI acylated with propionic anhydride which was found to be the most efficient transfecting agent compared to PEI acylated with acetic and butyric anhydrides, as reported in one of our earlier studies [27]. The nanoparticles prepared were characterized by measuring their size and surface charge by DLS, AFM and zeta potential, respectively. The *in vitro* gene silencing ability of these nanoparticles was then assessed on HEK 293 cells.

2. Experimental procedures

2.1. General methods

Polyethylenimine (PEI, M_W 750 kDa), high retention dialysis tubing (cut off = 12 kDa), 3-(4,5-dimethyl-thiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), agarose, HEPES, tris, EDTA, ethidium bromide (EtBr), bromophenol blue (BPB) and xylene cyanol (XC) were procured from Sigma chemical co., St. Louis, MO, USA. All other chemicals and reagents were procured locally. GFP protein expression was observed under Nikon Eclipse TE 2000-U inverted microscope, Kanagawa, Japan, fitted with C-Fl epifluorescence filter block B-2A consisting of excitation filter Ex 450–490 nm, Dichroic mirror DM 505 and barrier filter BA 520. Qiagen kit for plasmid isolation was purchased from Qiagen Inc., California, USA. Cell culture media, Dulbecco's modified Eagle's medium (DMEM), Fetal calf serum (FCS) were from GIBCO-BRL-Life Technologies, Web Scientific Ltd., UK, EGFP plasmid from BD Biosciences, USA. Both the sense and antisense sequences of the siRNA strands for the enhanced green fluorescent protein (EGFP) gene 5′-GGCAAGCUGACCCUGAAGUUCUU-3′ and 5′-GAACUUCAGGGUC AGCUUGCUU-3′, were obtained in purified form from Bangalore Genei, India.

2.2. Cell culture

The mammalian cell line, HEK 293 cells (Human embryonic kidney 293), were maintained as monolayer cultures in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum (FBS).

2.3. Plasmid purification

All the transfection experiments were carried out using the plasmid encoding enhanced green fluorescent protein gene (EGFP) under the control of cytomegalovirus (CMV) immediate early promoter. The plasmid was transformed into *Escherichia coli* bacterial strain DH5α and was extracted from the culture pellets using the Qiagen Endofree Maxi-Prep kit (Qiagen S.A., Courtaboeuf, France) as per manufacturer's instructions.

2.4. Preparation of nanoparticles of PEI

Polyethylenimine 750 kDa (1 g) was 30% acylated using the propionic anhydride (907.8 μl) as reported earlier [27,28]. Nanoparticles of acylated PEI were prepared by cross-linking with polyethylene glycol-bis(phosphate) (PEG-bis-P) which was synthesized from polyethylene glycol (M_W 8 kDa) following the procedure already reported by the author [26].

2.5. Determination of percentage of acylation

The percentage of amino groups acylated in PEI derivative were estimated through quantification of the free amino groups remaining on the polymer employing 2,4,6-trinitrobenzenesulfonic acid (TNBS) method [29]. The extent of acylation of PEI was also confirmed by ^{1}H NMR [28].

2.6. Characterization of nanoparticles

The acylated PEI–PEG nanoparticles prepared for the present study were characterized by the following procedures.

2.6.1. Atomic force microscopy

The size and surface morphology of the nanoparticles was determined by atomic force microscopy using NANO-R AFM System (Pacific Nanotechnology, USA) operating in Close Contact Mode. A Silicon Close Contact Mode Probe (Model: P-MAN-SICC-O, PACIFIC NANOTECHNOLOGY,USA) with substrate force constant of 40 N/m (nominal)and resonance frequency of 300 kHz (nominal) and tip radii <10 nm was used. Lyophilized powder (∼0.5 mg) of nanoparticles was dispersed by sonication in double distilled water (1 ml) to obtain a suspension, 2–3 μl of this solution was deposited on a "Piranha" cleaned glass slide and allowed to dry for overnight at room temperature. Subsequently, the glass surface containing the nanoparticles was imaged. Particle size was obtained using Nano Rule software. The height differences on the surface are indicated by the color code, lighter regions indicate higher heights.

2.6.2. Dynamic light scattering (DLS)

The hydrodynamic diameter of the acylated PEI–PEG nanoparticles was determined by dynamic light scattering (DLS) measurements. Nanoparticles were suspended in 1 ml of double distilled water and were sonicated prior to measurements. Nanoparticles size was determined using Zetasizer, Nano ZS (Malvern instruments, UK) employing a nominal 5 mW HeNe laser operating at 633 nm wavelength. The scattered light was detected at 173° angle. The refractive index (1.33) and the viscosity (0.89) of ultrapure water were used at 25 °C for measurements. All the data analysis was performed in automatic mode. Measured sizes were presented as the average value of 20 runs.

2.6.3. Zeta potential measurements

The acylated PEI–PEG nanoparticles were suspended in 1 ml 10 mM Tris, pH 7.4, followed by sonication and were subjected to zeta potential measurements on a Zetasizer Nano ZS. Zeta potential measurements were carried out in automatic mode and the values were presented as the average value of 30 runs. The Smoluchowski approximation was used to calculate zeta potential from the electrophoretic mobility.

2.7. Determination of siRNA loading efficiency

The loading efficiency of siRNA (%) adsorbed onto the PEI nanoparticles was calculated from the determination of free siRNA concentration in the supernatant recovered after nanoparticles centrifugation (13,000*g*, 15 min) by absorbance measurement at

260 nm. Supernatant recovered from unloaded PEI nanoparticles (without siRNA) was used as a blank. The loading efficiency ($E\%$) was calculated from the total concentration of the added amount of siRNA present in the system ($[siRNA]_T$) and that in the supernatant ($[siRNA]_s$) using the equation:

$$E(\%) = [DNA]_T - [DNA]_s / [DNA]_T \times 100$$

2.8. DNA retardation assays

The siRNA–nanoparticles complexes were prepared by taking a known amount of siRNA (0.5 μg) with varying amount of acylated PEI–PEG nanoparticles in 20 mM HEPES buffer, pH 7.2, containing 150 mM NaCl and by incubating for 30 min at room temperature. The resultant complexes were loaded onto a 0.8% agarose gel (Tris–acetic acid buffer, TAE) after mixing with loading buffer containing a tracking dye (xylene cyanol) and electrophoresed at 100 V for 45 min. in the same buffer. The bands corresponding to siRNA and siRNA–nanoparticles complexes were visualized under ultra violet light after staining the gels with ethidium bromide.

2.9. Biological activity of acylated PEI nanoparticles

Human embryonic kidney 293 (HEK 293) cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum. The cells were grown at 37 °C in humidified 5% CO_2 atmosphere. HEK 293 cells were seeded prior to transfection into 24-well plates at a density of 75×10^3 cells/well and were incubated for 16 h for adherence. After stipulated time the cells were washed once with serum free DMEM. Reporter gene encoding for green fluorescent protein (GFP) was used to assess the gene silencing efficiency of acylated PEI–PEG nanoparticles. The cells were transfected with pEGFP (0.5 μg) using Lipofectin according to manufacturer's protocol. After 4 h of transfection the medium was removed and the cells were washed with PBS and replenished with 200 μl of serum free medium. Fifty microliter medium without serum containing siRNA only, siRNA–nanoparticles or siRNA–Lipofectin complexes (with 0.5 μg siRNA each well) was then added to the cells followed by washing with PBS and re-supplementing with 300 μl of serum containing medium after 4 h incubation at 37 °C in humidified 5% CO_2 atmosphere. After 48 h, the transfected cells were observed under bright field and UV using GFP filter, at 10× magnification, under an inverted fluorescent microscope to observe the expression of green fluorescent protein.

2.10. Analysis of EGFP expression

The fluorescence intensity was measured to quantitate the GFP expression in the mammalian cells. The cells were washed twice with PBS and lysed by incubating for 30 min. with 400 μl buffer containing 10 mM Tris, 1 mM EDTA and 0.5% SDS, pH 7.4. The lysed cells were centrifuged at 300*g* for 5 min. and the supernatant was aspirated. Ten microliter lysate was used to estimate the expressed reporter gene product, green fluorescent protein (GFP), spectrofluorometrically at an excitation wavelength 488 nm and emission at 510 nm. Background fluorescence and auto-fluorescence were determined using mock treated cells. The total protein content in cell lysate from each well was estimated using Bradford's reagent (Bio-Rad) taking BSA as a standard. The level of fluorescence intensity of GFP was calculated by subtracting the background values and was normalized against protein concentration in cell extract. The data are reported assuming transfection of cells by pEGFP complexed with Lipofectin as 100% and represent mean ± standard deviation for triplicate samples.

2.11. Analysis of gene silencing using FACS measurements

To determine the extent of transfection, fluorescence activated cell sorter (FACS) analysis was performed. After 48 h of transfection the medium was aspirated from the 24-well plate and the cells were washed twice with 0.5% bovine serum albumin/phosphate buffered saline (BSA/PBS). Cells were re-suspended in 200 μl of 0.5% BSA/PBS and were subjected to FACS analysis. Data were obtained on BD-LSR (Beckton–Dickinson) and were analyzed by Cell Quest and Win MDI softwares.

2.12. Cytotoxicity

The toxicity of siRNA loaded acylated PEI–PEG nanoparticles was evaluated by MTT colorimetric assay [30]. HEK 293 cells were seeded onto 96-well plates at a density of 8×10^3 cells/well and incubated for 16 h for adherence. The cells were transfected as reported above in the transfection experiment with siRNA loaded acylated PEI–PEG nanoparticles. After 48 h, 50 μl MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (2 mg/ml in DMEM) was added to the cells and was incubated for another 2 h. The MTT containing medium was aspirated, and the formazan crystals formed by the living cells were dissolved in 100 μl isopropanol containing 0.06 M HCl and 0.5% SDS. Aliquots were drawn from each well after 1 h of incubation and the absorbance measured spectrophotometrically in an ELISA plate reader at 540 nm. Untreated cells were taken as control with 100% viability and cells without the addition of MTT were used as blank to calibrate the spectrophotometer to zero absorbance. The relative cell viability (%) compared to control cells was calculated by $[abs]_{sample}/[abs]_{control} \times 100$.

3. Results

3.1. Characterization of nanoparticles of acylated PEI

Commercially available high molecular weight branched 750 kDa PEI was derivatized with calculated amount of propionic anhydride to achieve 30% acylation leading to acylation of the primary and secondary amines. Propionic anhydride was added to PEI and the reaction mixture was incubated at 65 °C for 4.5 h. On estimation of the percentage of amine groups blocked using TNBS method, it was found to be only 23.2%. The relative methyl and methylene signals in ^1H NMR spectra were used to calculate the extent of primary and secondary amine acylation. The extent of actual acylation was found to be 22.6% as compared with the theoretical value of 30% in the acylated PEI derivative.

The ionic interaction between the positively charged amino groups of PEI and the negatively charged phosphate groups of PEG-bis-P, results in the formation of nanoparticles with 5% cross-linking designated as APP nanoparticles. AFM investigation of APP nanoparticles showed spherical and compact complexes with an average size of 100 nm (Fig. 1). The three-dimensional image revealed homogeneous population with a clear absence of aggregates even after 2 h. The nanoparticles maintained their morphology and shape even after binding to siRNA. The size of nanoparticles suspended in water was found to be around 110 nm by DLS measurements (Fig. 2). This size discrepancy is probably due to the two different methods used. DLS was performed on nanoparticles in water which makes them fully hydrated, whereas, AFM studies on samples dried to a glass slide surface. Moreover, the DLS measurement presents an average size range whereas, AFM visualises only a small number of nanoparticles. Owing to this reason, the use of different but complementary methods allows an overall evaluation to be made of both size and morphology.

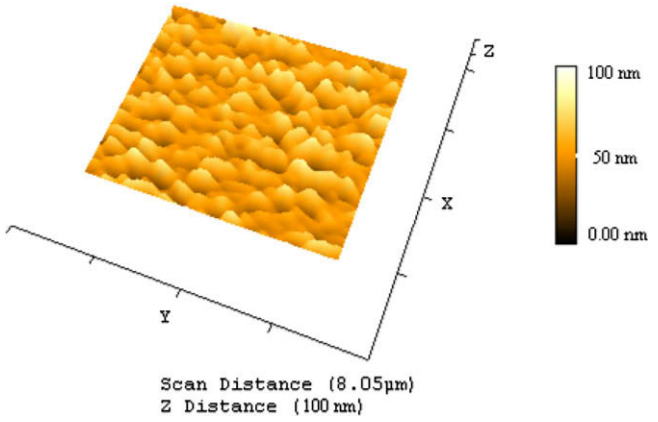

Fig. 1. Atomic force microscope image of APP nanoparticles in double distilled water. The average particle size is 100 nm.

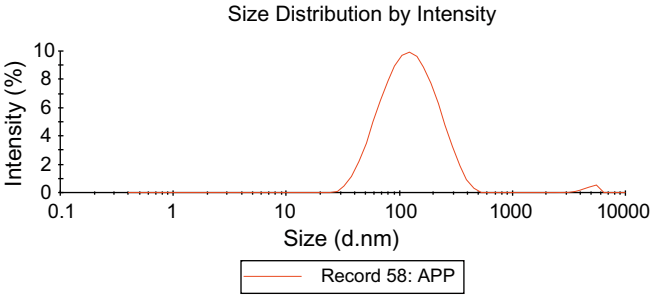

Fig. 2. Representative dynamic light scattering spectrum of APP nanoparticles in double distilled water. The average hydrodynamic diameter in this case is 110 nm.

Zeta potential measurement provides vital information about the surface charge present on the nanoparticles which was measured in the presence and absence of siRNA at physiological pH. On complexation of nanoparticles with siRNA zeta potential was found to decrease from 22.7 to 18.5 at physiological pH which is in accordance with what is expected (Fig. 3). The presence of significant amount of positive charge on the nanoparticles after siRNA complexation, is the major driving force behind the prevention of aggregation due to electrostatic repulsion between the cationic complexes. However, the zeta potential was −24.8 in 10% FBS which is the serum concentration used in complete medium.

3.2. Determination of siRNA loading efficiency

The amount of siRNA adsorbed onto APP nanoparticles was determined by spectrophotometrically measuring the optical density of the supernatant obtained after centrifugation of siRNA–nanoparticle complex. The amount of siRNA in filtrate calculated taking 1 O.D. at 260 nm equal to 50 µg of siRNA. The loading efficiency (*E*%) was found to be 86%.

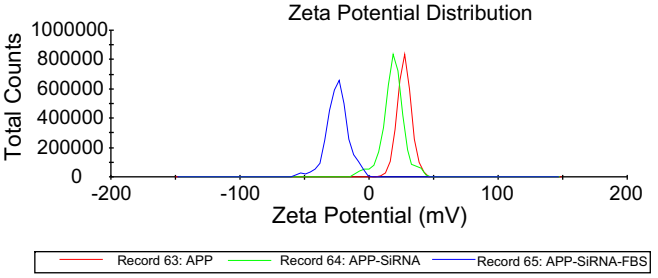

Fig. 3. Zeta potential of APP nanoparticles in PBS, pH 7.4 (record 63) is 22.7, siRNA loaded APP nanoparticles in PBS (record 64) is 18.5, APP nanoparticles in 10% FBS (record 65) is −24.8.

3.3. Gel retardation assay

The preparation of nanoparticle–siRNA complexes leads to the electro-neutralization of the negative charge of siRNA, which are unable to migrate under the influence of electric field during gel electrophoresis. In order to estimate the amount of nanoparticles required for complete electro-neutralization of siRNA, the complexes were prepared with siRNA at different weight ratios, maintaining the amount of siRNA constant and retardation was analyzed on 0.8% agarose gel. Taking native PEI 750 kDa as control complete retardation was observed at 0.4 µg/µg siRNA, whereas in the case of APP nanoparticles 5 µg was required (Fig. 4).

3.4. Biological activity of APP nanoparticles

Gene knockdown efficiency of APP nanoparticles was estimated by carrying out transfections with various siRNA–APP nanoparticle complexes in HEK 293 cells and compared to the commercially available transfection reagent, Lipofectin and native PEI polymer (750 kDa). The efficacy of nanoparticles for gene silencing was determined by using the plasmid carrying reporter gene, Green fluorescent protein (GFP). The qualitative analysis of level of gene silencing was carried out by observing under an inverted microscope after 48 h (Fig. 5). The siRNA–nanoparticle complexes showed comparable results to Lipofectin whereas naked siRNA revealed negligible silencing effect.

Quantitative analysis of gene silencing efficacy of nanoparticles was carried out by the estimation of GFP expression level in HEK 293 cells. The relative level of GFP expression in cells was determined by normalizing green fluorescence intensity values of extracted proteins from the formulations with different siRNA

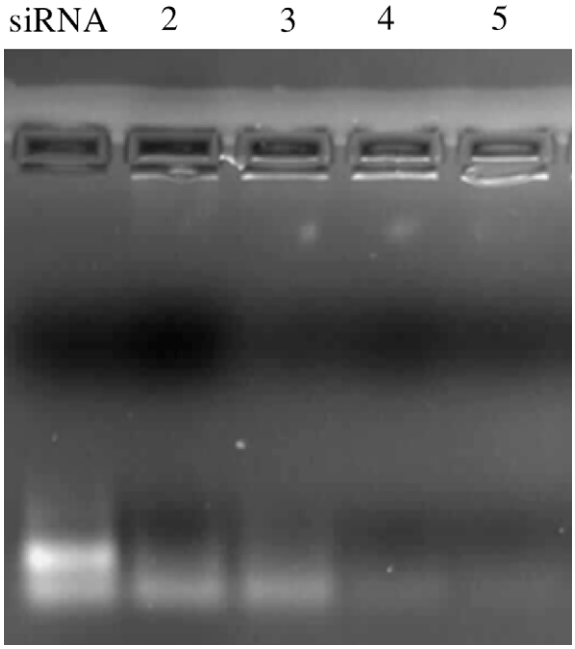

Fig. 4. DNA binding and retardation. siRNA, 0.5 µg was incubated with increasing amounts of APP in the presence of salt (150 mM NaCl) for 30 min. The samples were analyzed on a 0.8% agarose gel. Lane 1 is siRNA without nanoparticles, the values mentioned correspond to the amount of APP (µg) used in a 20 µl reaction to condense the siRNA by charge neutralization.

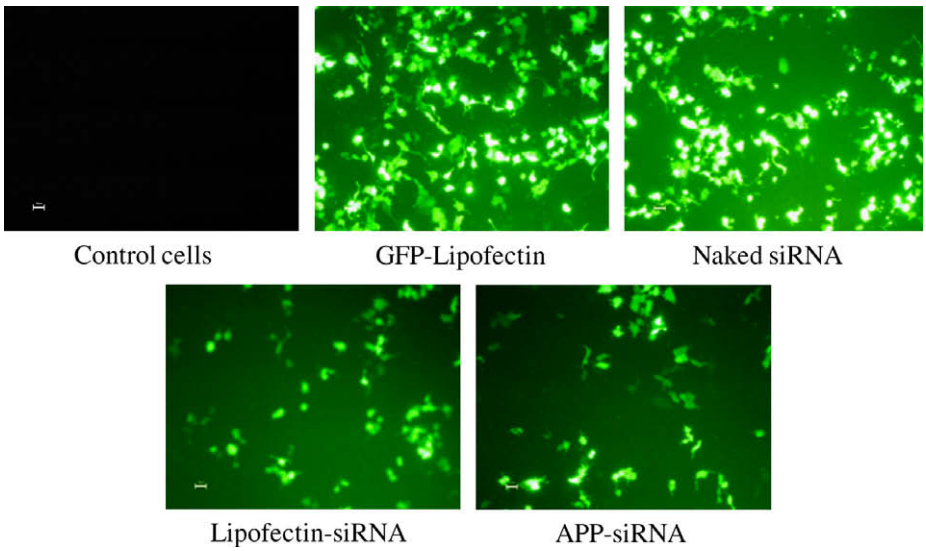

Fig. 5. Comparison of gene silencing efficiency of various siRNA formulations after 48 h. The green fluorescent protein (GFP) expression was observed under fluorescent microscope at 10× magnification. The data were recorded at optimal inhibition efficiency for APP nanoparticles, i.e. APP nanoparticles: siRNA at 30:1 ratio.

complexes, using non-treated cells as control. After 48 h, APP nanoparticles showed up to 83% inhibition of gene expression which was almost equal to that for siRNA–Lipofectin (81% inhibition) (Fig. 6).

To further establish the gene knockdown efficiency of APP nanoparticles flow cytometric analysis of HEK 293 cells was carried out. The result was consistent with that of the cellular fluorescence intensity assay (Fig. 7). The inhibition effect of siRNA–APP nanoparticles in HEK 293 cells was over 85% while that for siRNA–Lipofectin was 80%.

3.5. Cytotoxicity

The nanoparticles prepared by acylation followed by PEGylation of PEI bear lower positive charge as compared to the native PEI 750 kDa. To estimate the extent of toxicity caused by various siRNA complexes, MTT colorimetric assay was employed. HEK 293 cells treated with various siRNA complexes as described in transfection and gene silencing experiment. Native PEI polymer was quite toxic

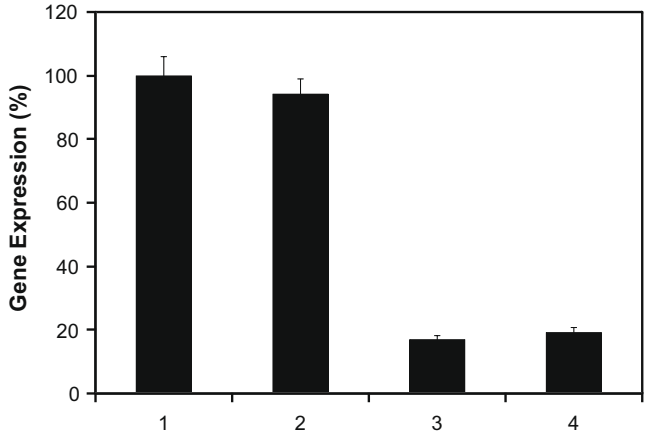

Fig. 6. Comparison of gene silencing efficiency of various siRNA formulations after 48 h. The level of GFP expression was estimated by the quantitation of green fluorescence after 48 h. The data were recorded at optimal inhibition efficiency for APP nanoparticles, i.e. APP nanoparticles: siRNA at 30:1 ratio. The assays were done in triplicate and the standard error is shown. (1) Lipofectin–pEGFP, (2) Naked siRNA, (3) APP–siRNA complex, (4) Lipofectin–siRNA.

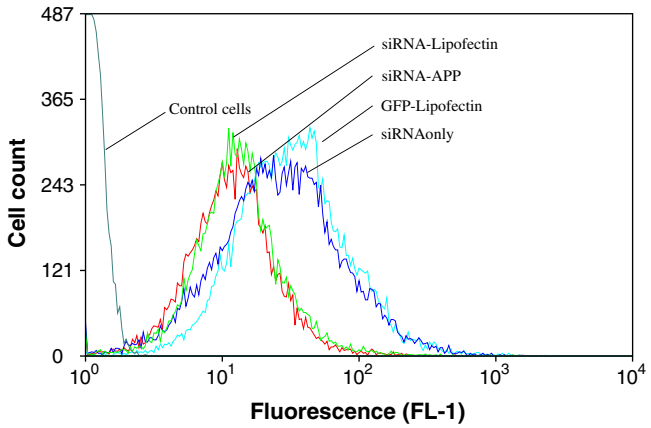

Fig. 7. Flow cytometric analysis of HEK 293cells expressing GFP as treated with various siRNA formulations after 48 h.

leading to high cell death which was evident on microscopic examination of the cells. The siRNA loaded APP nanoparticles showed very low toxicity after 48 h nearly 84% cells were viable and in case of naked siRNA cell viability was above 90% (Fig. 8). However, the treatment of cells with native PEI resulted in 51% cell viability.

4. Discussion

Recently, RNAi has evolved as one of the major strategies for sequence-specific silencing of gene expression and holds great promise for therapeutic treatment of a variety of human diseases. Nowadays the success of RNAi depends on three popular processes at cellular level, i.e. the delivery of exogenous dsRNA or siRNA, transient infection with viral or DNA vectors, and infection with recombinant viruses. An ideal system for siRNA delivery should effectively condense the siRNA molecules, ease the delivery of siRNA complexes to a wide variety of cell types, provide an efficient mechanism for escape from the endocytic vesicle and be biocompatible and/or biodegradable. These properties have been taken into account while developing various polymers based siRNA delivery systems. Polyethylenimine, a polycationic polymer has emerged as one of the most promising non-viral vectors with efficient DNA condensation and lysosome rupture properties. Till date,

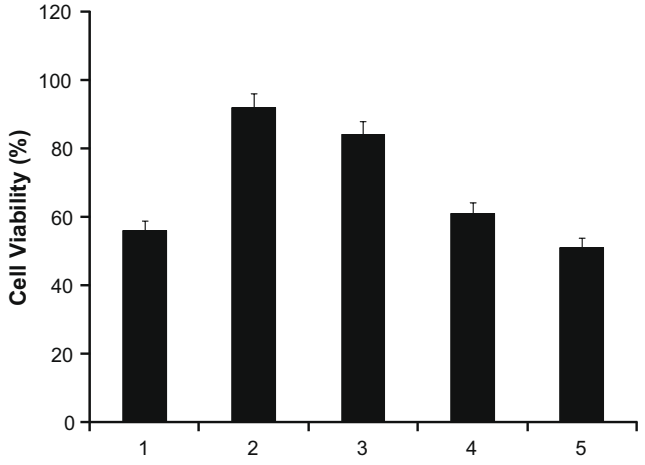

Fig. 8. Cell viability assay. HEK 293 cells were treated with various siRNA formulations under the transfection conditions. The assays were done in triplicate and the standard error is shown. (1) Lipofectin–pEGFP, (2) Naked siRNA, (3) APP–siRNA complex, (4) Lipofectin–siRNA, (5) PEI–siRNA complex ratio 2:1.

a large number of derivatives of PEI have been proposed, most of them focusing on modulation of the types of amino groups to enhance the transfection efficiency. In the present investigation, we have reported the siRNA delivery efficacy of nanoparticles prepared from high molecular weight PEI, acylated with propionic anhydride followed by cross-linking with derivatized PEG.

Nanoparticles-based transfecting agent has been found to restrict the polydispersity in size that may occur at the time of DNA condensation. Nanoparticles have been shown to have better sustainability than the polymer DNA complexes. Moreover, nanoparticles have also been reported to have higher intracellular uptake as compared to microparticles [31–33]. As reported earlier the nanoparticles were found to be spherical with uniform size distribution [27]. The high positive charge present on the nanoparticles as indicated by zeta potential studies reduced on complexation with siRNA was still enough, i.e. 18.5 to prevent aggregation of nanoparticle–siRNA complexes. However, the surface charge of APP–siRNA complex was reduced to negative value in the presence of 10% FBS (Fig. 3).

In PEI, every third atom is a nitrogen atom capable of undergoing protonation, thus conferring to the molecule a high polycationic nature, high DNA-condensing and a high buffering capacity [16]. The ability of APP nanoparticles to interact with siRNA was assessed by agarose gel electrophoresis studies. The complexes of APP nanoparticles and siRNA were prepared my simple mixing and incubation at room temperature. The association of APP nanoparticles with siRNA was strong enough to immobilize the siRNA by charge neutralization under the influence of electric current.

One of the major advantages of these complexes is that the siRNA is delivered to the cells without hampering the metabolic activity of cells as revealed by the results of MTT assay. As shown in Fig. 8, the cell viability was 84% when HEK 293 cells were treated with siRNA loaded APP nanoparticles for 48 h. Gene silencing experiment carried out by taking siRNA for reporter gene GFP resulted in up to 83% inhibition of gene expression (Fig. 6).

5. Conclusion

We have demonstrated that nanoparticles prepared from high molecular weight PEI by acylation with propionic anhydride followed by cross-linking with derivatized PEG could be efficiently used as siRNA delivery system for mammalian cells. The APP nanoparticles–siRNA complex was found to inhibit the GFP expression level up to 83%.

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