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Evaluation of Jeffamine®-cored PAMAM dendrimers as an efficient *in vitro* gene delivery system

Zeynep Aydin,[1*] Fahri Akbas,[1*] Mehmet Senel,[2] S. Naci Koc[3]

[1]Department of Genetics and Bioengineering, Faculty of Engineering, Fatih University, B. Cekmece, Istanbul 34500, Turkey
[2]Department of Chemistry, Faculty of Arts and Sciences, Fatih University, B. Cekmece, Istanbul 34500, Turkey
[3]Department of Chemical Engineering, Faculty of Engineering, Istanbul University, Avcilar, Istanbul 34500, Turkey

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Abstract: In this study, we investigated gene delivery properties of Jeffamine-cored polyamidoamine (PAMAM) dendrimers (JCPDs). The effects of dendrimer concentration, generation, and core size on the gene delivery have been analyzed. The experimental results showed that the JCPD effectively delivered plasmid DNA inside the HeLa cells, and the transfection efficiency improved considerably as the number of generation increased. The cytotoxicity of JCPD in different concentration was tested for HeLa cell line. JCPD was complexed with a lacZ gene carrying plasmid and tested for transfection efficiency using quantitative β-galactosidase expression assay. Additionally, confocal microscopy results revealed that JCPD effectively delivered green fluorescent protein-expressing plasmid into HeLa cells and produced fluorescent signal with satisfactory efficiency. The highest transfection efficiency was obtained from JCPDs G4 and G5, which mixed with expression plasmid vectors at a 10/1 weight ratio. These results indicated that under optimized conditions, JCPD can be considered as an efficient transfection reagent and can be effectively used for gene delivery applications. © 2012 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 100A: 2623–2628, 2012.

Key Words: PAMAM, transfection, gene delivery, cytotoxicity, Jeffamine®

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INTRODUCTION

In gene delivery studies, it is essential to find and develop an efficient and safe vector types. Different methods have been developed to transfer genes into cells by nonviral vectors. Viral vectors are efficient agents but causes some side effects, including cytotoxicity, mutagenesis, carcinogenesis, and immune response.[1] A nonviral vector, such as synthetic polymers, lipids, and peptides, considerably eliminates these problems. Nonviral vectors are in lower immunogenicity, safe, and can be easily prepared. Although nonviral carriers have such advantages, transfection efficiency are relatively very low when compared with viral vectors that limits its applications.[2] Therefore, to potentiate nonviral vectors, new modifications and approaches have been developed. For this purpose, several cationic lipid-mediated transferring strategies have been investigated. Cationic lipids bind to negatively charged phosphate backbone of DNA to be delivered into the cell. Similar to cationic lipids, cationic polymers also show affinity to negatively charged DNA through electrostatic interaction. Cationic polymers, such as polyethylenimine, polylysine, or polyamidoamine (PAMAM) and polypropylenimine dendrimers can transfer plasmid DNA across the cell membrane.[3–5] As a nonviral gene carrier, PAMAM dendrimers are most known polymers used because of their unique characteristics such as uniform size distribution, higher transfection efficiency, and lower cytotoxicity when compared with other cationic polymers.[6,7] PAMAM dendrimers include several surface amines that are capable of forming complexes through the electrostatic interaction with DNA (Table I). Thus, PAMAM dendrimers have been developed as nonviral carriers because of their suitable structure, surface functionality, and relatively high gene transfection efficiency. They are branched spherical polymer and can deliver single-stranded and double-stranded DNA or RNA of different size.[8] Dendrimers have an increased ionic interaction with DNA and produce very stable and highly soluble DNA complexes.[9] Modified forms of PAMAM dendrimers have also been generated to increase efficiency in transfection studies.[10,11]

The main objective of this study is to prepare a polyethylene oxide (Jeffamine)-cored PAMAM dendrimers that possess improved size of dendrimers. Assuming that the

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*These authors contributed equally to this work.
Correspondence to: F. Akbas; e-mail: fahriakbas@fatih.edu.tr
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TABLE I. Some Chemical and Physical Characteristics of Dendrimers

Generation	Molecular Weights (g/mol)			Number of Terminal Amino Groups	Number of Total Amino Groups
	Core A	Core B	Core C		
G0	440	3,000	5,000	3	3
G1	1,135	3,732	5,732	6	15
G2	2,506	5,103	7,103	12	39
G3	5,234	7,794	9,794	24	87
G4	10,760	13,320	15,320	48	183
G5	21,710	24,270	26,270	96	372

transfection efficiency limit of low-generation PAMAM dendrimers is due to small size, enhancement of transfection efficiency is expected due to the core size of the Jeffamine–PAMAM dendrimers. The cytotoxicity of the Jeffamine-cored PAMAM dendrimers (JCPDs) and dentriplex at different charge ratios was evaluated. The generation 4 and 5 JCPDs showed good transfection efficiency on the cell uptake in HeLa cell culture. The results indicated that under optimized conditions, JCPDs can be considered as an efficient transfection reagent and can be effectively used for gene delivery applications.

MATERIALS AND METHODS

Jeffamine® T series products (MW = 440, 3000, and 5000 g/mol) were gifts from HUNTSMAN International, LLC (Turkey). Methyl acrylate and ethylenediamine were obtained from Merck. Ketoprofen, Ibuprofen, and Diflunisal were obtained from Aldrich. All other chemicals were of analytical grade and used without further purification. Plasmid pTracer-CMV/Bsd/LacZ (Invitrogen) has a *lacZ* reporter gene inserted in the MCS. Activity of β-galactosidase, product of *lacZ*, can be determined quantitatively by a β-Galactosidase Enzyme Assay System with Reporter Lysis Buffer, which was purchased from Promega. Lipofectamine was obtained from Invitrogen. The FTIR–ATR spectra (4000–400 cm^{-1}) were recorded with a Bruker spectrometer. NMR spectra were recorded in $CDCl_3$ using a Bruker 400 MHz spectrometer.

General procedure: Synthesis of JCPDs G1–G5

Divergent synthesis of the Jeffamine-cored (with different MWs) amine-terminated PAMAM dendrimers were synthesized according to our previous study.[12] Dendrimer synthesis was carried out by initial Michael addition of metabolic solution of Jeffamine T series with excess methyl acrylate (1:10 molar ratio). The reaction mixture was stirred for 3 days at room temperature. The excess methylacrylate was removed under vacuum at 40–50°C temperature to afford the ester-functionalized derivatives. The reaction mixture was next submitted to the reaction sequence leading to the next-generation PAMAM dendrimers, consisting of the exhaustive amidation of the ester-functionalized Jeffamines to ethylenediamine (1:30 molar ratio), followed by Michael addition of the resulting amine with methyl acrylate (20 equiv of ester-terminated dendrimer). Excess reagents were removed under vacuum at 50–60°C temperature. Repetition of this two-step procedure ultimately leads to the next-generation PAMAM dendrimers (1, 2, 3, 4, and 5). The dendrimers, isolated in ~80 to 90% yield, were gummy in nature. The syntheses of the dendrimers were confirmed through ^1H NMR and FTIR spectroscopy. The GPC results of the dendrimers are given as Supporting Information in Table S1.

The FTIR, ^1H NMR results of dendrimer G-1: ^1H NMR ($CDCl_3$, δ); 1.09 (q, OCH_2CH_2O), 2.31 (m, NCH_2CH_2CO, 12H), 2.63 (m, $NHCH_2$ CH_2N, 12H), 2.76 (t, NCH_2CH_2COO, 12H), 3.23 (t, $NHCH_2CH_2N$, 12H), 4.79 (bs, NH_2, 12H), 7.02 (bs, $CONHCH_2$, 6H). FTIR (neat) cm^{-1}: 3347 (br, NH) and 1571 (amide C=O).

The FTIR, ^1H NMR results of dendrimer G-2: ^1H NMR ($CDCl_3$, δ); 1.11 (q, OCH_2CH_2O), 2.34 (m, NCH_2CH_2CO, 36H), 2.64 (m, $NHCH_2$ CH_2N, 36H), 2.78 (t, NCH_2CH_2COO, 36H), 3.26 (t, $NHCH_2CH_2N$, 36H), 4.87 (bs, NH_2, 24H), 7.02 (bs, $CONHCH_2$, 12H). FTIR (neat) cm^{-1}: 3360 (br, NH) and 1644, 1560 (amide C=O).

The FTIR, ^1H NMR results of dendrimer G-3: ^1H NMR ($CDCl_3$, δ); 1.11 (q, OCH_2CH_2O), 2.33 (m, NCH_2CH_2CO, 84H), 2.63 (m, $NHCH_2$ CH_2N, 84H), 2.78 (t, NCH_2CH_2COO, 84H), 3.25 (t, $NHCH_2CH_2N$, 84H), 4.84 (bs, NH_2, 48H), 7.08 (bs, $CONHCH_2$, 24H). FTIR (neat) cm^{-1}: 3365 (br, NH) and 1650, 1560 (amide C=O).

The FTIR, ^1H NMR results of dendrimer G-4: ^1H NMR ($CDCl_3$, δ); 1.13 (q, OCH_2CH_2O), 2.36 (m, NCH_2CH_2CO, 130H), 2.65 (m, $NHCH_2$ CH_2N, 130H), 2.74 (t, NCH_2CH_2COO, 130H), 3.28 (t, $NHCH_2CH_2N$, 130H), 4.86 (bs, NH_2, 96H), 7.08 (bs, $CONHCH_2$, 48H). FTIR (neat) cm^{-1}: 3360 (br, NH) and 1650, 1560 (amide C=O).

The FTIR, ^1H NMR results of dendrimer G-5: ^1H NMR ($CDCl_3$, δ); 1.12 (q, OCH_2CH_2O), 2.41 (m, NCH_2CH_2CO, 274H), 2.63 (m, $NHCH_2$ CH_2N, 274H), 2.78 (t, NCH_2CH_2COO, 274H), 3.32 (t, $NHCH_2CH_2N$, 274H), 4.86 (bs, NH_2, 192H), 7.12 (bs, $CONHCH_2$, 96H). FTIR (neat) cm^{-1}: 3360 (br, NH) and 1650, 1560 (amide C=O).

Preparation of dendrimer–DNA complexes

The JCPDs were dissolved in a 10 m*M* HEPES (pH 7.4) to a final concentration of 1 mg/mL. Dendrimer–plasmid DNA complexes were formed by incubating equal volumes of dendrimer and plasmid DNA at room temperature (20–25°C) for 20 min to obtain charge ratios from 2:1 to 10:1 (±). The final DNA concentration in the dendriplexes was 5 μg/mL. The charge ratios are presented as positive equivalents of cationic component to negative charge equivalents of the nucleic acid.

Agarose gel retardation assay

Polyplexes at various charge ratios ranging from 0.5:1 to 8:1 were prepared in HEPES buffered saline (10 m*M* HEPES, pH 7.4). After 30 min of incubation at room temperature for the complex formation, the samples were electrophoresed on a 1% (w/v) agarose gel and stained in an ethidium bromide solution (0.5 μg/mL). The location of the plasmid DNA was analyzed on a UV transilluminator.

Zeta-potential measurements of polyplexes

The zeta-potential values and size of JCPD G4 and G5 were determined by the Brookhaven Instruments ZetaPals system. Complexes were formed at a final concentration of 5 g/mL plasmid DNA in phosphate buffered saline (PBS; 50 m*M* PBS, 10 m*M* NaCl, pH 7.4) for zeta-potential experiments (Table II).

TABLE II. Zeta Electrical Potentials for Generation 4 and 5 JCPD

	Zeta Potential (mV)	
N:P Ratio	G4	G5
2:1	−26.2 ± 1.07	−27.87 ± 1.62
4:1	−26.62 ± 1.11	−23.09 ± 2.03
6:1	−23.95 ± 0.96	−20.46 ± 1.18
8:1	−18.83 ± 1.62	−13.36 ± 0.33
10:1	−25.57 ± 1.12	−21.25 ± 2.09

Cytotoxicity assay

Human HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS). The cells were routinely maintained on plastic tissue culture dishes (Grainer) at 37°C in an incubator under a humidified atmosphere containing 5% CO_2. All media routinely contained antibiotic–antimycotic agent (Biochrome). The cytotoxicity of the polymers was analyzed by 3-[4,5-dimethylthiazol]-2,5-diphenyltetrazolium bromide (MTT) assay. HeLa cells were seeded in a 96-well tissue culture plate at 10^6 cells per well in 90 μL DMEM medium containing 10% FBS. Cells achieved 70% confluence after 24 h. The medium was replaced with fresh DMEM medium without FBS before the addition of JCPD. The polymers were added to the cells with various concentrations (2–30 μg/ml) and incubated for 4 h at 37°C. Then, the polymer mixture was replaced with 100 μL of fresh DMEM medium supplemented with 10% FBS. The cells were incubated for an additional 24 h at 37°C. After incubation, 24 μL of 2 mg/mL MTT solution in PBS was added. The cells were incubated for an additional 4 h at 37°C, and then the medium was removed and 150 μL of dimethylsulfoxide was added to each well to dissolve the formazan crystals formed by living cells. Absorbance was measured at 570 nm using a microplate reader and recorded as a percentage relative to untreated control cells according to the following equation:

$$\text{Cell viability } (\%) = \text{OD570}^{(\text{sample})} / \text{OD570}^{(\text{control})} \times 100.$$

In vitro transfection experiment

HeLa cells were incubated with JCPD G4 and G5 derivatives at various charge ratios with plasmid DNA to determine the transfection efficiency of the polymers. Lipofectamine complexes were prepared under optimal conditions as positive controls. Transfection efficiency was evaluated as the expressed β-galactosidase activity divided by total cellular protein in the presence of 10% serum. Cells were seeded into 24-well cell culture plates at a density of 5×10^6 cells per well and grown overnight (~70% confluence). Immediately before transfection, the cells were rinsed with PBS and supplemented with 1 mL of fresh DMEM (Biochrome). The plasmid DNA (5 μg) and a range of concentrations of JCPD were each diluted into 500 μL of DMEM solution. JCPDs were added to the pDNA solution at room temperature and allowed to set for 30 min to allow dendrimer DNA complexes to form. The medium was removed, and the cells were then exposed to transfection mixtures for 6 h in the absence of serum at 37°C in a 5% CO_2 incubator. After 6-h transfection, mixtures were removed and incubated with 2 mL of fresh DMEM containing FBS (Biochrome). The cells were incubated for an additional 24 h at 37°C. Then, β-*galactosidase* gene expression was performed using β-Galactosidase Enzyme Assay System with Reporter Lysis Buffer. The β-galactosidase activity was detected spectrophotometrically (405 nm) using *o*-nitrophenol galactoside as the substrate. The transfection activity is expressed as nanomoles of *o*-nitrophenyl-β-D-galactopyranoside (ONPG)/min/mg protein. The β-galactosidase activity was normalized to the total protein concentration in the lysed cell samples, which was measured by the bicinchoninic acid (BCA) protein assay reagent kit (Pierce, Rockford, IL). Each experiment was carried out four times independently.

Transfection with JCPD/pEGFP-N1 and confocal microscopy

JCPD/pEGFP-N1 complexes were prepared by incubating equal volumes of dendrimer and plasmid DNA at room temperature (20–25°C) for 20 min to obtain charge ratios from 2:1 to 10:1 (±). The final DNA concentration in the dendriplexes was 5 μg/mL. The JCPD/pEGFP-N1 complex was incubated with HeLa cells. HeLa cells were seeded at a density of 5×10^4 cells per well in six-well plates 24 h before transfection. JCPD/pEGFP-N1 complexes were added to the cells and incubated for 6 h in 10% FBS containing media. After replacing the old media with fresh ones, the cells were further incubated for an additional 24 h at 37°C. Cells were fixed in 4% paraformaldehyde for 10 min, and the nuclei were stained with 10 μg/mL 4'-6-Diamidino-2-phenylindole (DAPI) (Invitrogen) for 10 min and then washed thrice with PBS. The cells on the slides were mounted with Fluoromount Aqueous Mounting Medium (Sigma) and covered with a coverslip. The fluorescent signal was analyzed using a Leica SP5 inverted confocal microscope. The transfection efficiency was calculated as the percentage of fluorescent cells out of the total number of cells. For control, naked pEGFP-N1 (1.0 μg in 200 μL fresh DMEM) was also incubated with HeLa cells using the same procedure described as above.

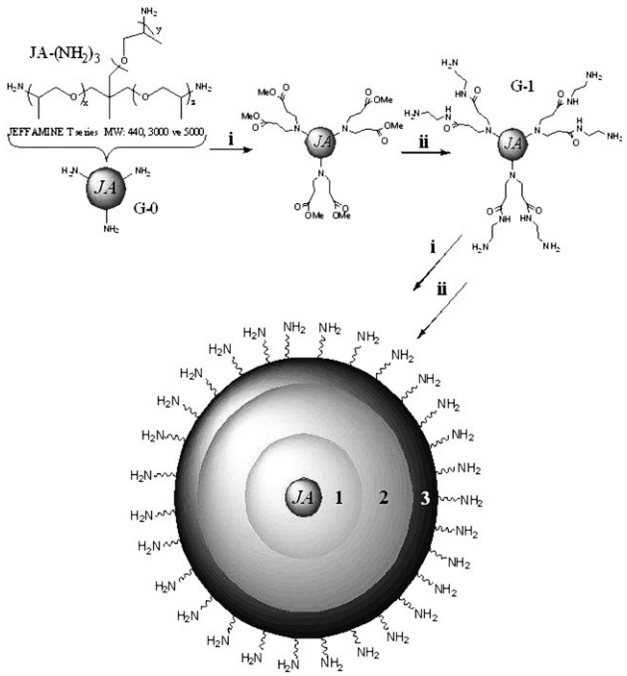

FIGURE 1. The synthetic pathway of Jeffamine-cored PAMAM dendrimers: (i) methyl acrylate in methanol at 40°C and (ii) ethylenediamine in methanol 40°C.

Statistical analysis

One-way ANOVA statistical analysis was used to evaluate the gene transfection efficiency between concentration and generation of the dendrimers. Results were expressed as mean ± standard error of the mean. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Preparation of JCPDs

The JCPDs were synthesized via divergent approach (Fig. 1) and characterized via ^{1}H NMR and FTIR spectral analysis, and the results agreed with that reported in the literature.[13] The prepared dendrimers were stored in their methanolic solution (20% w/w) in +4°C due to their highly hygroscopic properties.

Analysis of complex formation by agarose gel electrophoresis

To verify the complex formation of JCPD with plasmid DNA (pTracer-CMV/Bsd/LacZ), gel retardation assay was performed at various weight ratios of dendrimer/plasmid DNA complexes and analyzed in a 1% (w/v) agarose gel. Plasmid DNA was slightly retarded at a 1/1 weight ratio of JCPD/pDNA complex. The electrophoresis of JCPD/pDNA complex at this weight ratio showed faint DNA band [Fig. 2(A,B)]. The complete retardation of pDNA was observed for G4 at a 1/1 and higher weight ratios [Fig. 1(A)]. The complete retardation was observed for G5 at a 2/1 and higher weight ratios [Fig. 2(B)].

Zeta-potential and size measurements of the complexes with plasmid DNA

Zeta potential of dendriplexes was measured to elucidate the effect of the interaction between cationic dendrimer and anionic pDNA to surface charge of polyplex. Nanoparticles with zeta potential between −10 to + 10 mV are considered as approximately electroneutral, whereas zeta potential greater than +30 mV or less than −30 mV are considered strongly cationic or anionic.[14] As it is expected, cationic groups of the dendrimers and anionic groups of the pDNA are electrostatically interacted. Therefore, it can be concluded that G4 and G5 dendrimers with different N/P ratios have similar negatively charged zeta-potential values.

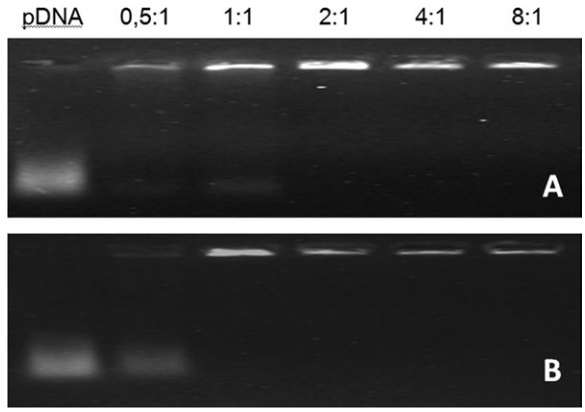

FIGURE 2. Gel retardation assay of JCPD/pDNA complexes at various weight ratios. Increasing amounts of JCPD were complexed with DNA. Mobility of DNA bound on JCPD was retarded on 1% (w/v) agarose gel.

Transfection experiments on cell lines

To evaluate the transfection efficiency, *in vitro* transfection assay was performed with JCPD/pDNA to HeLa cells. The JCPD G4 and G5 dendrimers and a plasmid encoding the β-galactosidase reporter gene were used. To optimize the transfection condition, JCPD/pDNA complexes were prepared at various weight ratios and transfected to HeLa cells (Table III). The β-galactosidase activity expressed with ONPG/min/mg protein was compared against control lipofectamine transfection efficiency (500 ng DNA mixed with 2.5 μL). JCPD G4 and G5 dendrimers were significantly less effective (∼40%) when compared with lipofectamine positive control (15% ± 1.3%). The highest transfection efficiency of JCPD/pDNA was obtained at a 10/1 weight ratio with G4 (T3G4: 6.75% ± 1%; T4G4: 8.9% ± 0.4%; T5G4: 9% ± 1%) and G5 (T3G5: 7.8% ± 1.1%; T4G5: 9% ± 1.3%; T5G5: 9.5% ± 1.4%; Fig. 3). One-way ANOVA test revealed that the level of gene expression was always

TABLE III. Optimization of the Transfection Condition Using Various Weight Ratios

	Concentration of Dendrimers (n*M*)	
N:P Ratio	G4	G5
2:1	188	94
4:1	301	150
6:1	301	150
8:1	301	150
10:1	376	188

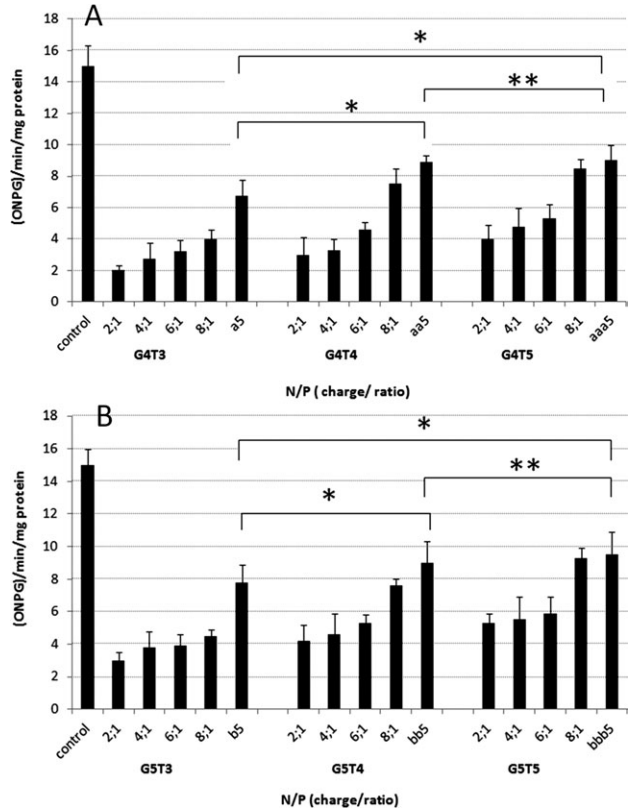

FIGURE 3. *In vitro* β-galactosidase activity (ONPG/min/mg protein) in HeLa cells 24 h after transfection with three variants (T3, T4, and T5). Lipofectamine used as positive control (500 ng DNA mixed with 2.5 μL). Variants of generation (A) G4 and (B) G5 JCPD at different N:P ratios. The data represent the mean ± SD of four independent experiments. Error bars represent standard deviation. Statistical differences at 1:10 ratio between variant are indicated with (*) for $p < 0.05$. No significant differences are indicated with (**). There are no differences between G4 and G5 variants at 1:10 ratio ($p > 0.05$).

significantly higher at the highest negativity/positivity (N:P) ratio used (10:1) for all the dendrimer generations tested ($p < 0.05$). At this N:P ratio, transfection efficiency results obtained for G5 (T5G5 variant) were same when compared with those obtained for G4 (T5G4 variant) without statistical significance ($p > 0.32$). These data show that variants do not statistically affect transfection efficiency.

Cytotoxicity assay

The toxicity of the JCPD/pDNA dendrimer complexes on HeLa cells was evaluated using the MTT assay for 24 h with various concentrations of JCPD (5, 10, 15, 25, and 30 μg/mL) and their DNA (5 μg/mL) complexes.

As shown in Figure 4, we observed that after incubation with the two types of JCPD at different concentrations for 24 h, the metabolic activity of HeLa cells decreased gradually with the increasing concentrations, suggesting a significant dose-dependent decrease in cell viability. Cell viability was above 50% at JCPD concentrations between 5 and 15 μg/mL, but decreased significantly at 20, 25, and 30 μg/mL. JCPD (G4 and G5) displayed a concentration- and generation-dependent toxicity. Toxicity was increased with increasing dendrimer concentration and followed the order of G5 > G4, confirming that a high density of cationic amines can exert damaging effect to cell membranes.

Confocal microscopy studies

The pEGFP-N1 plasmid was used for testing the expression of green fluorescent protein (GFP) in the cultured cells. JCPD/pEGFP-N1 complex was prepared at the condition displaying optimal transfection efficiency (Table III). The JCPD/pDNA complex was incubated with HeLa cells for 6 h in the presence of serum. After replacing with serum including fresh medium and further incubation for 24 h, the confocal images were obtained for GFP. The nucleus was stained in blue with DAPI. In general, as shown in figure, green florescence was observed in the cytoplasm. The results suggest that high level of pEGFP-N1 plasmid gene transfection and expression could be obtained with JCPD. Transfection efficiency was 8.6% ± 2.1% (for G4) and 11.4% ± 2.6% (for G5) for triplicate-cultured HeLa cells, which is considered as remarkable when compared with similar PAMAM studies.[18–20] (Qin et al. 2011; Yuan et al. 2010)

The use of nonviral carriers in gene transfection studies, especially in clinical applications, shows limited capacity.

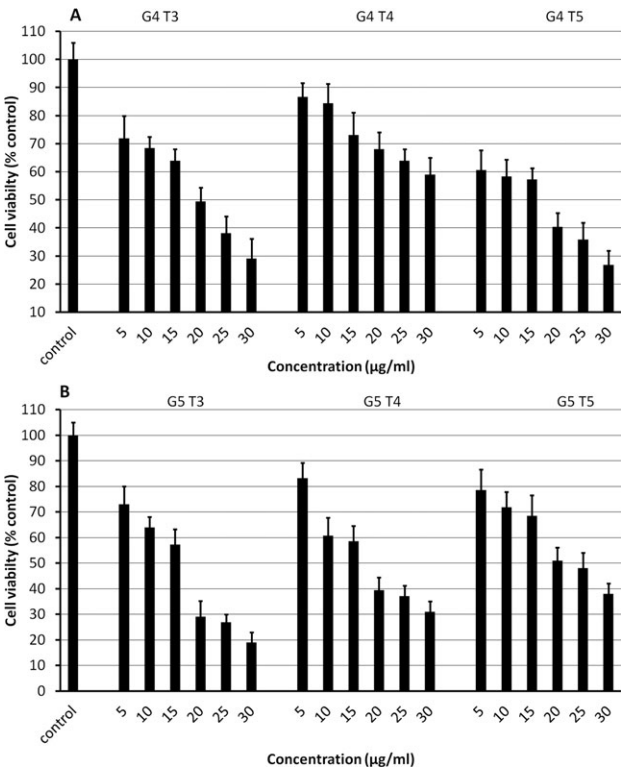

FIGURE 4. Viability of HeLa cells after incubation with (A) JCPD-G4 and (B) JCPD-G5 at different concentrations. The cellular viability is calculated as a percentage from the viability of the control cells (5 μg/mL of naked DNA). The viability of the control cells is considered to be 100%. The data represent the mean ± SD of four independent experiments. Error bars represent standard deviation. Among all generations and their variants, G5 is more toxic when compared with G4 ($p < 0.05$). A: For G4 variants, there is no significance between T3 and T5 ($p = 0.48$); however, T4 is less toxic when compared with others ($p < 0.001$). B: For G5 variants.

These low-efficiency problems arise from physicochemical properties of the polymeric or liposomal carriers, and the structures of cell membrane are the main barriers for gene delivery using nonviral carriers.[21] Thus, diverse approaches are undertaken by researchers to overcome these problems, and a new molecule has been developed to find out more efficient protocols. Dendrimers used in gene delivery systems bind with DNA by charge-based interactions. The PAMAM dendrimers carry positively charged primary amine groups on their surface and tertiary amine groups inside of the molecule. The surface amine groups participate in DNA binding, compact DNA into nanoscale particles, and promote the cellular uptake of DNA. The tertiary amine groups act as a proton sponge in endosomes and enhance the release of DNA into the cytoplasm.[22,23]

There is a correlation between the transfection efficiency and the molecular size and shape of the PAMAM dendrimers. Different cell lines have showed that transfection is strongly dependent on the cell type and that the DNA binding ability increased with the PAMAM generation number from G5 to G10.[8,24] Modification of PAMAM dendrimers also contributes to high levels of transfection. Researchers are still in search for ways of modifying dendrimers to improve their efficiency.[25] PAMAM dendrimers, as DNA carrier vector, have important advantages for *in vivo* applications, with no antigenicity and with lower cytotoxicity that can be excreted by urinary system.[26] The PAMAM dendrimers were proved to bind with the DNA to be transfected into cells *in vitro* and *in vivo*.

CONCLUSIONS

The JCPDs G4 and G5 formed complexes with plasmid DNA efficiently and plasmid DNA retarded completely at a 1/1 weight ratio for G4 and 2/1 weight ratio for G5 in gel retardation assay. Each JCPD dendrimer showed the ability to deliver plasmid DNA into HeLa cells, and the exogenous β-galactosidase and GFP gene had been successfully expressed. The highest transfection efficiency of JCPD/pDNA was obtained at a 10/1 weight ratio, which showed a slightly negative zeta potential. JCPD G5 showed higher transfection efficiency (11.4%) when compared with G4 (8.6%), and their cellular toxicity follows the sequence of G5 $>$ G4 order. In summary, the conjugation of Jeffamine to PAMAM produced an efficient transfection reagent. These results indicated that low generation of JCPD can also be used as nonviral gene delivery systems because of their large cores.

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