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Research Paper

Poly (amino ester) Composed of Poly (ethylene glycol) and Aminosilane Prepared by Combinatorial Chemistry as a Gene Carrier

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Purpose. Application of combinatorial chemistry and high throughput screening for the synthesis and evaluation of mini-library of novel biodegradable poly (β-amino ester)s (PAE)s composed of γ-aminopropyl-triethoxysilane (APES) and poly (ethylene glycol) diacrylate (PEGDA) for gene delivery efficiency and safety in 293T and HeLa cells in the presence of and absence of serum.

Materials and methods. PAEs were synthesized at different mole ratios of APES and PEGDA by Michael addition reaction and synthesis was confirmed by ^{1}H nuclear magnetic resonance (^{1}H-NMR). Ninety six ratios of polyplexes were evaluated for luciferase and MTS assay in 293T and HeLa cells in the presence of and absence of serum. Relationship between transfection efficiency and DNA binding ability of PAEs was studied by gel electrophoresis. Particle sizes and molecular weight of selected PAEs were measured by dynamic light scattering and gel permeation chromatography multi-angle light scattering, respectively.

Results. ^{1}H-NMR confirmed the synthesis of PAEs. In both cell lines, transfection efficiency and cell viability were increased for PAEs obtained from R106 (0.7:1, APES:PEGDA) to R121 (6:1, APES:PEGDA) with a marginal increase in APES concentration. Transfection pattern was uniform in the absence of and presence of serum. In both cell lines, PAE obtained from R121 demonstrated high transfection efficiency and low cytotoxicity as compared to polyethylenimine (25 KDa) and Lipofectamine. PAE obtained from R121 showed good DNA binding and condensation with average particle sizes of 133 nm.

Conclusion. Addition of PEGDA over APES resulted in a novel PAE which has high safety and transfection efficiency. Transfection and cytotoxicity are very sensitive to monomer ratios and mainly governed by concentration of amine monomer.

KEY WORDS: aminosilane; combinatorial chemistry; gene delivery; non-viral vector; poly (β-amino ester).

INTRODUCTION

The ultimate goal of gene therapy is to cure both inherited and acquired disorders in a straight forward manner by removing their causes that is by adding, correcting or replacing genes. A key problem in gene therapy is the efficient delivery of genetic material to the required cells within a patient without significant toxicity. A number of novel delivery strategies have been proposed to improve the efficiency of plasmid DNA transfection. However, the safety of the vector is still critical concern for any gene therapy. Ideal gene delivery system should be biodegradable, non-toxic, non-immunogenic, stable, target specific, and should assist gene expression. Although, till now, viral vectors are efficient gene delivery systems and have been tried in the majority of clinical trials, there have been a number of problems with their application in human gene therapy, such as poor targeting, rapid clearance, expensive, immunogenic and pathogenic. Non-viral vectors were designed on the potential of synthetic systems to bind and condense the genetic material effectively. Non-viral cationic polymeric systems are found to be efficient DNA carriers, both, *in vitro* and *in vivo*, and there is a good scope for the synthetic modification of this class of agents in order to manipulate their transfection efficiency, safety and target specificity (1).

All cationic polymers containing primary, secondary or tertiary amino groups are capable of condensing and complexing DNA under physiological conditions. Some examples

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ABBREVIATIONS: AEAPMS, N-β-(aminoethyl)-γ-aminopropyl-trimethoxysilane; APES, γ-aminopropyl-triethoxysilane; DMEM, Dulbaco's modified Eagles media; PAE, poly (β-amino ester); PEGDA, poly (ethylene glycol) diacrylate; PEI 25K, high molecular weight polyethylenimine (25 KDa); RLU/mg protein, Relative light units per milligram of protein; R106 to R121, reaction codes representing PAEs obtained from different mole ratios of APES and PEGDA.

include, poly (β-amino esters) (PAE) (2,3), poly(amido amine) dendrimers (4), polyethylenimine (PEI) (5). These polymers generally serve as gene carriers through electrostatic interactions for the intracellular delivery of preloaded genetic material. However, benefit owing to their cationic nature is overwhelmed by their high cytotoxicity profile. A number of approaches have been tried to overcome cytotoxicity limitation and to make non-viral cationic systems safe (1,6). One of the most successful approaches is the conjugation of nonionic hydrophilic polymer such as poly (ethylene glycol) (PEG) (7–9).

Although, organic materials are popular non-viral vectors for DNA delivery, there exists a continuing need for non-toxic, biodegradable, biocompatible systems that can transfect cells effectively and are easy to prepare economically. Recently a few inorganic systems such as, modified silica (10–13), modified gold (14), and calcium–magnesium phosphates (15) also received attention as non-viral vectors in gene delivery. These inorganic systems have advantages over organic ones *in vivo* with low polydispersity, good DNA condensation ability and high biocompatibility (16). These beneficial properties of inorganic moieties have given new prospective to the non-viral gene delivery system.

An organic polymer like PEG has number of advantages with biological aspect as well as formulation aspect. In biological aspect, it is hydrophilic, biocompatible, increases blood circulation time and reduces plasma clearance. It also prevents protein opsonization. In formulation aspect, it is soluble in most of the solvents. It can be easily derivatised and conjugated with many therapeutic moieties. Moreover, it is commercially available in variety of molecular weights. Alkoxy organosilanes with amine head are popular silane coupling agents. They are frequently used in attaching DNA to mica surface. Because of their inherent safety and biodegradability, they have also been reported in combination with silica nanoparticles in polynucleotide delivery (17). Alkoxy aminosilanes have organic and inorganic linkages and because of this unique property they may immerge as potential vectors for gene delivery.

The combinatorial chemistry and automated high-throughput synthesis has revolutionized modern drug discovery by rapid synthesis and evaluation with more precision. A key feature of these methods is that synthesis, storage, and cell-based testing are all performed without removing solvents, thereby, allowing high-throughput manipulations using simple, easily automated fluid handling systems. The effect of solvents is nullified by dilution to prevent their interference in cell studies.

In this study we used combinatorial chemistry technique to synthesize different PAEs composed of alkoxy aminosilane and poly (ethylene glycol) diacrylate (PEGDA) by Michael addition reaction and evaluated for their gene delivery efficiency. PEGDA was attached to γ-aminopropyltriethoxysilane (APES), which is water soluble and biocompatible alkoxy aminosilane derivative, by easily hydrolysable ester linkage. This PAE has number of beneficial properties like tertiary amine linkage which is useful for DNA complexation and endosomal escape. PEG in a backbone is helpful in reducing particle aggregation by charge masking and also useful for increasing circulation time. Hydrolysable ester linkage is essential for rapid intracellular break down of PAE into nontoxic monomers. Thus, cationic nature due to amino group bearing side chain, easily hydrolysable ester linkage and the presence of both, hydrophilic and hydrophobic groups on PAE makes it perfect candidate for the delivery of polynucleotide.

EXPERIMENTAL PROCEDURES

Synthesis of PAEs. PAEs were synthesized in 16 different mole ratios of APES to PEGDA as shown in Table I. Briefly, respective stoichiometric moles of APES (MW: 221.37, purity: 98%) (0.225 mM) (Sigma-Aldrich, USA) and PEGDA (Mn: 258, purity: 98%) (Sigma-Aldrich, USA) were separately dissolved in anhydrous ethanol (Sigma-Aldrich, USA) to make the final volume 1.5 ml. Then, PEGDA solution was slowly added to APES solution in 2 ml microtube (Axygen Scientific USA) at 50°C. Reaction was carried out at 50°C for 24 h in multiple angle incubator shaker. Reaction was sensitive to the moisture and hence care has been taken to prevent exposure to the atmospheric moisture. After completion of the reaction, samples were immediately stored at −20°C for further studies. The reaction scheme is shown in Fig. 1.

Confirmation of PAE synthesis. The confirmation of synthesis and the composition (Table II) (amine/acrylate) of the prepared PAEs were determined by measuring ^{1}H nuclear magnetic resonance (NMR) (600 MHz) (Advance™, Bruker, Germany). For measurement of NMR, selected PAE ratios were purified by ultrafiltration (Pall Life Sciences, USA) and samples were lyophilized. PAE was dissolved in D_2O at concentration of 7 mg/ml and ^{1}H-NMR was measured immediately.

Transfection in the absence of serum. 293T cells (1×10^4 cells/well) and HeLa cells (2×10^4 cells/well) were seeded into each well of a 96 well plate (SPL Life Sciences) and allowed to attach overnight in Dulbaco's modified Eagles media (DMEM) growth medium containing 10% fetal bovine serum and 100 U/ml penicillin. Polymer stock solutions (10 mg/ml) and working dilutions were prepared in water (at concentrations

Table I. Stoichiometric Mole Ratios of PAEs

Code for PAEs	Stoichiometric Mole Ratios of APES to PEGDA
R106	0.7:1
R107	1:1
R108	1.1:1
R109	1.2:1
R110	1.4:1
R111	1.6:1
R112	1.8:1
R113	2:1
R114	2.5:1
R115	3:1
R116	3.5:1
R117	4:1
R118	4.5:1
R119	5:1
R120	5.5:1
R121	6:1

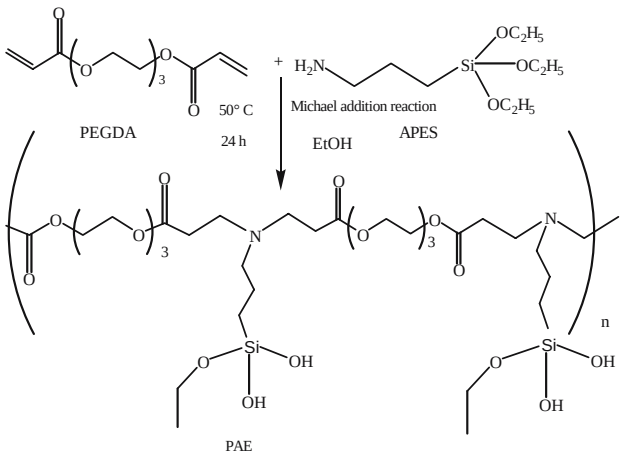


Fig. 1. Proposed reaction scheme for PAE copolymer.

necessary to yield the different PAE/DNA weight ratios from 10:1 to 110:1) and 10 μl of 25 mM sodium acetate buffer (pH 5.5) was added. 1.5 μg of pCMV-Luc DNA was used to form complexes. The mixtures were incubated for 30 min to allow the complex formation and then diluted with serum free media to make final volume of 150 μl. The complexes were transferred to each well of 96 well plates and incubated with cells for 4 h. Then the media were changed with fresh media containing serum and incubated for 72 h at 37°C and 5% CO_2. Relative light units (RLU) were measured with chemiluminometer (EG&G Berthold, Germany). Controlled experiments were performed with DNA, PEI 25 K and Lipofectamine. PEI 25 K transfection was performed as described above while Lipofectamine transfection was done as per vendor's protocol. RLU were normalized to protein concentration and protein quantification was determined by the BCA (Promega, USA) method. Assay was performed in triplicates.

Transfection in the presence of serum. Luciferase assay was performed in the presence of serum in a similar way as described under transfection in the absence of serum in 293T and HeLa cells. PAE/DNA complexes were prepared in a similar way as described above by 30 min incubation. After complex formation dilutions were made with serum containing media (10% FBS) (Hyclone, South Logan, UT, USA) to make final volume of 150 μl. The complexes were transferred to each well of 96 well plate and all subsequent steps were performed in an exactly same way as described above.

Cell viability assay. MTS assay was performed to measure cell viability of synthesized PAEs in 293T and HeLa cells. Cells were seeded in 96 well plates at an initial density of 1×10^4 cells/well for 293T and 2×10^4 cells/well for HeLa cells, in 0.2 ml growth medium and incubated to reach 80% confluence. To facilitate handling, polymer stock solutions (10 mg/ml) were prepared in water. Assay was performed without adding plasmid in order to measure maximum possible cytotoxicity of PAEs. Working dilutions of each polymer were prepared in water (at concentrations equivalent to yield different PAE/DNA weight ratios from 10:1 to 110:1) and 10 μl of 25 mM sodium acetate buffer (pH 5.5) was added. Final volume was adjusted to 100 μl by DMEM media. After incubation for 24 h, the media were changed with growth media containing 20 μl of Cell Titre 96 Aqueous One Solution Reagent (Promega, USA). Finally, after incubation for 2 h, absorbance was measured at 540 nm using ELISA plate reader (GLR 1000, Genelabs Diagnostics, Singapore). The assay was performed in triplicates. Cell viability (%) was calculated according to the following equation:

$$\text{Cell viability}(\%) = \left[\text{OD}_{540(\text{sample})} / \text{OD}_{540(\text{control})}\right] \times 100.$$

PAE/DNA complex formation. Gel electrophoresis was performed to confirm complex formation ability of selected PAE with DNA. Complex formation was induced at various mass ratios (5:1 to 80:1) of PAE to DNA and final volume with agarose gel loading dye mixture 6× (Biosesang, Korea) was 13 μl. The complexes were loaded onto 0.8% agarose gels with EtBr (0.1 μg/ml) and run with tris acetate buffer at 100 V for 40 min. DNA retardation was observed by irradiation with UV light.

Particle sizes and molecular weight measurement. The particle sizes of selected ratios of PAE/DNA complexes were measured by using electrophoretic light scattering spectrophotometer (ELS 8000, Otsuka Electronics, Osaka Japan) with 90° scattering angle. Molecular weights of selected ratios of PAE were measured by size exclusion chromatography with multi-angle light scattering.

RESULTS AND DISCUSSION

Synthesis of PAE. We successfully synthesized PAEs composed of APES and PEGDA by Michael addition reaction in which APES was attached with PEGDA by easily hydrolysable ester linkage. APES is a water soluble organosilane derivative having primary amine and ethoxysilane functionalities, separated by propyl moiety. It has unique organic (Si–C, C–C, C–N, C–H) and inorganic (Si–O) linkages which may exhibit beneficial properties of both organic

Table II. Composition of Selected PAEs by ^{1}H-NMR

Code for PAEs	Moles of APES to PEGDA	Composition of Amine (mol%)	Composition of Acrylate (mol%)	Amine/Acrylate
R106	0.7:1	29.4	70.6	0.42
R107	1:1	36.9	63.1	0.59
R108	1.1:1	37.7	62.3	0.61
R114	2.5:1	48.3	51.7	0.93
R118	4.5:1	59.6	40.4	1.48
R120	5.5:1	63.0	37.0	1.70
R121	6:1	66.4	33.6	1.98

and inorganic vectors. Primary amine on APES makes it suitable for Michael addition reaction. Michael addition reaction is a reaction of choice for PAE synthesis owing to its simplicity and high yield (18). Our previous works reported the success of PAEs composed of different polycations like spermine (19) and low molecular weight PEIs (20,21) for safe and efficient gene delivery.

In this work, we used combinatorial chemistry to evaluate PAEs composed of APES and PEGDA in different stoichiometric mole ratios for safe and efficient gene delivery. Our previous experiences suggest, high transfection efficiency is governed by nature of cationic monomer and properties of copolymer backbone. The absence of titrable amines in copolymer makes complex inefficient for endosomal escapes and exhibit very low transfection efficiency (22). In addition, higher shielding of cationic charge also reduces transfection efficiency drastically. We observed decrease in transfection efficiency with increase in molecular weight of PEGDA probably because of higher charge shielding effect with increase in molecular weight of PEGDA (23). Hence, for this study we used low molecular weight PEGDA (258 Da) which has already been proven very effective when conjugated with low molecular weight PEI (23). In addition to PAE composed of APES and PEGDA, we also synthesized and evaluated PAE composed of N-β-(aminoethyl)-γ-aminopropyl-trimethoxysilane (AEAPMS) and PEGDA for its transfection efficiency and safety (data not shown). However, poor reproducibility of reaction with AEAPMS and very low transfection ability concentrated our focus on APES based PAEs for gene delivery.

Combinatorial chemistry and high through-put screening allowed us to synthesize and evaluate mini-library of APES and PEGDA based PAEs for its gene delivery efficiency and cytotoxicity at a single setup. High molecular weight is prerequisite for any polymer to give good transfection, theoretically, the maximum molecular weight should occur at stoichiometric equivalence (amine/diacrylate=1 mole/mole). In addition, as reported by Anderson *et al.*, amine terminated copolymers have better transfection efficiency than acrylate terminated when prepared with slight excess of amine monomer (24). Hence, we selected all ratios of APES and PEGDA near stoichiometric equivalence with small increase in mole ratio of APES. As mole ratio of APES increased from R106 to R121, amine terminated PAEs were formed. For selected PAEs (Table II), 4 (R106, R107, R108 and R114) of the 7 selected PAEs had amine/diacrylate ratio <1, indicating more incorporation of acrylate monomer and acrylate terminated polymers, and 3 (R118, R120 and R121) of the 7 had amine/diacrylate ratios >1, indicating amine terminated polymers. Homogenous reaction and improved yield were obtained by using anhydrous ethanol as a solvent. The yield was around 60% for selected ratios after purification by ultrafiltration. The obtained PAEs were readily soluble in water because of its hydrophilic nature. Proton NMR spectroscopy confirmed the synthesis of PAEs between APES and PEGDA. The peaks for methyl protons, δ=1.3–1.4 ppm [Si–O–CH_2–CH_3], and methylene protons, δ=3.36 ppm [Si–O–CH_2–], δ=0.8–0.9 ppm [–CH_2–Si–O], δ=1.8–2.0 ppm [Si–CH_2–CH_2–] of APES were clearly appeared. The methyl protons of tertiary amine, formed during reaction, δ=2.4–3.2 ppm [–N (CH_2)$_3$–] and peaks of PEG ethylene protons, δ=3.6–3.8 ppm (–OCH_2CH_2–) and ester protons, δ=4.3 ppm (–OCH_2–) were also detectable in ^{1}H-NMR of polymer as per expectation (Fig. 2).

Luciferase expression in the absence of serum. Transfection efficiency is a key parameter for any gene delivery vector. Many vectors with high transfection efficiency are highly toxic while vectors with low toxicity are poor in transfecting cells. Optimum balance between these two parameters is a key to the success in gene therapy. Combinatorial and high throughput approach allowed us to investigate transfection efficiency of 96 polyplexes at the same setting to select best ratio with maximum transfection efficiency and safety. Transfection efficiency is a sensitive parameter which may vary significantly even with small change in monomer concentration (24). Sixteen ratios of PAEs (R106 to R121) with marginal change in amine monomer concentration were administered in six weight ratios (ranging from 10:1 to 110:1) of PAE: DNA, respectively. All 96 polyplex ratios were evaluated for luciferase assay in 293T and HeLa cells in the presence of and absence of serum.

In serum free media, it was observed that luciferase expression was high and cell line dependent. Although 293T cells exhibited ten folds higher transfection than HeLa cells, transfection pattern was uneven probably because of higher sensitivity of 293T cells for transfection and cytotoxicity. In 293T cells, PAEs obtained from R106 to R113 showed some transfection at lower weight ratios but it was suddenly decreased with increased weight ratios which may be because of low cell viability at these ratios. PAEs obtained from R114 to R119 showed intermediate transfection while PAEs obtained from R120 and R121 gave good transfection (Fig. 3a). However, in HeLa cells slightly different transfection pattern was observed. PAEs obtained from R106 to R115 failed to give significant transfection. On the other hand, PAEs obtained from R116 to R119 showed intermediate transfection which was slowly increased from R116 to R119. Transfection was highest with PAEs obtained from R120 and R121 and it was increased with increasing weight ratios till 90:1 after that it again decreased due to increased cytotoxicity (Fig. 3b).

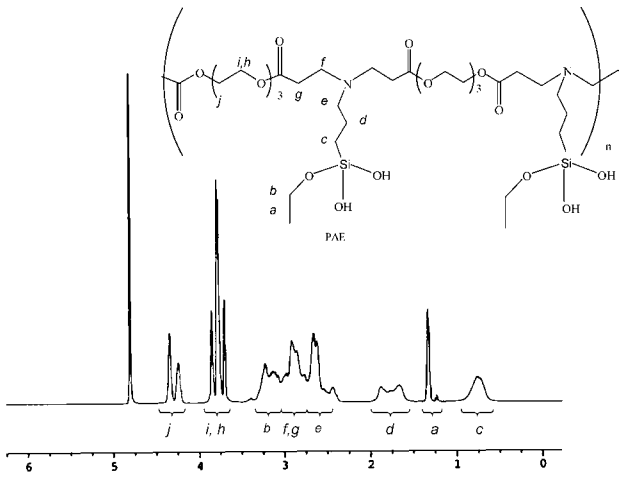


Fig. 2. ^{1}H NMR spectrum (600 MHz) of PAE in D_2O.

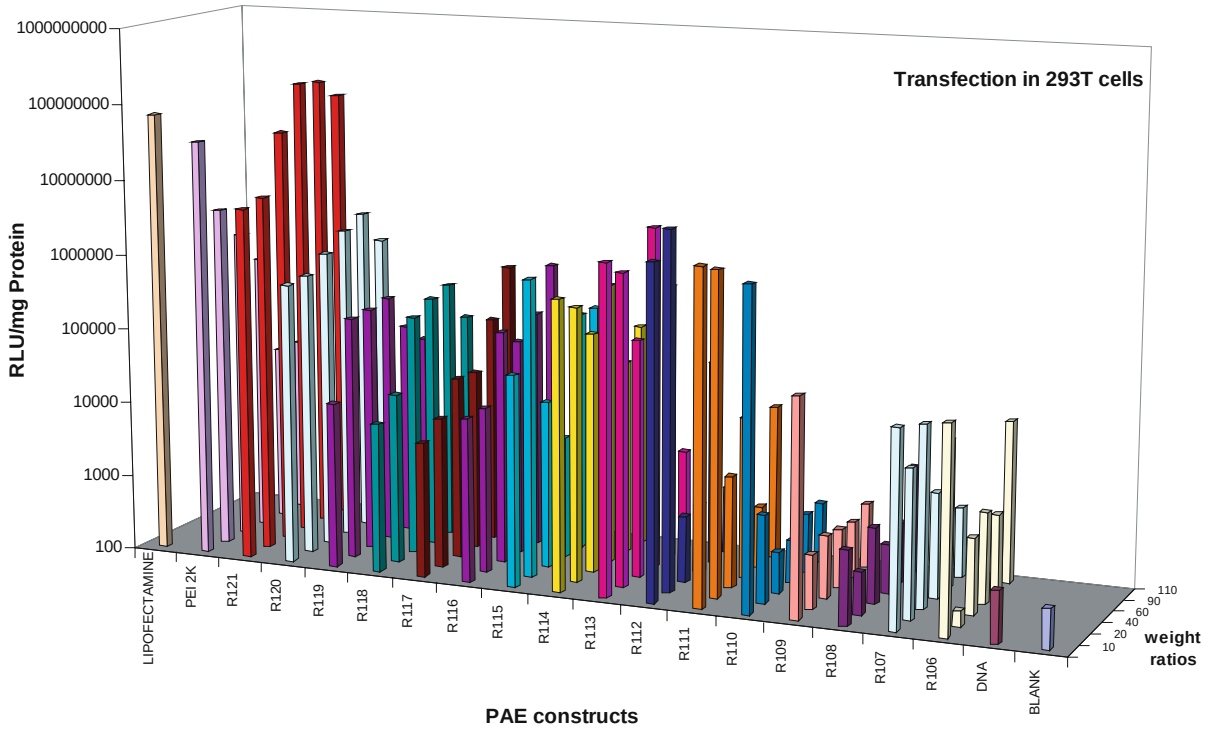
Overall in both cell lines, it was observed that transfection was increased with increasing APES concentration while it is low and non-uniform at low APES monomer concentration (Fig. 3). As it is illustrious, increase in amine monomer i.e. APES, increases net cationic charge which allows PAE to complex and condense DNA effectively and promotes its rapid uptake by endocytosis. Although, exact reason is unclear for non-uniform transfection at lower ratios, we suppose this is because of toxic behavior of PAEs with lower APES concentration as indicated by cytotoxicity data. Also, PAEs obtained with lower APES concentration (R106 to R114) failed to exhibit significant increase in transfection even if PAE/DNA ratio increased ten times. In contrast, PAE ratios with increasing APES concentration (R117 to R121), exhibited significant change in transfection even with small increase in PAE/DNA weight ratio. This study indicated that transfection efficiency is primarily controlled by amine monomer concentration in copolymer and secondarily controlled by polymer/DNA weight ratio. Thus, optimum amine monomer concentration in copolymer with optimum polymer/DNA weight ratio is an important requirement for high transfection. Ten folds higher transfection in 293T cells than HeLa cells also elucidated the cell line dependency of transfection efficiency (Fig. 3a and b). In the absence of serum, PAE obtained from R121 showed higher transfection than PEI 25K and Lipofectamine in 293T cells while it is higher than PEI 25K and comparable with Lipofectamine in HeLa cells. Five out of 16 PAEs demonstrated good transfection potential as compared PEI 25K in both cell lines. Moreover, transfection efficiency of PAE (R121) at mass ratio 60:1, 90:1 and 110:1, was 10 fold greater than that of PEI 25K. At these ratios transfection was also higher than that of Lipofectamine in 293T cells and comparable in HeLa cells.

Luciferase expression in the presence of serum. High transfection in the presence of serum protein is an important requirement for non-viral vectors in order to use them effectively for *in vivo* studies. It is commonly observed that many non-viral vectors show good transfection efficiency in the absence of serum, however, when serum is added, the proteins in serum interfere with the DNA-cationic polymer complex and reduce their availability to the cells resulting in poor transfection. In order to check effect of protein interference on transfection efficiency of PAEs, we studied all 96 ratios of polyplex for luciferase assay in serum (10% FBS) containing media in 293T cells (Fig. 4a) and HeLa cells (Fig. 4b). In the presence of serum although small decrease in transfection was observed, the transfection pattern was similar as in the absence of serum. In both cell lines, PAEs obtained from ratios R106 to R116 showed low and irregular transfection while PAEs obtained from R117 to R120 showed intermediate transfection with gradual increase from R117 to R120. Even though, transfection was lowered in the presence of serum, still it was significantly high with PAEs obtained above R117 (Fig. 4). Moreover, in consistent with transfection data in the absence of serum, PAEs obtained above R117 exhibited increase in transfection with increase in PAE/DNA weight ratios till 90:1 and after that it slowly decreased. PAE obtained from R121 at weight ratios 40, 60 and 90 showed high transfection efficiency as compared to PEI 25K and Lipofectamine in both cell lines. This ability of PAEs to give significant transfection in the presence of serum is attributed to the ability of PEG to prevent protein opsonization (25).

Cell viability assay. A critical concern in gene therapy is the safety of a vector which is forcing tug of war between viral and non-viral systems. Even non-viral vectors like high molecular weight PEI, dendrimers, cationic liposomes etc. are highly toxic due to lack of easily cleavable linkage. Introduction of biodegradable linkage is essential for the breakdown of macromolecules into small easily clearable units, once DNA is released in the cytosol. Since PAEs are easily biodegradable owing to the rapid hydrolysis of ester bond *in-vivo*, they are becoming polymers of choice in gene delivery. We evaluated cytotoxicity of our mini-library of PAEs in 293T and HeLa cells by MTS assay. In order to measure maximum possible cytotoxicity, PAEs were administered in increasing concentrations to 293T cells (Fig. 5a) and HeLa cells (Fig. 5b). In both cell lines, PAEs obtained from R106 to R113 exhibited very high cytotoxicity which further increased with increase in weight ratios, while PAEs obtained from R114, R115 and R116 showed good cell viability at lower ratios but significant cytotoxicity at higher weight ratios. Excellent cell viability and uniform transfection pattern were observed with PAEs obtained from R117 to R121. Slight cytotoxicity was observed at higher mass ratios (90:1 and 110:1) but it was not significant (viability above 80% in all ratios). This study indicated that cytotoxicity was highly sensitive to monomer ratio and varied drastically even with small change in monomer concentration. This cytotoxicity study further reaffirms the findings of Aknic *et al.* who observed that PAEs synthesized with higher diacrylate monomer were highly toxic as compared with excess amine monomer (26). Exact reason for this is unclear; however, same results are more broadly applicable for different PAEs (26). The plausible explanation is, hydrophobic diacrylates are alkylating agents and highly toxic to the cells if present in excess. At lower APES ratios, acrylate terminated copolymer may predominate and exhibit high cytoxicity. While at higher APES ratios cytotoxicity of PAEs were reduced due to excess of amine monomer which increased amine contents and consumed diacrylate significantly to form amine terminated copolymer (24). As the ratio of amine monomer i.e. APES increased, cytotoxicity was reduced and transfection efficiency was increased. Very high cytotoxicity was observed with all ratios of PEI 25K. Also cytotoxicity of Lipofectamine was higher than that of PAEs obtained above R118. Thus, PAEs with higher APES ratio displayed remarkable cell viability and found to be safe as compared with Lipofectamine and PEI 25K, even if concentration reached 10 folds greater than those at which PEI 25K is cytotoxic.

Complexation study. For any non-viral vector, DNA condensation is an inevitable prerequisite which provides information about the transfection potential of the system. It was observed from transfection data, increased APES concentration increased transfection efficiency and we expected this increase is due to enhanced capacity of PAE to complex and condense negatively charged DNA. We confirmed this prediction by gel retardation assay. We selected three ratios R106, R120 and best performed ratio R121 for gel retardation assay. In gel electrophoresis, it was

a



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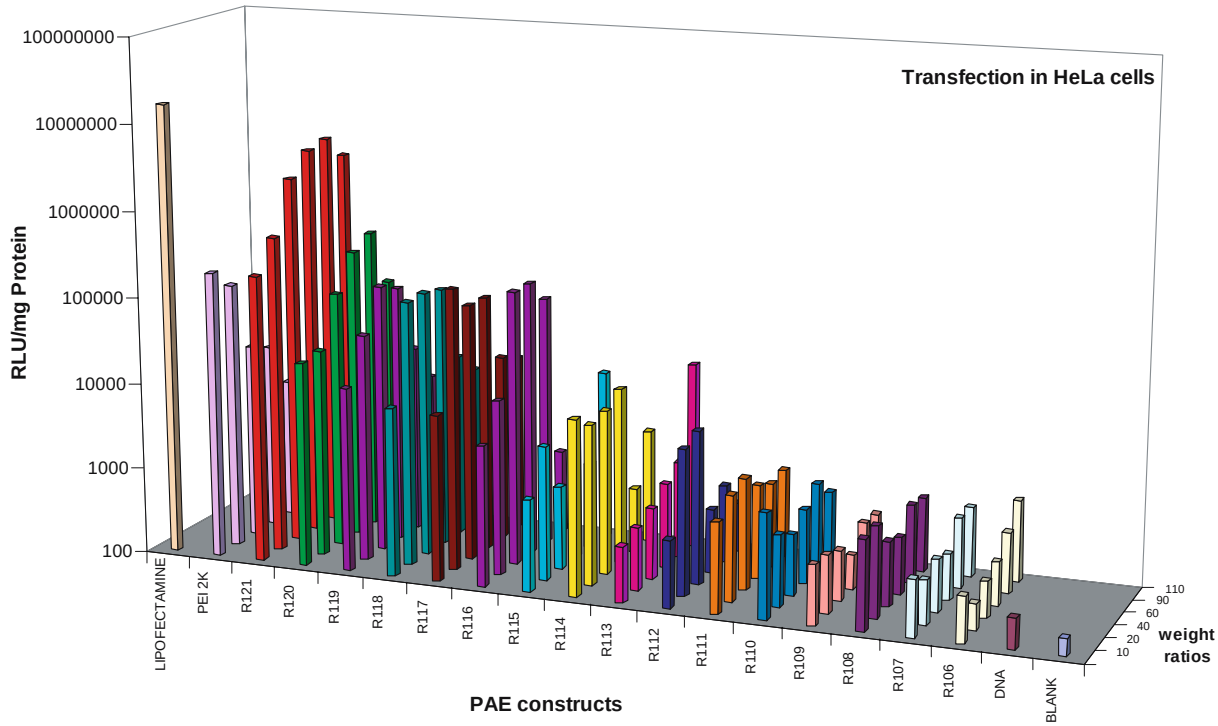

Fig. 3. Transfection efficiency of PAE/DNA complexes in serum free-media at various mass ratios in **a** 293T cells and **b** HeLa cells.

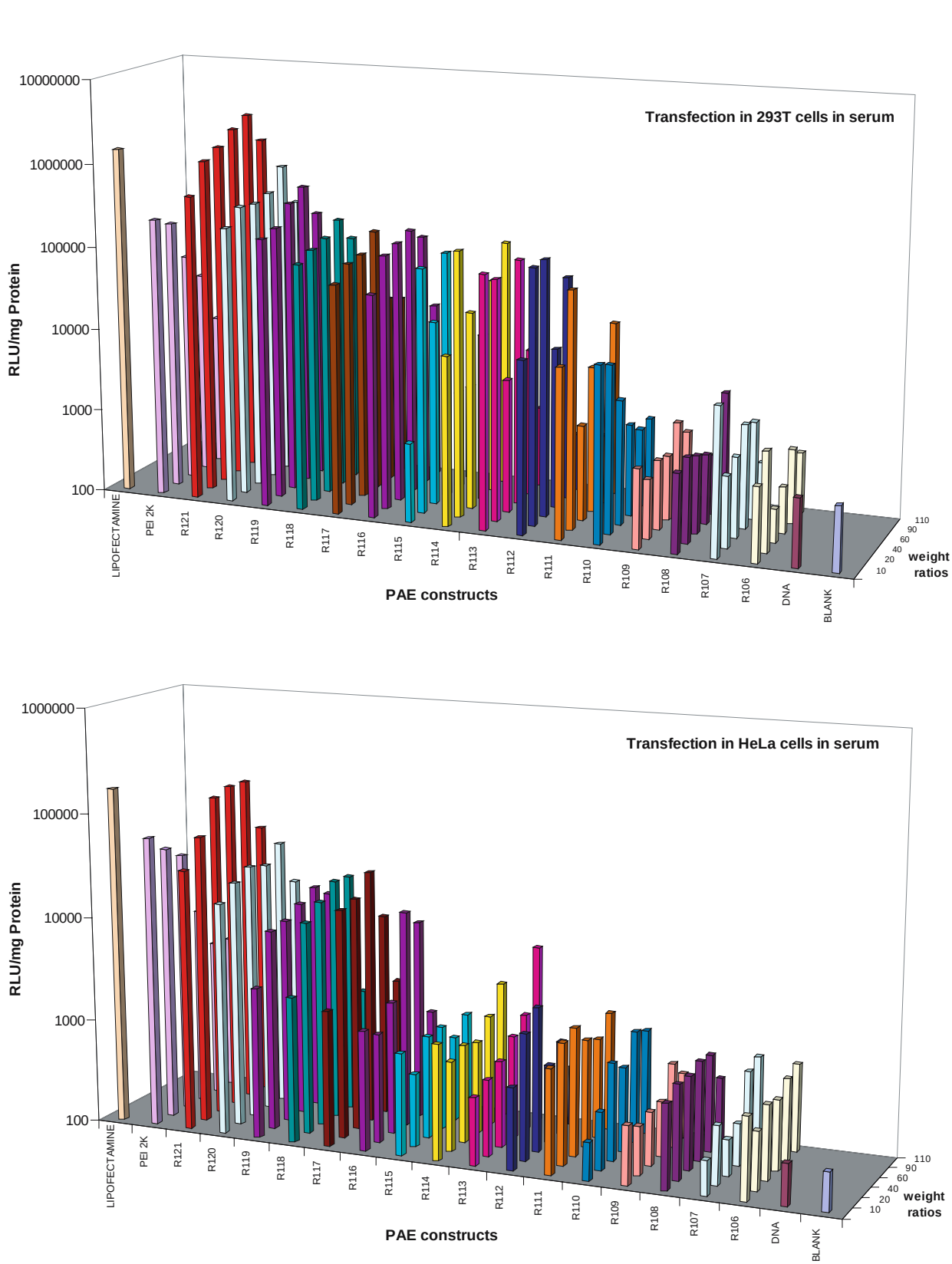

Fig. 4. Transfection efficiency of PAE/DNA complexes in serum containing media at various mass ratios in **a** 293T cells and **b** HeLa cells.

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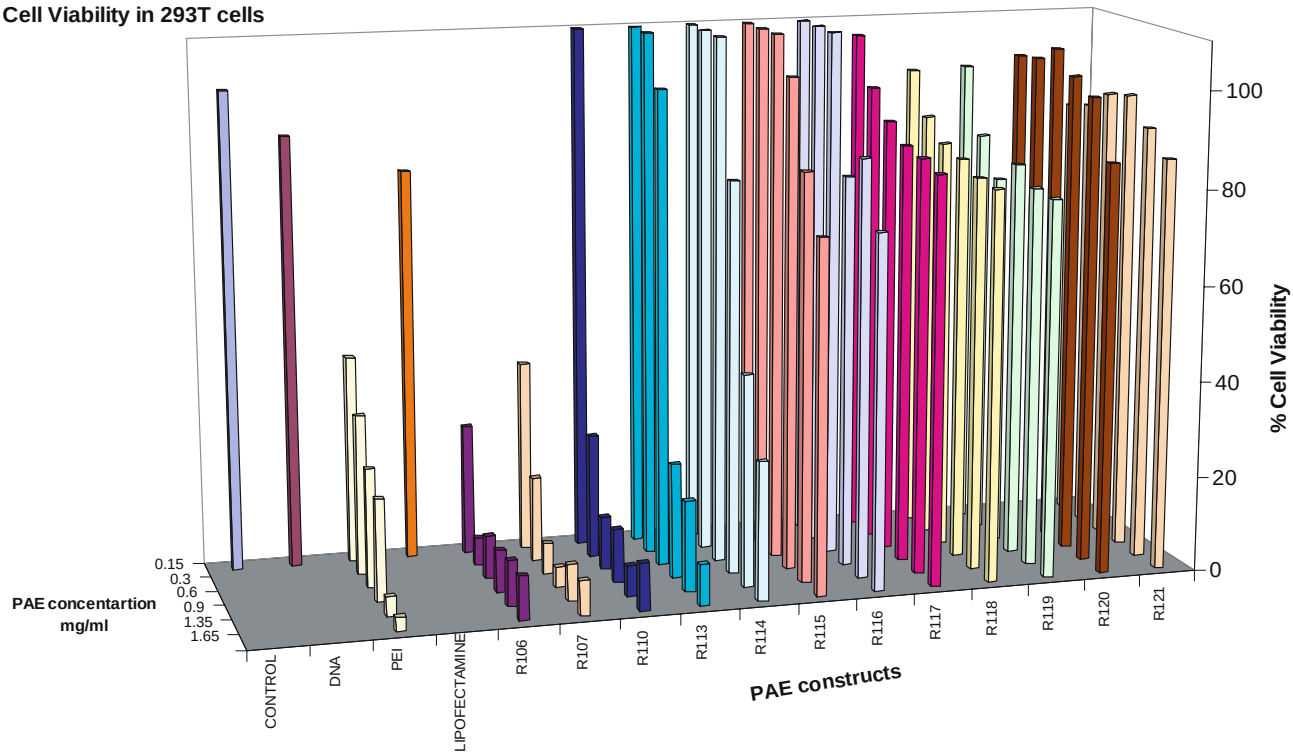

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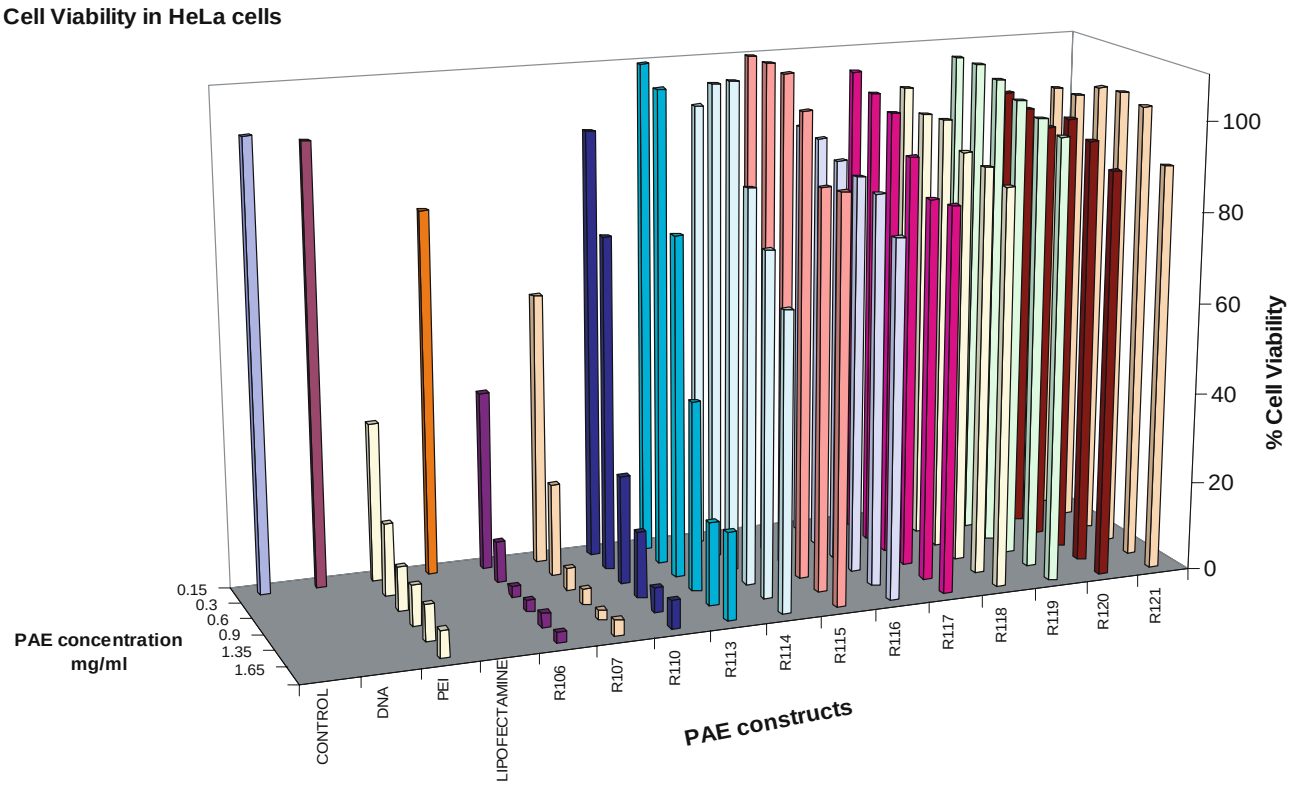

Fig. 5. Cytotoxicity of PAEs at various concentrations in **a** 293T cell line and **b** HeLa cell line.

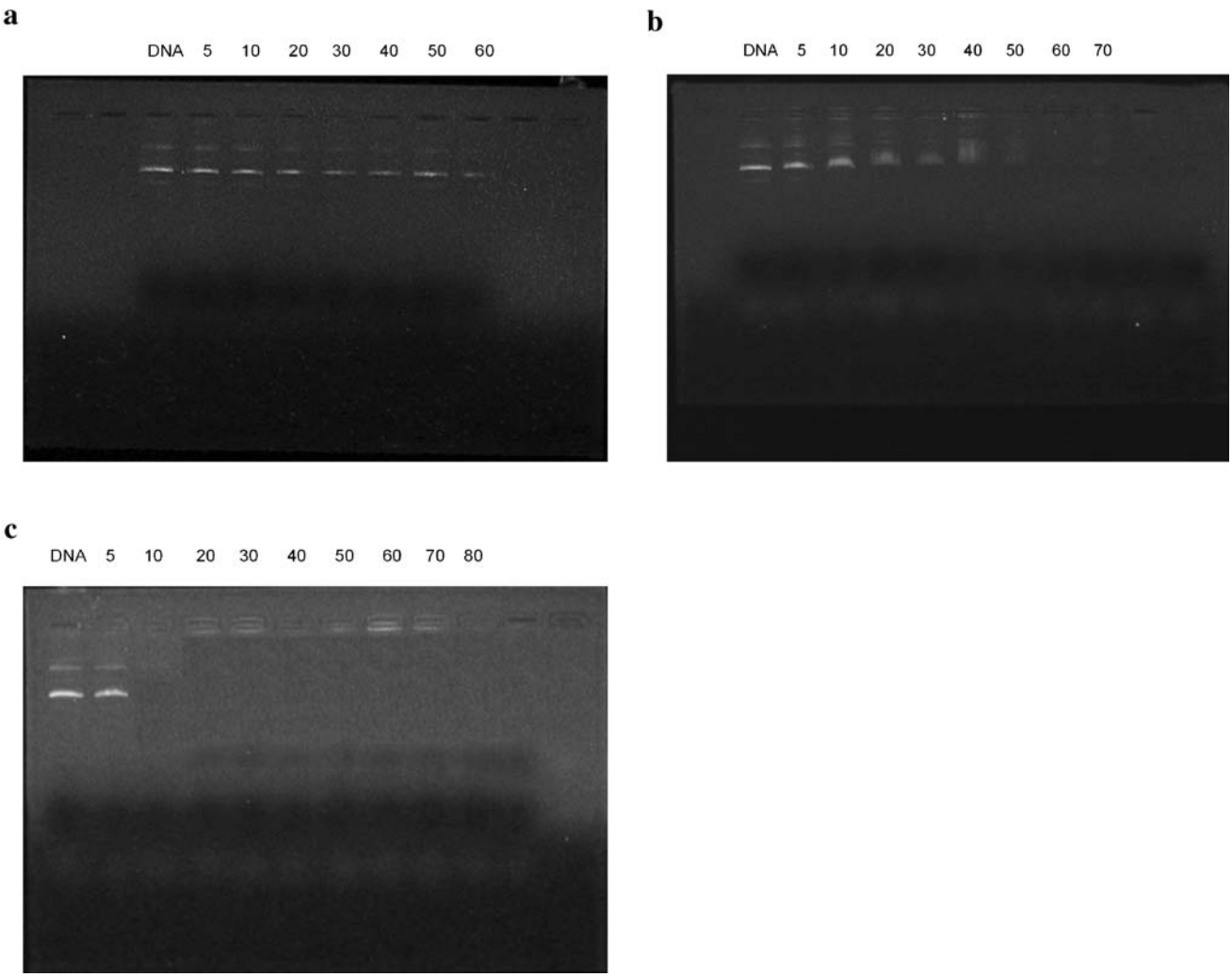

Fig. 6. Agarose gel electrophoresis of PAE/DNA complexes (pGL3 control); **a** R106, **b** R120 and **c** R121.

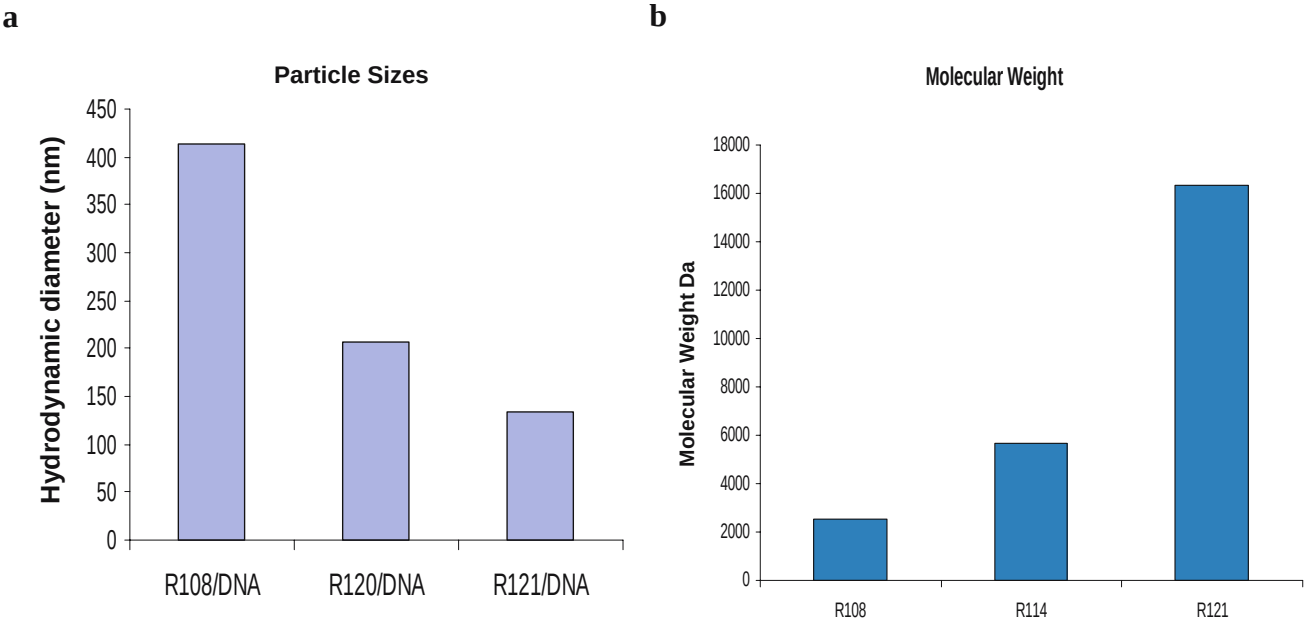

Fig. 7. **a** Particle sizes of three PAE/DNA complexes at weight ratio 110:1 and **b** molecular weight of three PAEs.

observed that PAE obtained from R106 was unable to bind and retard DNA efficiently even at 60:1 weight ratio and hence poorly transfect cells (Fig. 6a). Although PAE obtained from R120 showed significant transfection at 10:1 weight ratio, its DNA retardation ability was less at this ratio, however, with increase in weight ratios its DNA retardation ability was increased (Fig. 6b). PAE obtained from R121 showed good DNA complexation and retardation at different weight ratios ranging from 10:1 to 80:1 of PAE/DNA (Fig. 6c). Thus, gel retardation data clearly indicated that increase in amine monomer increased DNA binding and condensing ability of PAEs.

Particle sizes and molecular weight of PAE. In order to reach target cell and deliver plasmid inside cell, DNA carrying vector has to cross number of barriers. These barriers may vary from centimeter (in blood circulation) to nanometer size ranges (intracellular). The primary method of cellular entry for nanoparticles is by endocytosis. For non-receptor-mediated endocytic uptake, cationic polymers should have high molecular weight and should able to condense DNA in nanometer scale. For any non-viral vector these two properties are crucial in getting cellular entry via non-receptor-mediated endocytosis for high transfection. For efficient endocytosis and gene transfer, the complex must be small (less than 200 nm) and compact. It was observed that molecular weight of PAEs obtained from R106 to R121was increased from R106 to R121. Our best performed PAE obtained from R121 has molecular weight 16 KDa and showed good ability to condense DNA in nanometer range with average particle sizes of 133 nm while that of R120 polyplex is 203 nm. PAE with higher diacrylate content (R108) showed lower molecular weight with higher average particle sizes of 414 nm (Fig. 7).

CONCLUSIONS

Herein, we have demonstrated synthesis and screen of mini-library of novel PAEs composed of APES and PEGDA by using combinatorial chemistry and high throughput screening. Evaluation of this mini-library has revealed many important properties of PAEs which govern their transfection efficiency and safety. PAEs based on APES and PEGDA are efficient in transfecting 293T and HeLa cells in the presence of and absence of serum. However, transfection efficiency and safety of PAEs are highly sensitive to monomer ratio. Even small increase in APES monomer concentration has significantly increased transfection efficiency and cell viability. High transfection and cell viability can be achieved after optimum balance between monomer concentration and PAE/DNA weight ratio. However, transfection efficiency and safety of PAE are mainly governed by monomer ratio than PAE/DNA weight ratio. PAE obtained from R121 exhibited excellent cell viability and good transfection efficiency in 293T and HeLa cells in the presence of and absence of serum. Also it showed good DNA binding and condensing ability into nanometer scale. Thus, addition of PEGDA over APES resulted in a novel PAE which has high potential in gene delivery due to its degradability, DNA condensing ability, low cytotoxicity and high transfection efficiency.

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