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Supramolecular Host–Guest Pseudocomb Conjugates Composed of Multiple Star Polycations Tied Tunably with a Linear Polycation Backbone for Gene Transfection

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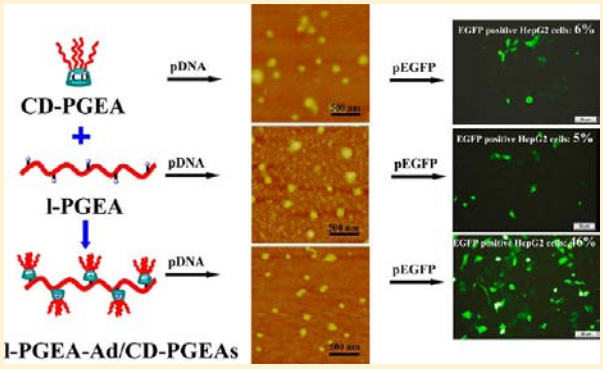
ABSTRACT: A series of novel supramolecular pseudocomb polycations (l-PGEA-Ad/CD-PGEAs) were synthesized by tying multiple low-molecular-weight β-cyclodextrin (CD)-cored, ethanolamine-functionalized poly(glycidyl methacrylate) (PGEA) star polymers (CD-PGEAs) with an adamantine-modified linear PGEA (l-PGEA-Ad) backbone via the host–guest interaction. The pseudocomb carriers were studied in terms of their DNA binding capabilities, cytotoxicities, and gene transfection efficiencies in the HepG2 and HEK293 cell lines. The pseudocomb l-PGEA-Ad/CD-PGEAs exhibited better plasmid DNA-condensing abilities than their counterparts, CD-PGEA and l-PGEA. Meanwhile, the pseudocomb carriers displayed low cytotoxicity, similar to CD-PGEA and l-PGEA. Moreover, the gene transfection efficiencies of the pseudocomb carriers were much higher than those of CD-PGEA and l-PGEA at various PGEA nitrogen/DNA phosphate molar ratios. Such supramolecular preparation of pseudocomb gene carriers could provide a flexible approach for adjusting the structure and functionality of supramolecular polymers via the proper use of non-covalent interactions.



INTRODUCTION

The development of safe and effective polycationic vectors is of crucial importance for gene therapy.[1] In comparison with viral vectors and cationic lipids, polycations as the major type of nonviral gene delivery vectors show low host immunogenicity and can be produced on a large scale. A large number of polycations, including polyethylenimine (PEI),[2] polyamidoamine,[3,4] chitosan,[5,6] and cyclodextrin (CD)-based cationic vectors,[7,8] have been reported to deliver nucleic acids. We found that ethanolamine (EA)-functionalized poly(glycidyl methacrylate) (PGEA) with plentiful flanking secondary amine and hydroxyl groups can produce good gene transfection efficiencies in some cell lines while exhibiting low toxicity.[9−11] More recently, high-molecular-weight comb-shaped PGEA gene carriers composed of a low-molecular-weight PGEA backbone and side chains were proposed by a combination of atom transfer radical polymerization (ATRP) and ring-opening reactions. [11]

Because of their dynamic tunability,[12−14] supramolecular polymers based on non-covalent interactions[15,16] have been increasingly explored as nonviral gene vectors in preclinical studies[17,18] and clinical trials.[19] Their easy self-assembly process and appropriate association strength in binding of conjugates provide significant benefits for functional drug and gene delivery with facile preparation and optimization of supramolecular carriers.[20−23] Among varieties of supramolecular structures, one typical model is the easily assembled and well-demonstrated molecular recognition system based on CD and adamantane (Ad).[8,14,17−20] The application of CD as the host component is particularly appealing because of its excellent biocompatibility, nonimmunogenicity, and low toxicity in animal and human bodies.[13,24,25] Furthermore, novel star gene carriers having CD cores have been developed by derivatizing the hydroxyl groups on the outside surfaces of the CDs to serve as initiation sites for the growth of cationic branches.[26−28] Star polymers consisting of β-CD cores and cationic poly(2-dimethylaminoethyl methacrylate) (PDMAEMA) arms can efficiently mediate gene transfection.[27] The effects of the number of arms and the arm length on gene delivery has also been investigated.[28] The transfection efficiency of star polycations with a suitable number of arms was found to depend on the high molecular weight, and the high cytotoxicity of PDMAEMA-based carriers limits their effective applications.

In this work, by the synergistic combination of the advantages of low-molecular-weight β-CD-cored PGEA (CD-PGEA) star polymers and the dynamic tunability of supramolecular polymers, we prepared a novel class of supramolecular pseudocomb PGEA gene carriers via the host–guest interaction between the CD-PGEA host and three different adamantine-modified linear PGEA (or l-PGEA-Ad) guests

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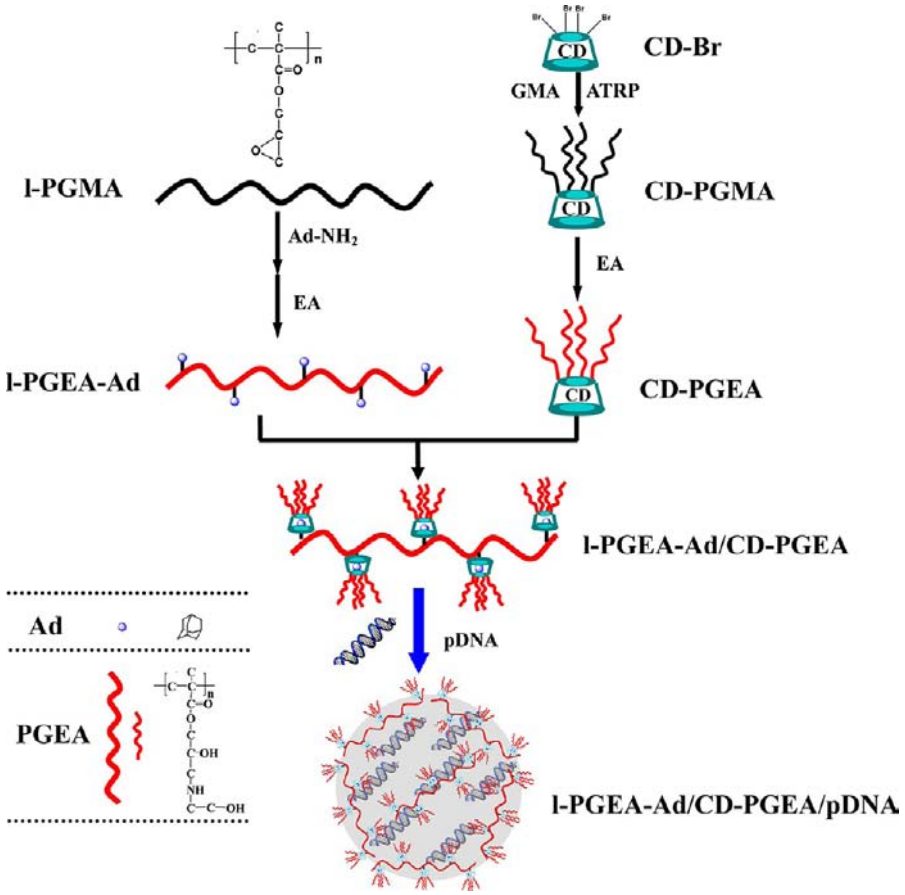

Figure 1. Schematic illustration of the preparation of l-PGEA-Ad/CD-PGEA/pDNA complexes.

(Figure 1). The supramolecular preparation of these pseudocomb l-PGEA-Ad/CD-PGEAs provides a flexible approach for adjusting the structure and functionality of supramolecular polymers using non-covalent interactions.

EXPERIMENTAL PROCEDURES

Materials. β-CD (99%), branched PEI ($M_w \approx 25$ kDa), EA (>98%), amantadine (Ad-NH_2, 98%), glycidyl methacrylate (GMA, >98%), *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA, 99%), 2-bromoisobutyryl bromide (BIBB, 98%), ethyl bromoisobutyrate (99%), and copper(I) bromide (CuBr, 99%) were obtained from Sigma-Aldrich (St. Louis, MO). GMA was used after removal of the inhibitors in a ready-to-use disposable inhibitor-removal column (Sigma-Aldrich). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), penicillin, and streptomycin were purchased from Sigma Chemical (St. Louis, MO). The HepG2 cell line was purchased from the American Type Culture Collection (ATCC, Rockville, MD).

Synthesis of l-PGMA and CD-PGMA via ATRP. Linear poly(GMA) (l-PGMA) was first prepared via ATRP of GMA as described in our previous publication.[9] The l-PGMA was synthesized using a [GMA (12 mL)]:[ethyl bromoisobutyrate]:[CuBr]:[PMDETA] molar feed ratio of 140:1:1:1.5 at 50 °C in 12 mL of tetrahydrofuran (THF). The reaction was performed in a 50 mL flask equipped with a magnetic stirrer under typical ATRP conditions. GMA, THF, ethyl bromoisobutyrate, and PMDETA were introduced into the flask, and the reaction mixture was degassed by bubbling argon for 20 min. Next, CuBr was added to the mixture, and the flask was then sealed with a rubber stopper under an argon atmosphere. The polymerization was allowed to proceed under continuous stirring at 50 °C to produce l-PGMA from 2 h of ATRP (M_n = 1.1 × 10^4 g/mol, PDI = 1.35). The reaction was stopped by dilution with THF, and the catalyst complex was removed by passing the blue dilute polymer solution through a short aluminum oxide column. After removal of THF in a rotary evaporator, PGMA was precipitated in excess *n*-hexane. The crude polymer was purified by reprecipitation twice in hexane to remove the reactant residues prior to being dried under reduced pressure.

As shown in Figure 1, in this work the target number of arms of the CD-cored star PGMA (CD-PGMA) was four. The starting bromoisobutyryl-terminated CD (CD-Br) with about four initiation sites was used. The detailed preparation procedures and characterization were described in our previous publication.[5] For the preparation of star CD-PGMA via ATRP, a [GMA (4 mL)]:[CuBr]:[PMDETA] molar feed ratio of 100:1:1.5 was used at room temperature in 6 mL of anhydrous *N*,*N*-dimethylformamide (DMF) containing 0.2 g of CD-Br. The reaction was conducted in a 25 mL flask equipped with a magnetic stirrer and under typical ATRP conditions.[5,9] CD-Br, PMDETA, and GMA were introduced into the flask containing 6 mL of anhydrous DMF, and the reaction mixture was degassed by bubbling nitrogen for 10 min. Next, CuBr was added to the mixture under a nitrogen atmosphere, and the flask was then sealed with a rubber stopper under a nitrogen atmosphere. The polymerization was allowed to proceed under continuous stirring at room temperature for 10 min (M_n = 8.4 × 10^3 g/mol, PDI = 1.32). The final reaction mixture was

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precipitated with excess methanol and washed with deionized water prior to lyophilization.

Preparation of l-PGEA-Ad/CD-PGEA Complexes. The introduction of adamantine (Ad) onto l-PGMA was performed via direct ring-opening reactions of the epoxy groups of l-PGMA with Ad-NH_2 to produce Ad-coupled l-PGMA (l-PGMA-Ad). Three l-PGMA-Ad polymers with different l-PGMA:Ad ratios were prepared by adjusting the l-PGMA:Ad-NH_2 feed ratio. With the predetermined epoxy:Ad molar feed ratio (2:1, 5:1, or 10:1), Ad-NH_2 (5.25, 2.10, or 1.05 mmol) in 3 mL of DMF was added dropwise at ambient temperature into the flask containing l-PGMA (containing 10.50 mmol of GMA units) in 10 mL of DMF. The reaction was allowed to proceed at 30 °C for 24 h to produce l-PGMA-Ad, which was directly prepared for the subsequent synthesis of l-PGEA-Ad. Different EA-functionalized PGMAs (PGEAs) were prepared by reacting l-PGMA, l-PGMA-Ad, and CD-PGMA with EA. The PGMA (0.3 g) was dissolved in 7 mL of DMF, and 5 mL of EA and 1 mL of triethyleneamine were then added. The reaction mixture was stirred at 37 °C for 5 days to produce the corresponding PGEA. The final reaction mixture was precipitated with excess diethyl ether, and the crude product was purified by dialysis against distilled deionized water (DDW) for 24 h using a dialysis membrane (molecular weight cutoff = 3500 Da) prior to lyophilization.

For the preparation of l-PGEA-Ad/CD-PGEA complexes, a 1:1 molar feed ratio of CD and Ad units was used. CD-PGEA (containing 0.05 mmol of CD units) in 10 mL of H_2O was added dropwise at ambient temperature into a flask containing l-PGEA-Ads (containing 0.05 mmol of Ad units) in 10 mL of H_2O. The mixture was stirred for 12 h, dialyzed in water for 24 h, and freeze-dried to yield l-PGEA-Ad/CD-PGEA.

Polymer Characterization. The molecular weights of the polymers were measured by gel-permeation chromatography (GPC), and their chemical structures were determined by NMR spectroscopy. GPC measurements of PGMAs were performed on a Waters GPC system equipped with Waters Styragel columns, a Waters 2487 dual-wavelength UV detector, and a Waters 2414 refractive index detector. THF was used as the eluent at a low flow rate of 0.5 mL/min at 25 °C. ^{1}H NMR spectra were measured by accumulation of 1000 scans with a relaxation time of 2 s on a Bruker ARX 300 MHz spectrometer using $CDCl_3$ (for PGMAs) or D_2O (for PGEAs) as the solvent. The chemical shifts were referred to the solvent peaks (7.20 ppm for $CDCl_3$ and 4.70 ppm for D_2O, respectively). Two-dimensional (2D) nuclear Overhauser effect NMR spectroscopy (NOESY) experiments were performed at 500 MHz in D_2O on a Bruker Avance DRX 500 MHz NMR spectrometer.

Characterization of Polycation/pDNA Complexes. The plasmid (encoding *Renilla* luciferase) mainly used in this work was pRL-CMV (Promega, Cergy Pontoise, France), which was cloned originally from the marine organism *Renilla reniformis*. The plasmid DNA (pDNA) was amplified in *Escherichia coli* and purified according to the supplier's protocol (Qiagen, Hilden, Germany). The purity and concentration of the purified DNA were determined by absorption at 260 and 280 nm and by agarose gel electrophoresis. The purified pDNA was resuspended in tris-EDTA (TE) buffer and kept in aliquots at a concentration of 0.5 mg/mL. All of the polycation stock solutions were prepared at a nitrogen concentration of 10 mM in distilled water. Solutions were filtered via sterile membranes (0.2 μm average pore size) and stored at 4 °C. Polycation:DNA ratios are expressed as molar ratios of nitrogen (N) in PGEAs to phosphate (P) in DNA (N/P ratios). An average molecular weight of 325 Da per phosphate group of DNA was assumed. All of the polycation/pDNA complexes were formed by mixing equal volumes of polycation and pDNA solutions to achieve the desired N/P ratio. Each mixture was vortexed and incubated for 30 min at room temperature. Each polycation was examined for its ability to bind pDNA through agarose gel electrophoresis using procedures similar to those described previously.[5] The particle sizes and ζ potentials of the polycation/pDNA complexes were measured using a Zetasizer Nano ZS (Malvern Instruments, Southborough, MA) using previously described procedures.[5] The polyplex morphology was visualized using an atomic force microscopy (AFM) system with a Dimension model 3100 microscope with a Nanoscope IIIa controller (Veeco, Santa Barbara, CA). The samples were imaged in tapping mode with setting of 512 pixels/line and a scan rate of 1 Hz. Image analysis was performed using Nanoscope software after removal of the background slope by flattening of the images.

Cell Viability. The cytotoxicities of the polycations were evaluated using the MTT assay in the HepG2 and HEK293 cell lines. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), penicillin (100 units/mL), and streptomycin (100 μg/mL) at 37 °C under 5% CO_2 at 95% relative humidity. The cells were seeded in a 96-well microtiter plate at a density of 10^4 cells/well and incubated in 100 μL of DMEM per well for 24 h. The culture media were replaced with fresh culture media containing 10 μL of polyplex solutions at various N/P ratios, and the cells were incubated for 24 h. Next, 10 μL of sterile-filtered MTT stock solution in phosphate-buffered saline (PBS) (5 mg/mL) was added to each well to give a final MTT concentration of 0.5 mg/mL. After 5 h, the unreacted dye was removed by aspiration. The produced formazan crystals were dissolved in dimethyl sulfoxide (100 μL/well), and the absorbance was measured using a Bio-Rad model 680 microplate reader at a wavelength of 570 nm. The cell viability relative to control cells cultured in medium without polycations was calculated as $(A_{test}/A_{control}) \times 100\%$, where A_{test} and $A_{control}$ are the absorbance values of the test wells (with polycations) and control wells (without polycations), respectively. For each sample, the final absorbance was the average of those measured from six wells in parallel.

Transfection Assay. Transfection assays were first performed using plasmid pRL-CMV as the reporter gene in the HepG2 and HEK293 cell lines in the presence of serum. In brief, the cells were seeded in 24-well plates at a density of 5×10^4 cells in 500 μL of medium per well and incubated for 24 h. The polycation/pDNA complexes (20 μL/well containing 1.0 μg of pDNA) at various N/P ratios were prepared by addition of the polycation into the DNA solution followed by vortexing and incubation for 30 min at room temperature. At the time of transfection, the medium in each well was replaced with 300 μL of fresh normal medium supplemented with 10% FBS. The complexes were added to the transfection medium and incubated with the cells for 4 h under standard incubator conditions. The medium was then replaced with 500 μL of fresh normal medium supplemented with 10% FBS. The cells were further incubated for an additional 20 h under the same conditions, resulting in a total transfection time of 24 h. The cultured cells were washed twice with PBS and lysed in 100 μL of cell-culture lysis reagent (Promega). Luciferase gene expression was quantified using a commercial kit (Promega)

and a Lumat LB 9507 luminometer (Berthold Technologies, Bad Wildbad, Germany). Protein concentrations in the cell samples were analyzed using a bicinchoninic acid assay (Biorad Lab, Hercules, CA). Gene expression results were expressed as relative light units (RLUs) per milligram of cell protein lysate (RLU/mg protein). PGEA-mediated gene transfection was also assessed at the optimal N/P ratio with plasmid pEGFP-N1 encoding enhanced green fluorescent protein (EGFP) (BD Biosciences, San Jose, CA) as the reporter gene in the HepG2 cell line using the same procedures as described above. The transfected cells were imaged using a Leica DM IL fluorescence microscope. The percentage of EGFP-positive cells was determined using flow cytometry (FCM) (Beckman Coulter).

RESULTS AND DISCUSSION

Synthesis and Characterization of l-PGEA-Ad/CD-PGEA Carriers. The syntheses of the l-PGEA-Ad guest and CD-PGEA host are illustrated in Figure 1. l-PGMA (M_n = 1.1 × 10^4 g/mol, PDI = 1.35) was first synthesized via ATRP.[9] Next, three adamantine-modified l-PGMAs (l-PGMA-Ads) with different epoxy:Ad ratios (2:1 for l-PGMA-Ad-1; 5:1 for l-PGMA-Ad-2; and 10:1 for l-PGMA-Ad-3) were obtained via ring-opening reactions with amantadine (Ad-NH_2). The low-molecular-weight β-CD-cored PGMA star polymer CD-PGMA (M_n = 8.4 × 10^3 g/mol, PDI = 1.32) was also prepared via ATRP. The obtained l-PGMA, l-PGMA-Ads, and CD-PGMA were reacted with EA to produce the corresponding l-PGEA, l-PGEA-Ads, and CD-PGEA vectors.

Typical ^{1}H NMR spectra of different PGMAs and PGEAs are shown and analyzed in detail in Figure 2. For l-PGMA and CD-PGMA (Figure 2A), the signals at 3.8 and 4.3 ppm correspond to the methylene protons adjacent to the oxygen moieties of the ester linkages (**a**, CH_2−O−C═O). The peaks at 3.2 ppm (**b**) and 2.63 and 2.84 ppm (**c**) can be assigned to the protons of the epoxide ring. The ratio of the areas of peaks **a**, **b**, and **c** were about 2:1:2, indicating that the epoxy groups of the PGMA remained intact throughout ATRP, consistent with the results of earlier reports.[9,11] For CD-PGMA, the chemical shifts in the 3.6−4.0 ppm region are mainly associated with the inner methylidyne and methylene protons of the glucose units of β-CD (**A**, C*H*−O and CH_2−O). Signal **A** associated with the CD core was very obvious because of the considerable contribution of CD to the overall star polymer structure. For l-PGEA, l-PGEA-Ad, and CD-PGEA (Figure 2B), peaks **b** and **c** associated with the epoxide rings of PGMA disappeared completely after the ring-opening reactions of PGMA with EA, and peaks **a** at 3.8 and 4.3 ppm shifted and combined to form a single peak at 3.95 ppm (**d**). The new peak at 3.7 ppm (**e**) is associated with the C*H*−OH methylidyne and O−CH_2 methylene protons. The strong peak at 2.7 ppm is mainly attributable to the methylene protons (**f**, NH−CH_2). The above results indicated that the oxirane rings of PGMA were opened by EA under the present reaction conditions. For l-PGEA-Ad [Figure 2B(b)], the obvious chemical shifts in the 1.6−2.0 ppm region are mainly associated with the inner methylidyne and methylene protons on the Ad units (**B**, C*H* and CH_2). Moreover, similar to CD-PGMA, the obvious signal **A** associated with the CD core was also observed for CD-PGEA [Figure 2B(c)].

To obtain the supramolecular l-PGEA-Ad/CD-PGEAs, the three l-PGEA-Ad guests were complexed with the CD-PGEA host in deionized water by host−guest interactions between the CD and Ad species. The typical NOESY spectra of the l-PGEA-Ad-1/CD-PGEA and l-PGEA-Ad-2/CD-PGEA complexes (Figure 3) show that the signals of the Ad protons are correlated with the signals of the inner protons of CD. This result demonstrated the successful complexation between the CD and Ad moieties. Furthermore, the much stronger correlated signals from l-PGEA-Ad-1/CD-PGEA indicated that the higher Ad content in l-PGEA-Ad-1 produced a greater number of CD-PGEAs tied to l-PGEA-Ad-1.

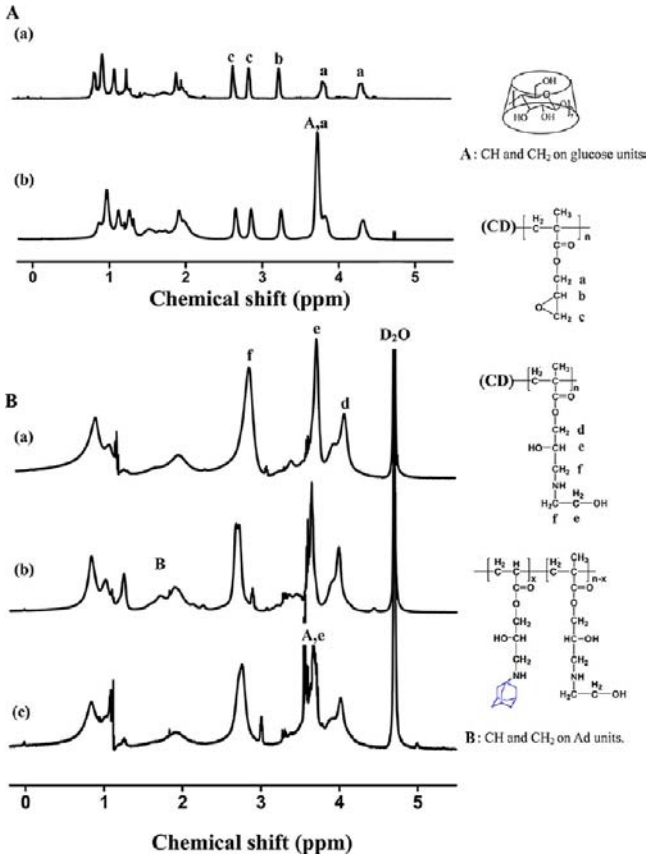

Figure 2. ^{1}H NMR spectra (300 MHz) of (A) l-PGMA (a) and CD-PGMA (b) in $CDCl_3$ and (B) l-PGEA (a), l-PGEA-Ad (b), and CD-PGEA (c) in D_2O.

Biophysical Characterization of Polycation/pDNA Complexes. The ability of l-PGEA-Ad/CD-PGEAs to condense pDNA into particulate structures was confirmed by agarose gel electrophoresis and particle size and ζ potential measurements. Figure 4 shows the gel retardation results for polycation/pDNA complexes with increasing N/P ratios. All of the l-PGEA-Ad/CD-PGEAs can compact pDNA completely within the N/P ratio of 1.5, while CD-PGEA and l-PGEA just condensed pDNA when the N/P ratio was up to 2.5. The above results indicated that the pDNA-condensing ability of l-PGEA-Ad/CD-PGEAs is better than those of CD-PGEA and l-PGEA alone, probably as a result of the high-molecular-weight supramolecular pseudocomb structure of the l-PGEA-Ad/CD-PGEAs. The (a) particle sizes and (b) ζ potentials of the polycation/pDNA complexes at various N/P ratios are shown in Figure 5. All of the polycations can efficiently compact pDNA into small nanoparticles. The particle size decreased with increasing N/P ratio. The surface charge of the complex became positive upon the complete self-assembly of polycations and pDNA. The positive net surface charge should produce good affinity for anionic cell surfaces and facilitate cell uptake.

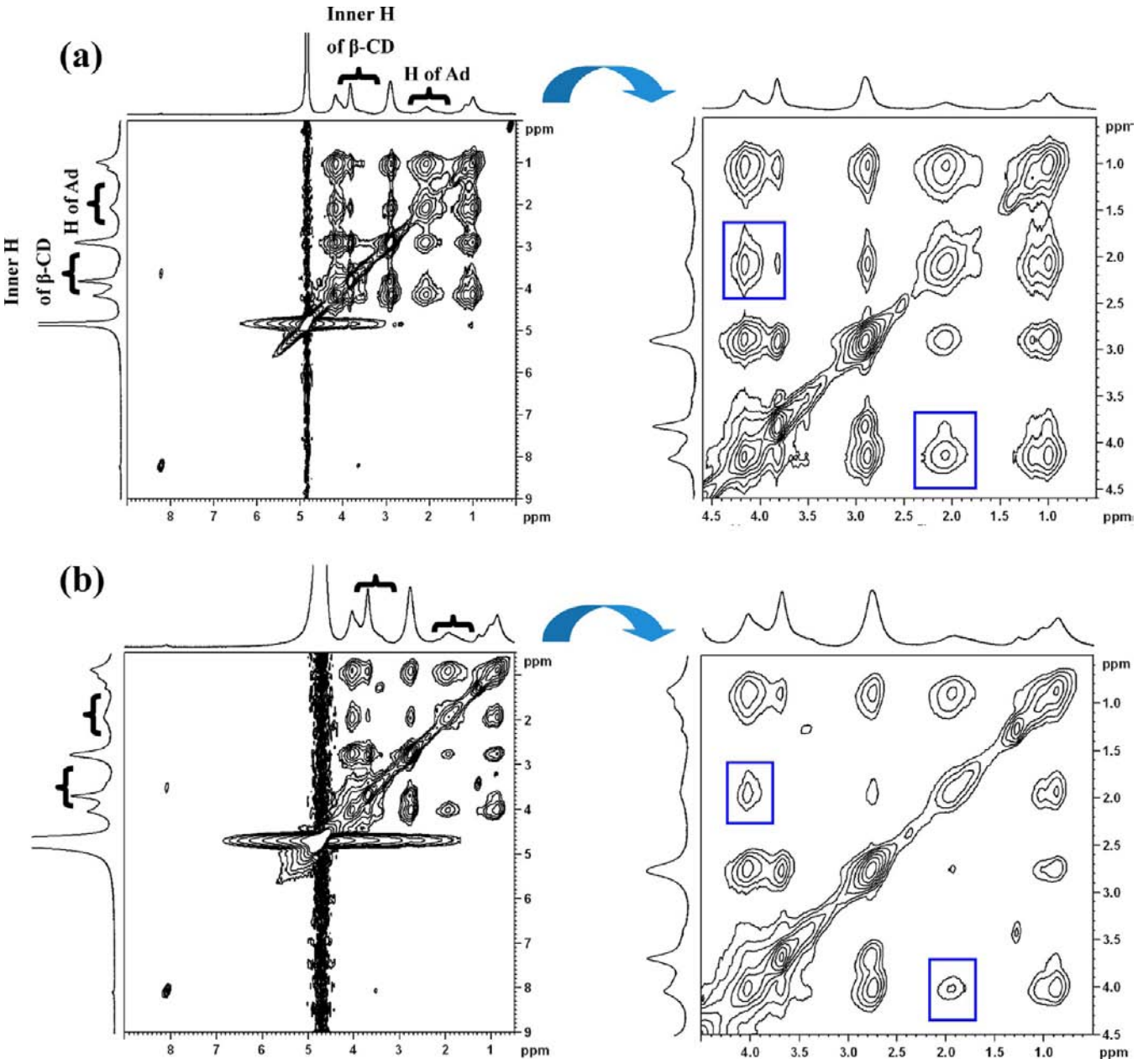

Figure 3. 2D NOESY ^{1}H NMR spectra of the (a) l-PGEA-Ad-1/CD-PGEA and (b) l-PGEA-Ad-2/CD-PGEA complexes.

Figure 6 shows representative AFM images of the (a) CD-PGEA/pDNA, (b) l-PGEA/pDNA, and (c) l-PGEA-Ad-2/CD-PGEA/pDNA complexes at N/P ratios of 15. The images clearly reveal that the compacted complexes exist uniformly in the form of nanoparticles.

Cell Viability Assay. The cell viability of the polycation/pDNA complexes as a function of N/P ratio was evaluated in HepG2 and HEK293 cells by MTT assay (Figure 7). In comparison with the control PEI/pDNA complexes, low-molecular-weight CD-PGEA and l-PGEA exhibited much lower cytotoxicities. The l-PGEA-Ad/CD-PGEAs showed the expected low cytoxicity, similar to that of CD-PGEA. It is well-known that the cytotoxicity of polycations increases with molecular weight.[29] The above results indicated that the supramolecular pseudocomb polymers can achieve low/compromised toxicity despite their high molecular weights.

In Vitro Gene Transfection Assay. The in vitro gene transfection efficiencies of the polycation/pDNA complexes were first assessed using luciferase as a gene reporter in HepG2 and HEK293 cells in complete serum media (Figure 8). The transfection efficiency generally first increased at lower N/P ratios and then decreased slightly with increasing N/P ratio. At lower N/P ratios, pDNA could not be condensed efficiently by the polycations, while the presence of increasing concentrations of free polycations at higher N/P ratios would result in increasing cytotoxicity. l-PGEA-Ad/CD-PGEAs exhibited gene transfection efficiencies much higher than that of PEI (25 kDa) at its optimal N/P ratio of 10. This observation demonstrated that the supramolecular pseudocomb polycations possessed great transfection ability. As expected, l-PGEA-Ad/CD-PGEAs exhibited significantly higher gene transfection efficiencies than CD-PGEA and l-PGEA at various N/P ratios. This may be attributed to the better pDNA-condensing abilities of the

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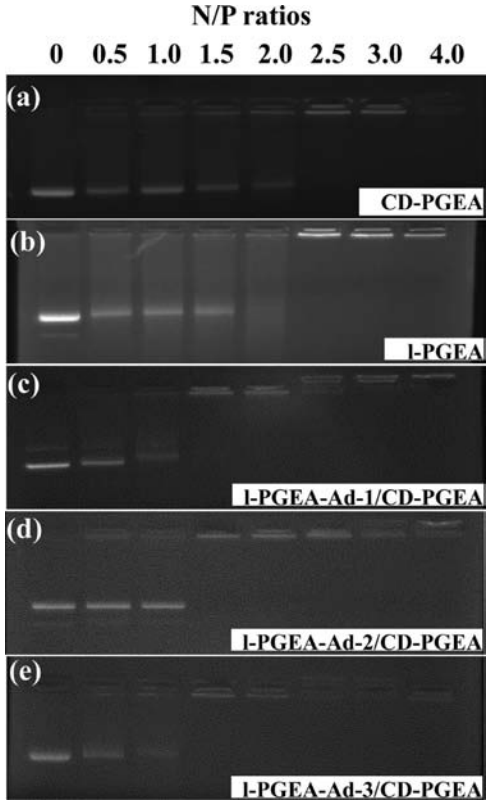

Figure 4. Electrophoretic mobility of pDNA in complexes with the PGEA polycations at various N/P ratios.

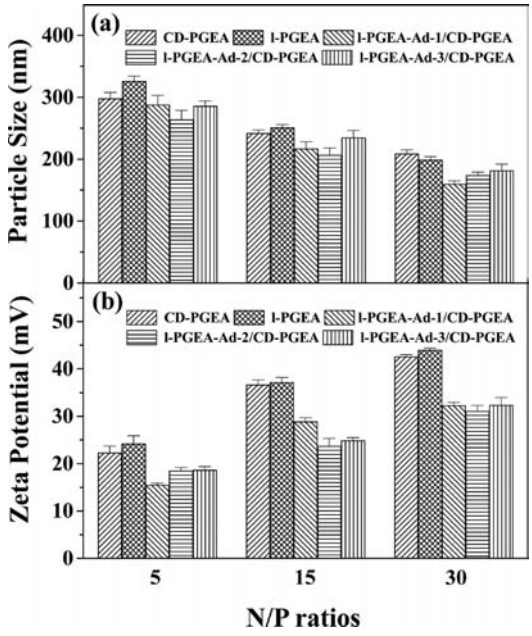

Figure 5. (a) Particle sizes and (b) ζ potentials of the PGEA polycation/pDNA complexes at various N/P ratios (mean ± SD, n = 3).

supramolecular carriers in comparison with CD-PGEA and l-PGEA and their similar low toxicities. In addition, the transfection efficiency mediated by l-PGEA-Ad-2/CD-PGEA was much higher than those mediated by the other l-PGEA-Ad/CD-PGEAs at various N/P ratios. Compared with l-PGEA-Ad-3/CD-PGEA, l-PGEA-Ad-2/CD-PGEA with higher amount

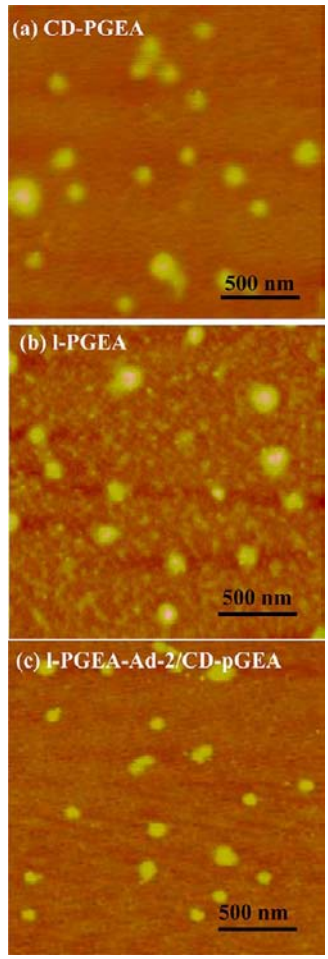

Figure 6. AFM images of (a) CD-PGEA/pDNA, (b) l-PGEA/pDNA, and (c) l-PGEA-Ad-2/CD-PGEA/pDNA complexes at N/P ratios of 15.

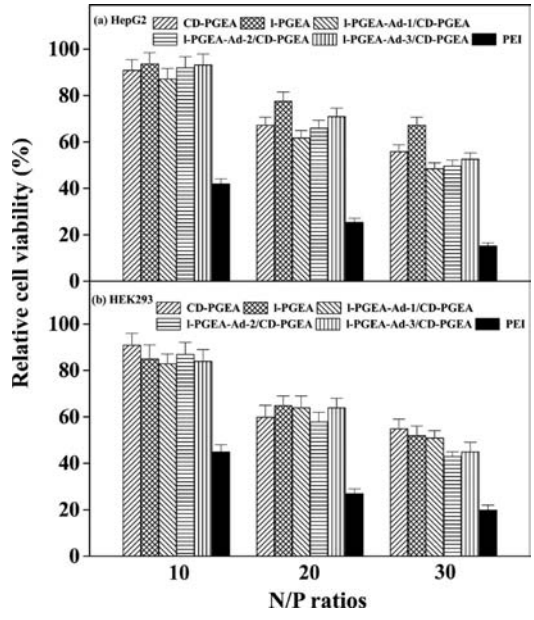

Figure 7. Cell viabilities of the polycation/pDNA complexes in comparison with those of PEI (25 kDa) at different N/P ratios in the (a) HepG2 and (b) HEK293 cell lines.

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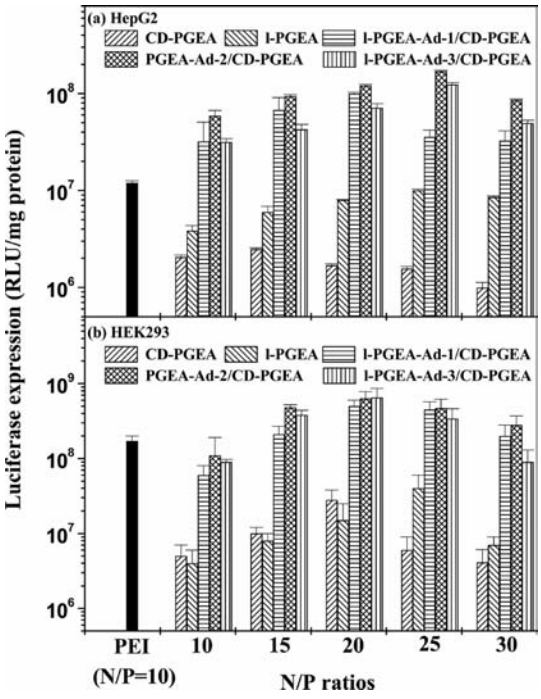

Figure 8. In vitro gene transfection efficiencies of the l-PGEA-Ad/CD-PGEA/pDNA complexes at various N/P ratios in comparison with that mediated by PEI (25 kDa) at its optimal N/P ratio of 10 (mean ± SD, n = 3) in (a) HepG2 and (b) HEK293 cells.

of tied CD-PGEA would have increased binding ability and complex stability, probably leading to the higher transfection efficiency. On the other hand, the excess Ad content of l-PGEA-Ad-1/CD-PGEA resulted in a dense layer of CD-PGEAs tied to the l-PGEA backbone, reducing the flexibility of the l-PGEA-Ad-1/CD-PGEA vector and leading to the lower transfection ability. The above results indicated that l-PGEA-Ad/CD-PGEAs with a suitable amount of CD-PGEA possess the best gene transfection ability.

In an attempt to confirm the gene delivery capability of l-PGEA-Ad/CD-PGEAs, direct visualization of gene expression of EGFP in HepG2 cells was also performed using fluorescence microscopy. Representative images of EGFP gene expression mediated by vectors at their optimal N/P ratios are shown in Figure 9. The fluorescence signals obtained with delivery of plasmid EGFP mediated by l-PGEA-Ad-2/CD-PGEA were significantly stronger than those obtained with CD-PGEA and l-PGEA. The optimal percentages (determined using flow cytometry[5]) of the EGFP-positive HepG2 cells for l-PGEA-Ad-2/CD-PGEA, CD-PGEA, and l-PGEA were 46%, 6%, and 5%, respectively. The above results suggested that the HepG2 cells treated with l-PGEA-Ad-2/CD-PGEA/pEGFP complexes showed much higher expression levels, consistent with the results of luciferase expression (Figure 8a). In addition to the HepG2 cell lines, the l-PGEA-Ad/CD-PGEAs also have great ability to transfect other cell lines, including HEK293 cells.

CONCLUSIONS

Novel supramolecular pseudocomb l-PGEA-Ad/CD-PGEA polycations composed of multiple CD-PGEAs tied tunably to the l-PGEA backbone were successfully prepared for highly efficient gene delivery. The l-PGEA-Ad/CD-PGEAs possessed the favorable properties of novel CD-cored star polymers, low-toxicity PGEA, and supramolecular polymers. Such pseudo-

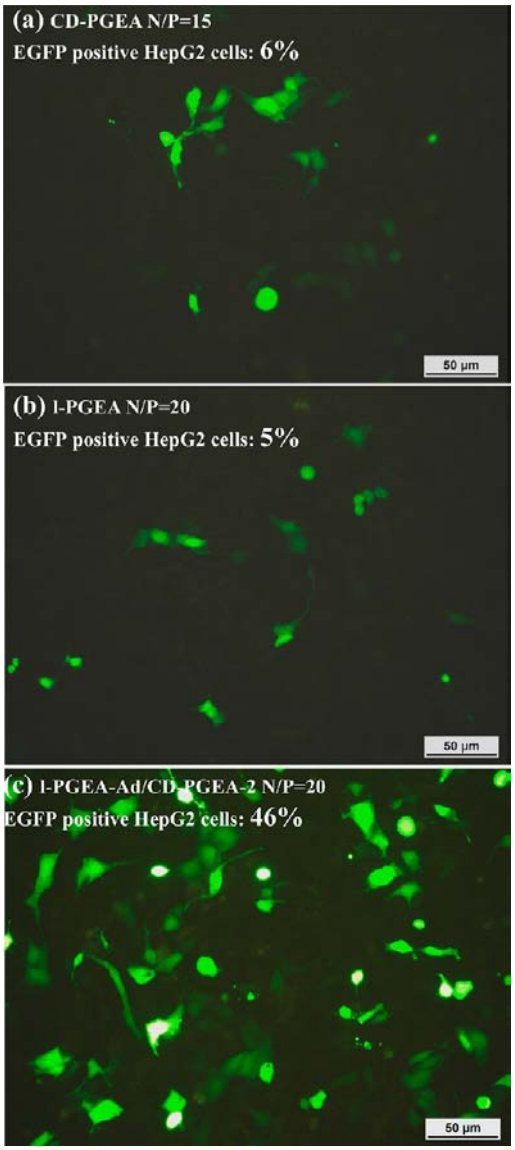

Figure 9. Representative images of EGFP expression mediated by (a) CD-PGEA, (b) l-PGEA, and (c) l-PGEA-Ad-2/CD-PGEA at their corresponding optimal N/P ratios in HepG2 cells.

comb PGEA gene carriers exhibited good ability to complex pDNA and enhanced gene transfection efficiencies. Furthermore, the flexible host−guest complexation of Ad-containing backbones (with tunable tying sites) and CD-cored star polymers with tunable polymer arms could be used to construct a wide class of supramolecular pseudocomb polymers. Thus, the present work demonstrates that the facile preparation of supramolecular pseudocomb polycations by properly tying CD-cored star polycations to a cationic low-toxicity derivatized PGMA backbone provides a new strategy for designing and developing new gene delivery systems.

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Notes

The authors declare no competing financial interest.

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