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ARTICLE TYPE

A well-defined coil-comb polycationic brush with "star polymer" as side chains for gene delivery†

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Novel well-defined coil-comb polycationic brushes were synthesized *via* atom transfer radical polymerization and used as gene carriers. The side chains of the comb block were composed of star polymers with cyclodextrin as the core and poly(2-dimethylaminoethyl methacrylate) (PDMAEMA) as the arm. The DNA binding capability of the brushes was characterized, and the cytotoxicity and transfection efficiency were investigated in COS-7 and 293T cells. With such super-high grafting density of PDMAEMA, the brushes effectively condensed pDNA into spherical nanoparticles of 100-200 nm in size and exhibited significantly higher transfection capability compared to the single star polymer. In some cases, they also showed comparable or higher transfection efficiency and lower cytotoxicity than PEI25K. The results indicated these brushes had good promise for the potential gene therapy. Actually, this work also provides a versatile method to prepare polymer brushes with such unique structure and flexible functionality, which has wide applications in the fields of biomedical devices, electronics and catalysts.

Introduction

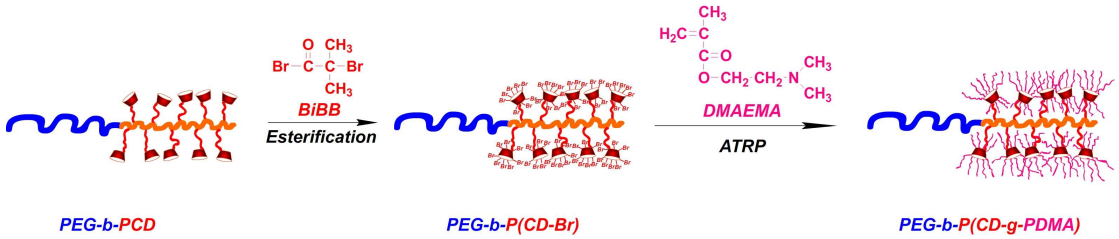
Gene therapy provides a promising paradigm for the treatment of various genetic diseases, cancers, viral infection, and cardiovascular disorders[1-5]. Development of efficient delivery vehicles and methods is the key challenge in realizing the gene therapy. Viral gene vectors achieved high protein expression or transfection efficiency, but suffered from immunogenicity, low loading capacity, and challenge of scale-up[6, 7]. Alternatively, non-viral vectors with enhanced biosafety and biocompatibility are receiving increasing attention, because they offer a high flexibility regarding the size of the delivered nucleic acid and can be synthesized in large quantities at low cost. A great number of polycations have been reported to be capable of delivering genes. These materials include polyethylenimine (PEI)[8], poly((2-dimethyl amino)ethyl methacrylate) (PDMAEMA)[9], poly(*L*-lysine) (PLL)[10] or poly(*L*-histidine)[11], polyamidoamine (PAMAM)[12], chitosan[13], and other polysaccharide-based cationic carriers[14-16].

Low cytotoxicity and high transfection efficiency are the most challenging tasks in the design of gene delivery vectors. Polymer brushes represent a new class of materials with interesting properties which attract considerable attention in recent years. They are defined as dense layers of chains tethered to a surface or molecule that the distance between neighboring grafts is less than twice of the gyration radius (R_g) of a free polymer chain in solution[17, 18]. With such high grafting density, the polymer chains are usually forced to stretch out from the surface due to the geometrical constraint. During the past decade, polymer brushes have inspired many interesting developments in the fields of electronics[19, 20], catalysts[21], and biomedical devices. Polycationic brushes consisting of densely grafted cationic chains can produce remarkable condensing capability to the oppositely charged polymer, which might effectively reduce the working concentration. Besides, the high osmotic repulsion within the brush layer can also enhance the colloidal stability. When used as the gene delivery vectors, they tend to increase the condense capability, cellular uptake and transfection efficiency with lower cytotoxicity simultaneously[22-28]. For example, Huang and coworkers synthesized monodispersed brush-like polymers and introduced it into siRNA delivery system. This system acquired high gene silence efficiency and good biocompatibility[29]. Liu and coworkers synthesized a cationic polymer brush onto nanodiamond (ND-brushes). The ND-brushes could not only efficiently deliver plasmids into COS-7 cells, but also mediate higher expression than PEI25k with lower cytotoxicity[30]. Xu and coworkers prepared several well-defined PDMAEMA chains onto natural polysaccharides. These obtained copolymers showed capable of mediating much more efficient gene transfection and much low cytotoxicity in comparison with high-molecular-weight PDMAEMA[31-33].

Star-shaped polycations represent a type of interesting non-viral gene carriers due to their dense molecular architecture with moderate flexibility[34-36]. Star polymers consisting of cyclodextrin (CD) cores and polycationic arms can not only efficiently mediate gene transfection[33,37-40], but also co-delivery hydrophobic drug *via* the inclusion interaction[41] due to the unique properties of CDs[42]. Combination of the advantages of both brush-like

polycations and CD-based star polycations may provide a new strategy for the design of non-viral gene delivery vectors.

In this paper, a novel kind of well-defined coil-comb polycationic brush was synthesized by atom transfer radical polymerization and used as gene carriers (Scheme 1). The side chains of the comb block of the copolymer were composed of star polymers with cyclodextrin as the core and PDMAEMA as the arms. Using such "grafting from" approach, super-high grafting density of PDMAEMA chains with well-defined compositions was achieved. In comparison with the single CD-based star polymer, the newly designed polycationic brushes exhibited significantly better capability to deliver pDNA to COS-7 and 293T cells and achieved very high levels of gene expression even at low N/P ratios. Notably, in some cases, they showed comparable or higher transfection efficiency and lower cytotoxicity than PEI25K.



Scheme 1 Synthesis of the coil-comb polycationic brushes *via* ATRP.

Experimental

Materials

2-(Dimethylamino) ethyl methacrylate (DMAEMA, Aldrich, 98%) was purified by passing through a basic alumina column. β-cyclodextrin (β-CD, Sinopharm Chemical Regent Co.,Ltd) was recrystallized twice from water and dried in vacuum at 80 °C overnight. CuCl (Tianjin, P. R. China, AR) was dissolved in concentrated HCl, precipitated by dilution with water, washed with ethanol and ethyl ether for three times, and then dried under vacuum. $CuCl_2$ (Tianjin, P. R. China, AR) was baked at 120 °C to remove the crystal water. 2-Bromoisobutyryl bromide (BiBB, Aldrich, 98%), *N,N,N′,N″,N″*-pentamethyldiethylenetriamine (PMDETA, Aldrich, 99%), bipyridine (bpy) (Sinopharm Chemical Reagent Co., Ltd), and anhydrous *N*-methyl-2-pyrrolidone (NMP, Alfa, 99.5%) were used without further purification. Dulbecco's modified Eagle medium (DMEM) and fetal bovine serum (FBS) were obtained from Thermo Scientific. Phosphate buffered saline (PBS) and trypsine-EDTA solution were bought from Solarbio Technology Co., Ltd. The plasmids used in this study were pGL3-control (Promega, USA) encoding luciferase and pEGFP-C1 (Promega, USA) encoding enhanced green fluorescence protein (EGFP). All plasmid DNAs were amplified in *Escherichia coil* and purified according to Endo Free Plasmid Kit (Tiangen). COS-7 and 293T cells were gifts from Nankai University. All other chemicals were purchased from Tianjin Jiangtian Chemical Technology Co.,Ltd and used as received. The preparation of PEG-*b*-PCD block copolymer and the control single star polymer (CD-*g*-PDMA) are described in Electronic Supplementary Information (ESI). The molecular strucutures of each chemicals is also displayed in ESI (Scheme S2†).

Synthesis of dibolck copolymer initiator PEG-*b*-P(CD-Br)

PEG-*b*-PCD (0.012 mmol, 0.247 g) was dissolved in dry NMP and cooled to 0°C. BiBB (13.4 mmol, 1.66 mL) diluted in NMP was added dropwise. The reaction mixture was stirred at 0°C for 4 h and then at 25°C for an additional 48 h. After completion of stirring, the mixture was transferred to a dialysis bag (MWCO 7000) and further purified by dialysis against sodium bicarbonate aqueous solution and distilled water, respectively. The hydrophobic product was recovered by filtration and then dried under vacuum.

Synthesis of coil-comb polycationic brushes PEG-*b*-P(CD-*g*-PDMA) *via* ATRP

The coil-comb polycationic brushes were synthesized by ATRP of DMAEMA from PEG-*b*-P(CD-Br) initiator. Typically, CuCl (0.784 mmol, 7.7 mg) and bpy (0.157 mmol, 24.5 mg) were dissolved in 2 mL of acetone/water (95/5, v/v) in a two-neck flask and degassed with three freeze-pump-thaw cycles. A solution of PEG-*b*-P(CD-Br) (0.0005 mmol, containing 0.784 mmol of initiator sites, 22.0 mg) and DMAEMA (4.704 mmol, 0.793 mL) in 3 mL of acetone/water (95/5, v/v) was added into the solution through a syringe under Ar atmosphere, and the mixture was then degassed with another two freeze-pump-thaw cycles. The reaction mixture was stirred at 35°C for 20 h and then purified by dialysis against distilled water for 2 days with 10 exchanges.

Characterization

^{1}H NMR analysis was carried out on a Varian UNITY-plus 400M spectrometer. The carbon and hydrogen element analysis was performed on Elementar Vario ELIII automatic elemental analyzer. The bromine content was measured by oxygen flask combustion-mercuric nitrate titration method. The absolute molecular weights and molecular weight distributions of the polymers were determined by gel permeation chromatograph (GPC) equipped with a Hitachi L-2130 HPLC pump, a Hitachi L-2490 refractive index detector and a Malvern multiple detector. DMF with 0.01 M of LiBr was used as eluent at a flow rate of 1.0 mL min^{-1}. Polymer solutions were injected through three Shodex KD-800 columns with 5000K-5K (100000 Å), 40K-500 and 5K-500 molecular ranges at 50°C. The sizes of polymer/pDNA polyplexes were measured by a Malvern Zetasizer Nano ZS instrument at 25°C. Atomic force microscopy (AFM) images were collected on a Nanoscope V atomic force microscope (Digital Instruments Inc.)

Preparation of Polymer/plasmid DNA polyplexes

Polycationic brushes were dissolved in MQ water with a concentration of 5 mg mL^{-1}, filtered by sterile membranes (0.22

μm) and stored at 4°C. The plasmid DNA was resuspended in *tris*-EDTA (TE) buffer and kept in aliquots of 0.5 mg mL^{-1} in concentration. Polymer and pDNA solutions were diluted in PBS solution (1 mM, pH 6.0) individually prior to mixed together to obtain polymer/pDNA polyplexes at various N/P ratios. Polyplexes were formed by slowly dropping polymer solution into an equal volume of pDNA solution. The mixed solution was vortexed and incubated at room temperature for 30 min. Polyplexes were freshly prepared before each individual experiment.

Gel Retardation Analysis

Polymer/pDNA polyplex (10 μL, containing 0.2 μg of pDNA) was mixed with 2 μL of loading buffer and loaded on a 0.8% agarose gel containing SYBR Green. Electrophoresis was performed in *tris*-acetate (TAE) running buffer at a voltage of 120 V for 0.5 h on a GE HE 33 system. DNA bands were visualized and photographed on GE LAS 4500 system.

Cell viability

COS-7 and 293T cells were seeded in 96-well plates at a density of 7000 cells/well and 15000 cells/well, respectively. The cells were cultured in DMEM, supplemented with 10% FBS at 37°C, under 5% CO_2. After incubated for 24 h, the culture media were replaced with fresh media containing polymer with concentrations ranging from 5 to 300 μg mL^{-1}. The media were removed after 24 h，and then 10 μL of CCK-8 regents (Dojindo Laboratories) and 90 μL fresh media were added to each well. After 2 h, the plate was gently shaken for 2 min to dissolve formazan crystals. The absorbance was measured using a multifunctional ELISA plate reader (Thermo Varioskan Flash) at 450 nm. All experiments were carried out in triplicate. Cell viability was calculated by equation (1):

$$\text{Cell viability (\%)} = \frac{A_{Sample} - A_{Blank}}{A_{Control} - A_{Blank}} \times 100\% \quad (1)$$

Where A_{Sample} and $A_{Control}$ represent the absorbance of CCK-8 regents determined for cells treated with different samples and for control cells (untreated), respectively. A_{Blank} is the absorbance of CCK-8 regents without cells.

The cytotoxicity of polymer/pDNA polyplexes was evaluated by the similar procedures. The cells were incubated in 96-well plates for 24 h, and then the media were replaced with serum-free media containing 8 μL polyplexes solution with varying N/P ratios. The cells were incubated at 37°C for 4 h. Afterwards, the media were replaced with fresh DMEM containing 10% FBS and incubated for additional 44 h. The cell viability was determined as described above.

***In vitro* transfection assay**

COS-7 and 293T cells were seeded in 24-well plates in 500 μL of DMEM supplemented with 10% FBS at a density of 4×10^4 cells/well and 8×10^4 cells/well, respectively. The cells were incubated at 37°C under 5% CO_2 for 24 h. At the time of transfection, the medium in each well was replaced by 500 μL of serum-free medium containing polyplexes solutions with varying N/P ratios. After 4 h, the transfection medium was replaced with fresh DMEM containing 10% FBS. Following an additional 44 h, the transfected cells were observed under inverted fluorescence microscope (Leica) for EGFP fluorescence visualization or subjected to luciferase activity assay. For luciferase activity assay, the cells were washed with PBS twice and then lysed in 150 μL of reporter lysis buffer for 20 min followed by freeze-thaw cycles to ensure complete lysis. The cell lysate was centrifugated at 12000 rpm at room temperature for 1 min. The supernatant was collected and the relative luciferase light units (RLU) were measured on a multifunctional ELISA plate reader (Thermo Varioskan Flash) using luciferase assay kit (Promega). The results were normalized to the total protein content using the bicinchoninic acid (BCA) protein assay (Thermo Scientific).

Statistical analysis

All data were presented as mean±standard deviation. The results were compared using one-way ANOVA, and differences were considered to be significant when $p<0.05$.

Results and discussion

Synthesis and characterization of coil-comb polycationic brushes *via* ATRP

PEG-*b*-PCD diblock copolymer was firstly synthesized by ATRP of methylacrylate-substituted CD monomer (MCD) from PEG-Br macroinitiators. ^{1}H NMR result (Fig. 1a) shows that the signal at 4.8 ppm (signal *C1-H*) corresponds to the protons on C1 in CD moieties. The average degree of polymerization (DP_n) of the MCD calculated from the integral ratio of signal *C1-H* to signal *G* is about 11. According to the GPC results, the molecular weight (M_n) of the block copolymer is 27898 and the polydispersity (PDI) is 1.17. The CD moieties were then reacted with 2-bromoisobutyrly bromide. ^{1}H NMR spectrum in Fig. 1b shows that the signal *d* at 1.9 ppm represents the methyl protons ($(C\underline{H}_3)_2$-C(Br)-) of the 2-bromoisobutyryl groups. The initiator site (Br) number calculated from ^{1}H NMR result (Fig. 1b) is 143 per chain (13 per CD), which is consistent to the result measured by elementary analysis (132 per chain (12 per CD)). GPC results display that the M_n and PDI of the initiator block copolymer are 79260 and 1.40, respectively. After esterification, the pendent hydrophilic CD moieties were changed to hydrophobic initiator cores.

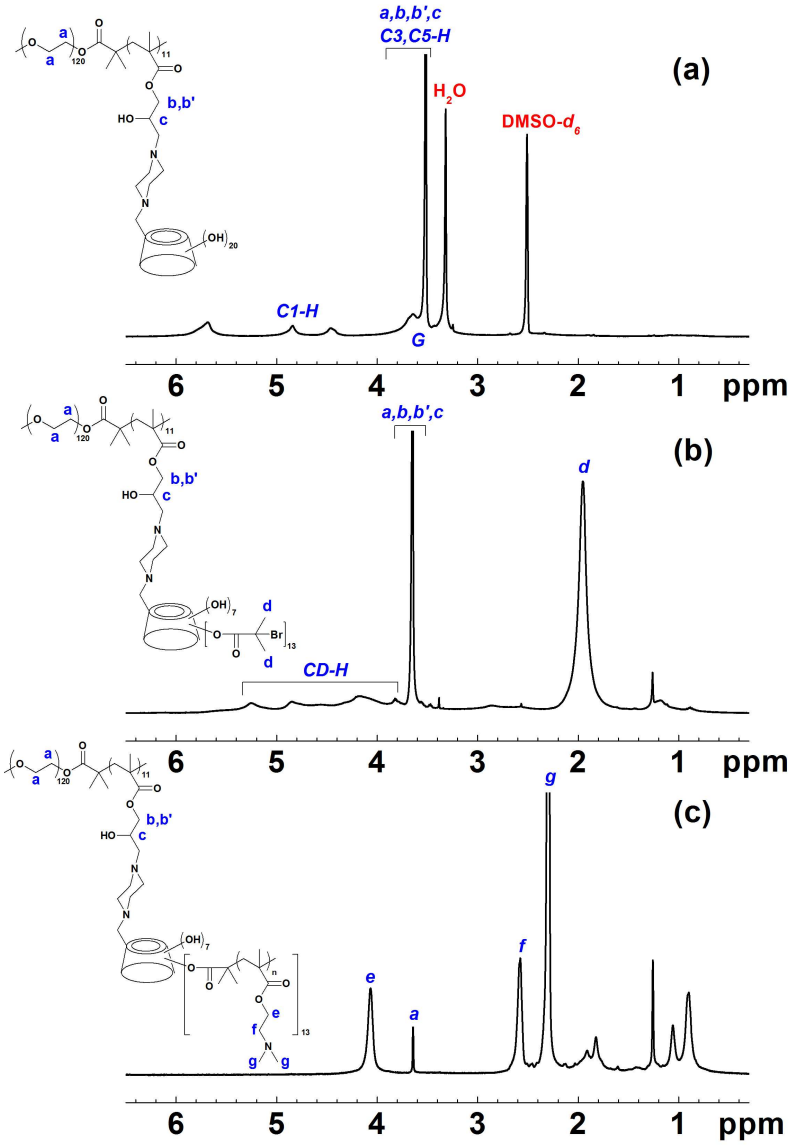


Fig. 1 ^{1}H NMR spectra of PEG-*b*-PCD diblock copolymer in DMSO-d_6, PEG-*b*-P(CD-Br) macroinitiator in $CDCl_3$ and PEG-*b*-P(CD-*g*-PDMA) coil-comb polycationic brush in $CDCl_3$.

Using CuCl/bpy as the catalyst and acetone/water as the solvent, ATRP of DMAEMA was conducted from PEG-*b*-P(CD-Br) initiator block copolymer, and thus well-defined coil-comb polycationic brushes with "star polymer" as side chains (abbreviated to "brush") were obtained. Fig. 1c shows the ^{1}H NMR spectrum of the PEG-*b*-P(CD-*g*-PDMA) brush. The chemical shifts at 2.3 and 2.6 ppm are attributed to the methyl ($(C\underline{H}_3)_2$-N-) and the methylene ($-C\underline{H}_2$-$N(CH_3)_2$) groups on the tertiary amine, respectively. The chemical shift at 4.1 ppm is assigned to the methylene group ($-C\underline{H}_2$-OOC-) adjacent to the carboxylate group of PDMAEMA. The proton signal *a* at about 3.6 ppm is mainly attributed to the methylene protons ($-C\underline{H}_2$-$C\underline{H}_2$-O-) on the PEG units. The signals corresponding to the protons on CD moieties are invisible due to the minor contribution of CD to the overall polymer structure. The molecular weight and PDI of the polycationic brushes were then determined by GPC and the results are displayed in Table 1. In our work, all brushes display relatively narrow polydispersity, which indicates that well-defined coil-comb polycationic brushes were successfully synthesized without any intermolecular coupling reactions.

Table 1 Characterization of PEG-*b*-P(CD-*g*-PDMA) brushes and the control star polymer.

Sample	M_n (g/mol) [a]	PDI[a]	$M_{PDMAEMA}$ [b]	DP_n (per chain) [c]
brush1	4.75×10^5	1.35	3.96×10^5	18
brush2	8.51×10^5	1.26	7.72×10^5	34
brush3	1.45×10^6	1.14	1.37×10^6	61
star polymer	3.65×10^4	1.04	3.35×10^4	17

[a] Determined from GPC results. [b] $M_{PDMAEMA}$ represents the molecular weight of PDMAEMA calculated by the difference between the molecular weight of the brush and initiator measured from GPC. [c] DP_n represents the average number of DMAEMA unit on one PDMAEMA chain, which calculated from the following equation: $DP_n = M_{PDMAEMA}$/the number of initiator site per polymer/molecular weight of DMAEMA.

Preparation and characterization of polyplexes

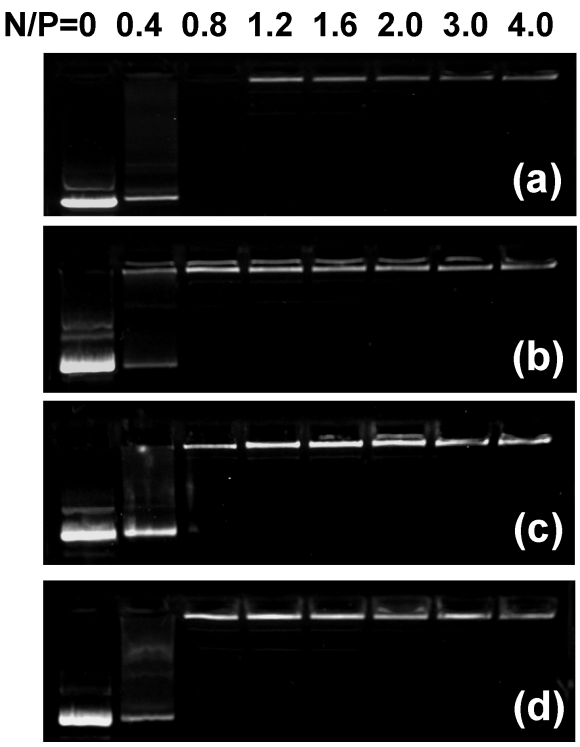


Fig. 2 Agarose gel electrophoresis retardation assays of brush1/pDNA (a), brush2/pDNA (b), brush3/pDNA (c), and star/pDNA (d).

A successful gene delivery system requires that DNA must be condensed by polycation into nanoparticles small enough to facilitate cellular uptake. The abilities of the brushes and the single star polymer carriers to condense DNA were evaluated by an electrophoresis retardation assay at different N/P ratios from 0.4/1 to 4/1 and the results are shown in Fig. 2. As expected, all brushes can completely inhibit pDNA migration when the N/P ratio is larger than 0.8/1, similar to that of the single star polymer. These results indicate that the brush carriers exhibit good capability of condensing pDNA to form stable polyplexes.

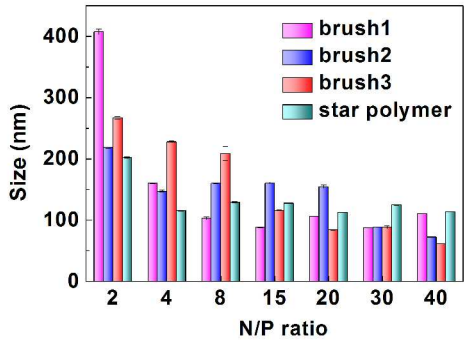


Fig. 3 Particle sizes of the polyplexes at various N/P ratios.

The particle sizes of the brush/pDNA polyplexes with different N/P ratios were determined and compared with the star/pDNA polyplex. As shown in Fig. 3, the hydrodynamic diameter (D_h) of the brush/pDNA and star/pDNA polyplexes are less than 200 nm when the N/P ratio increases to higher than 8/1. This suggests both of the brushes and star polymer can efficiently condense pDNA into nanoparticles and these polyplexes are suitable for delivery of pDNA into the cells. The morphology of the brush alone and the polyplexes at N/P ratio of 10/1 was then investigated under AFM and the images are shown in Fig. S3† and Fig. 4. Clearly, both of the brush and polyplexes display spherical shape under AFM. However, the polyplexes show large size (in the range of 80-120 nm) than the brush alone (about 20 nm), and smaller size than the naked pDNA (about 1 μm in the diameter of the major axis[43]), implying that pDNA is tightly condensed into the brush/pDNA polyplexes.

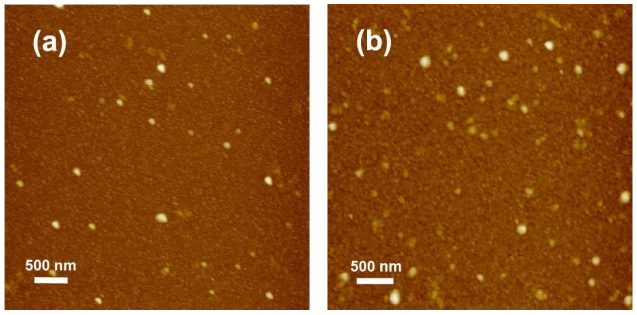


Fig. 4 AFM images of brush1/pDNA (a) and brush3/pDNA (b) polyplexes at N/P ratio of 10/1.

In vitro cytotoxicity evaluation

Cytotoxicity is one of the most important factors to evaluate the possibility of polymer materials being used as gene delivery system. In this work, the cytotoxicity of both of the cationic polymers and polyplexes was evaluated in COS-7 and 293T cells.

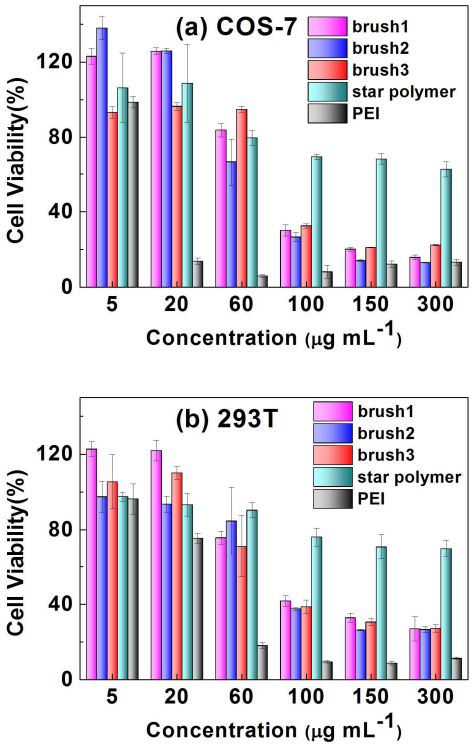


Fig. 5 Cytotoxicities of cationic polymers in COS-7 (a) and 293T (b) cells.

As shown in Fig. 5, the viabilities of the cells for all cationic polymers decrease with the increase of polymer concentration, demonstrating the cytotoxicities of all cationic polymers are dose-dependent. All brushes exhibit significantly lower cytotoxicity than PEI ($p<0.05$) at the same concentration, although they have a great quantity of amino group. This is attributed to the introduction of biocompatible CD. However, the cytotoxicities of the brushes are higher than that of the single star polymer at a higher concentration of more than 100 μg mL^{-1}, which is probably due to the high molecular weight of the polycationic brushes[44]. Interestingly, when the polymer concentration is below 20 μg mL^{-1}, brush1 and brush2 promote the proliferation of COS-7 cells, and brush1 and brush3 promote the proliferation of 293T cells, while this phenomenon is not observed in the case of single star polymer. These results suggest the brushes have no significant cytotoxicity at low concentrations. In addition, the brushes exhibit slightly cell-type dependent cytotoxicity. It seems that the brushes have lower cytotoxicity in COS-7 cells than in 293T cells when polymer concentration is lower than 60 μg mL^{-1}, but have higher cytotoxicity in COS-7 cells than in 293T cells with further increasing polymer concentration.

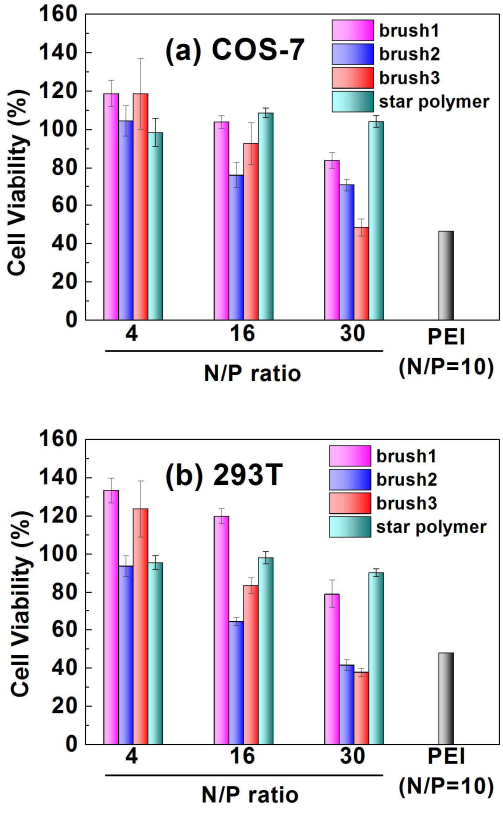


Fig. 6 Cytotoxicities of the polymer/pDNA polyplexes in COS-7 (a) and 293T (b) cells as a function of N/P ratios.

The cytotoxicities of the polymer/pDNA polyplexes at different N/P ratios are also determined in this study and the results are shown in Fig. 6. As compared to PEI25K, most brush/pDNA polyplexes show lower cytotoxicity both in COS-7 and 293T cell at the N/P ratios used in the following transfection experiments. Clearly, cytotoxicities of all brush/pDNA polyplexes increase with increasing the N/P ratio. Compared to star/pDNA polyplex, the brush/pDNA polyplexes exhibit comparable cytotoxicities at the low N/P ratio (≤16) and enhanced cytotoxicities at the high N/P ratio (≥16), which is perhaps due to the high molecular weight of PDMAEMA[44]. Similar to the polycationic brushes, brush/pDNA polyplexes also exhibit promoting effect on cell proliferations at low N/P ratio of 4/1. In addition, brush1/pDNA polyplex exhibits lowest cytotoxicity in three brush/pDNA polyplexes, due to its shortest length of PDMAEMA chains[31]. Interestingly, brush3/pDNA polyplex, the one with longest length of PDMAEMA chains displays lower cytotoxicity than brush2/pDNA polyplex below the N/P ratio of 16/1. This might be attributed to the "brush effect". That is, when PDMAEMA chains increase to a certain length, the chains stretch to nearly full length due to the high osmotic pressure and electrostatic repulsion[45]. The cationic amino groups in the internal section cannot touch negatively charged cell membranes[40]. Therefore, the interactions between brushes and cell membrane are weakened and subsequently decrease cytotoxicity. Moreover, all of the brush/pDNA polyplexes exhibit cell-type dependent cytotoxicity. It seems that COS-7 cells are more sensitive to brush1/pDNA, while 293T cells are more sensitive to brush2/pDNA and brush3/pDNA.

In vitro luciferase gene transfection

Using PGL3-control plasmid as a reporter gene, the *in vitro* transfection efficiencies of brush/pDNA polyplexes in COS-7 and 293T cells were measured.

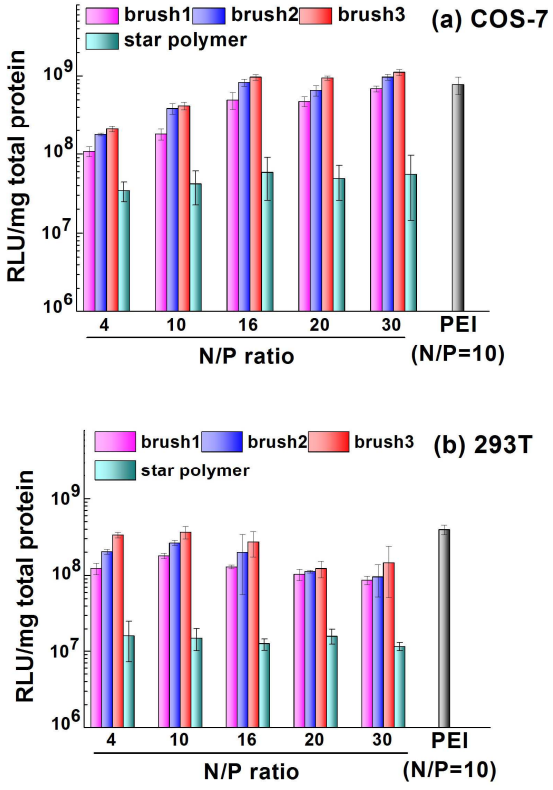


Fig. 7 *In vitro* luciferase expression of the polymer/pDNA polyplexes in COS-7 (a) and 293T (b) cells at different N/P ratio.

As shown in Fig. 7a, all the brush/pDNA polyplexes exhibit strong activities in transfecting COS-7 cells even at the lowest N/P of 4/1. The brush vectors display the comparable or higher transfection capabilities than PEI25K at the N/P ratio higher than 16/1. More important, all the brush/pDNA polyplexes exhibit significantly higher transfection efficiencies compared to the star/pDNA polyplex ($p<0.05$). These results indicate that such unique brush-shaped architecture can further increase the binding interaction than the single star-shaped PDMAEMA, and thus enhance the interaction with pDNA or cellular membranes. Moreover, the transfection efficiencies of brush/pDNA polyplexes increase with the increase of N/P ratio, which is attributed to the larger amounts of free polycations that increase the cellular uptake and help genes to escape from endosome[46, 47]. In addition, the transfection efficiency of brush/DNA polyplex follows the order of brush1<brush2<brush3, which indicates the arm length of PDMAEMA chains is a factor that influenced transfection efficiency of brushes. With longer length of PDMAEMA, the pDNA binding capability and polyplex stability increase, thus the higher transfection efficiency is achieved. Similar trends have also been found by Xu[32, 33].

We further measured luciferase expression efficiencies of brush/pDNA polyplexes in 293T cells to investigate their transfection capability in different-type cell, and the results are shown in Fig. 7b. Similar to that in COS-7 cells, the transfection efficiencies of all brush/pDNA polyplexes are remarkably higher than that of star/pDNA polyplexes ($p<0.05$). The transfection efficiency of brush/pDNA polyplex increases with increasing the arm length of brushes, and brush3/pDNA polyplex exhibits the comparable transfection efficiency to that of PEI25K at low N/P ratio of 4/1 and 10/1. Unlike in COS-7 cells, all brush/pDNA polyplexes exhibit the decreased transfection efficiencies in 293T cells with the increase of N/P ratio. As above discussed (Fig. 6), 293T cells are more sensitive to most brush/pDNA polyplexes than COS-7 cells, and cell viability decreases with the increase of N/P ratio, thus resulting in the decreased transfection efficiencies. Among these brush vectors, brush3 exhibits best transfection capability and acceptable toxic in both of COS-7 and 293T cells, suggesting that brush3 has a promising potential as an effective vector for gene delivery.

In vitro EGFP gene transfection

We further assess the ability of brush3/pDNA polyplex to transfect COS-7 cells using plasmid pEGFP-C1 encoding EGFP DNA, which allows direct visualization of transfection efficiency by fluorescence microscopy. Fig. 8 gives the transfection images mediated by brush3/pDNA, star/pDNA and PEI25K/pDNA polyplexes. Compared to the star/pDNA polyplex, significant enhanced fluorescence signals are observed in the case of brush3/pDNA polyplex. This result suggests that brush3/pDNA exhibits higher transfection capability than the single star polymer, which is consistent with that of luciferase expression. It further demonstrates the brush could increase the endosomal escape of the complexed pDNA by the "proton sponge" effect compare to the star-shape PDMAEMA.

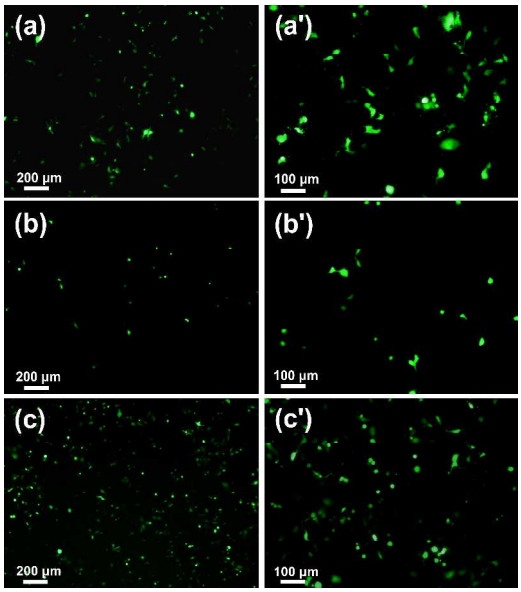


Fig. 8 Fluorescent images of EGFP expression mediated by brush3/pDNA (a and a'), star/pDNA (b and b') and PEI25K/pDNA (c and c') polyplexes at N/P ratio of 20/1 (containing 2 μg of pDNA) in COS-7 cells.

Conclusions

In summary, well-defined coil-comb polycationic brushes with "star polymer" as side chains were synthesized by ATRP using CuCl/bpy as catalyst in acetone/water. Plasmid DNA can be effectively condensed by the polycationic brushes to form spherical polyplexes with the size less than 200 nm. The brush/pDNA polyplexes exhibited significantly enhanced gene transfection efficiency compared to the single star polymer. In addition, they also showed comparable or higher transfection efficiency than PEI25K in some cases, but lower cytotoxicity than PEI25K at the same N/P ratio. These results demonstrated the polycationic brushes with such unique architectures had great promise for the potential gene therapy. Actually, this work also provides a versatile method to prepare polymer brushes with super-high grafting density and flexible functionality, which has potential applications in the fields of drug and gene carriers, biosensors, antifouling surfaces, electronics and catalysts.

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Notes and references

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The well-defined polycationic brush with super-high grafting density of PDMAEMA showed higher transfection capability than single star polymer and PEI25K.