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PAPER

A highly fluorescent cationic bifunctional conjugate

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It was of crucial importance to modify perylene-3,4,9,10-tetracarboxylic acid bisimides (PBIs) for the design of new perylene-based bio-dye agents with strong fluorescence. Recently, we reported that ethanolamine (EA)-functionalized poly(glycidyl methacrylate) (or PGEA) can produce good transfection efficiency, while exhibiting very low toxicity. Herein, the low-toxic PGEA was proposed to be conjugated with PBIs *via* facile atom transfer radical polymerization for the well-defined highly fluorescent cationic bifunctional conjugate (PBI–PGEA). The obtained PBI–PGEA exhibited good water-solubility properties, characteristic spectroscopic patterns of PBIs, and excellent photostability. The PBI–PGEA conjugate can be used as an efficient cell bio-dye for rapid (2–5 min) cell labeling at low concentrations (0.06–0.12 mg mL^{-1}). Such a fast labeling process did not induce obvious cytotoxicity, avoiding possible side-effects to the cells. In addition, the PBI–PGEA still possessed good gene transfection efficiency in different cell lines. With the strong fluorescence in water and good transfection properties, the developed bifunctional PBI–PGEA should possess more potential in bioimaging and gene delivery.

1. Introduction

Because of their outstanding thermal and photophysical stabilities, perylene-3,4,9,10-tetracarboxylic acid bisimides (PBIs) have been widely used to develop perylene-based bio-dye agents with high fluorescence.[1–3] PBIs possess extremely low solubility with strong aggregation in water. Great efforts have been made to increase the water solubility of PBIs by modifying the perylene core with bulky substituents[4] or by conjugating hydrophilic species (including cyclodextrin,[5] poly(ethylene glycol) (PEG),[3,6,7] poly(vinyl alcohol)[8] and polyglycerol-dendron[2]) at the imide positions. It should be pointed out that the bulky substituents of the perylene core would shift the fluorescence color of the fluorophore and did not preserve the green–yellow emission of the parent core-unsubstituted derivative. For the fluorescent cell labeling application, the uncharged hydrophilic species-functionalized PBIs mainly depend on the cellular uptake process. The hydrophilic nature, especially for PEG, does not benefit rapid cellular uptake.[9] It generally takes several hours to complete the cell labeling process.[3] Further improvement of the cell labeling process of PBIs is still required to extend their biomedical applications.

We recently reported that ethanolamine (EA)-functionalized poly(glycidyl methacrylate) (PGMA), or PGEA with plentiful flanking secondary amine and hydroxyl groups, can produce good transfection efficiency in different cell lines, while exhibiting very low toxicity.[10,11] In this present work, we report the highly fluorescent cationic bifunctional conjugate (PBI–PGEA) by modifying PBIs with low-toxicity cationic PGEA. The novel fluorescent PBI–PGEA can be used as an efficient bio-dye for rapid (2–5 min) cell labeling. Such a fast labeling process did not induce cytotoxicity, avoiding possible side-effects to the cells. In addition, the PBI–PGEA also possessed good gene transfection efficiency in different cell lines. The present bifunctional PBI–PGEA conjugate should possess more potential applications in biomedical fields.

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2. Experimental section

Materials

Branched polyethylenimine (PEI, M_w ~25 000 Da), glycidyl methacrylate (GMA, 98%), ethanolamine (EA, 98%), *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA, 99%), 2-bromoisobutyryl bromide (BIBB, 98%), and copper(I) bromide (CuBr, 99%) were obtained from Sigma-Aldrich Chemical Co., St. Louis, MO. GMA was used after removal of the inhibitors in a ready-to-use disposable inhibitor-removal column (Sigma-Aldrich). *N*,*N*-bis(2-[2-hydroxyethoxy]ethyl) perylene-3,4,9,10-tetracarboxylic acid bisimide (PBI–OH) was synthesized according to the literature.[12] 3-(4,5-Dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT), penicillin, and streptomycin were purchased from Sigma Chemical Co., St. Louis, MO. C6 and HEK293 cell lines were purchased from the American Type Culture Collection (ATCC, Rockville, MD).

Synthesis of PBI–PGEA

As shown in Fig.1, PBI–OH was first modified with PGMA based on facile atom transfer radical polymerization (ATRP).

The starting bromoisobutyryl-terminated PBI (PBI–Br) was synthesized according to the following procedures. About 570 mg (about 1 mmol) of PBI–OH was suspended in 100 mL of anhydrous methylene chloride with stirring and then kept in an ice bath. About 2.1 g (about 9 mmol) of BIBB in 10 mL of methylene chloride was added drop-wise into the flask through an equalizing funnel for a period of 10 min at 0 °C. After this addition, the flask was sealed with continuous stirring for 2 h. The reaction was allowed to proceed at room temperature for another 24 h to produce the crude PBI–Br initiator. The resultant $(C_2H_5)_3N{\cdot}HBr$ and unreacted PBI–OH were removed by centrifugation. After removal of the solvent by rotary evaporation, the crude polymer was purified by passing through a silica gel column using $CHCl_3$ as eluent.

The PBI–PGMA polymers (Fig.1) were synthesized using a molar feed ratio [GMA (4 mL)] : [CuBr (34.6 mg, 0.24 mol)] : [PMDETA (62.4 μL, 0.36 mmol)] of 120 : 1 : 1.5 in 10 mL of THF containing 0.1 g (0.24 mol) of PBI–Br at 50 °C. The used THF solvent was purified by distillation. The reaction was performed in a 25 mL flask equipped with a magnetic stirrer and under the typical conditions for ATRP. GMA, PBI–Br, and PMDETA were introduced into the flask containing 10 mL of THF. After PBI–Br had dissolved completely, the reaction mixture was degassed by bubbling argon for 30 min. Then, CuBr was added into the mixture under an argon atmosphere. The reaction mixture was purged with argon for another 10 min. 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 for 12 (PBI–PGMA1 from 12 h of ATRP, $M_n = 1.06 \times 10^4$ g mol^{-1}, PDI = 1.2) to 24 h (PBI–PGMA2 from 24 h of ATRP, $M_n = 1.48 \times 10^4$ g mol^{-1}, PDI = 1.3). The reaction was stopped by exposing to air. The PBI–PGMA was precipitated in excess methanol to remove the catalyst complex. The crude polymer was purified by re-

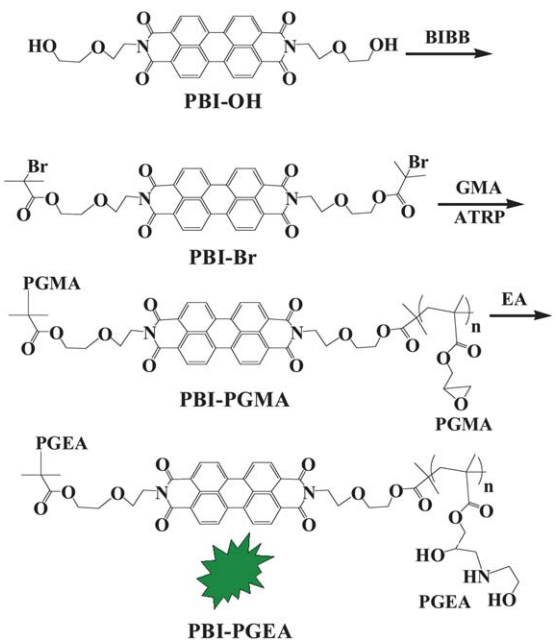

Fig. 1 Preparation processes of fluorescent ethanolamine-functionalized poly(glycidyl methacrylate) (PGEA).

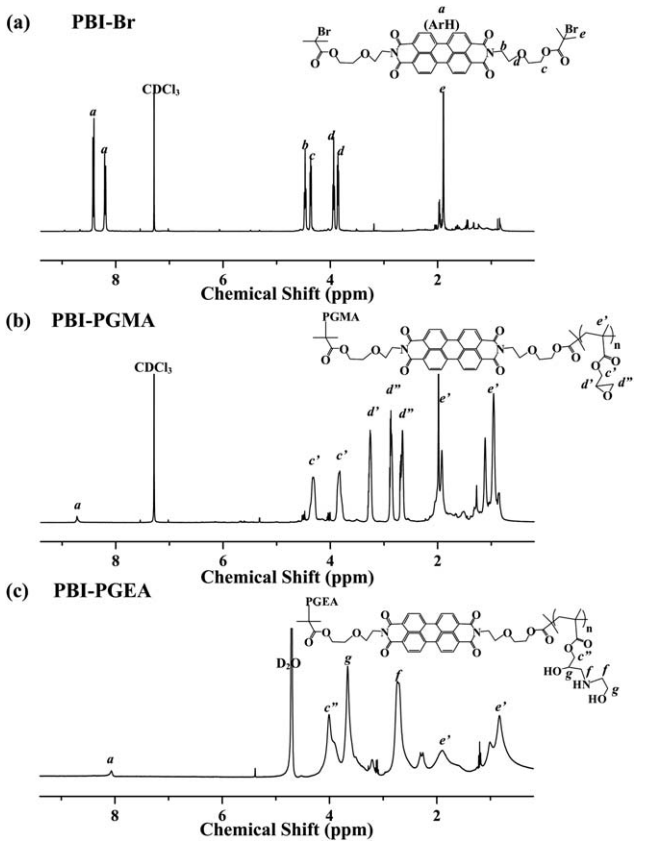

Fig. 2 Typical ^{1}H NMR spectra of (a) PBI–Br (in $CDCl_3$), (b) PBI–PGMA (in $CDCl_3$), and (c) PBI–PGEA (in D_2O).

precipitation cycles into methanol to remove the reactant residues, prior to being dried under reduced pressure.

For the preparation of PBI–PGEA, 0.4 g of PBI–PGMA was dissolved in 7 mL of DMF (HPLC grade). 4 mL of EA and 2 mL of triethyleneamine was then added. The reaction mixture was stirred at 50 °C for 72 h to produce PBI–PGEA. The final reaction mixture was precipitated with excess diethyl ether. The crude produce was purified by 24 h dialysis against DDW using a dialysis membrane (MWCO 3500) prior to lyophilization.

Polymer characterization

The polymers were characterized by gel permeation chromatography (GPC), nuclear magnetic resonance (NMR) spectroscopy, UV-Vis absorption spectroscopy and fluorescence emission spectroscopy. GPC measurements were performed on a Waters GPC system equipped with Waters Styragel columns, a Waters-2487 dual wavelength (λ) UV detector, and a Waters-2414 refractive index detector. THF was used as the eluent at a low flow rate of 1.0 mL min^{-1}. Monodispersed PEG standards were used to generate the calibration curve. ^{1}H NMR spectra were measured on a Bruker ARX 300 MHz spectrometer, using *d*-chloroform (CCl_3D) as the solvent with 1000 scans at a relaxation time of 2 s. UV-Vis absorption spectra in the wavelength range of 200 to 900 nm were obtained from a Shimadzu UV-3101PC spectrophotometer. Fluorescence emission spectra in the wavelength range from 510 to 700 nm were measured on a HITACHI F4600 fluorescence spectrophotometer.

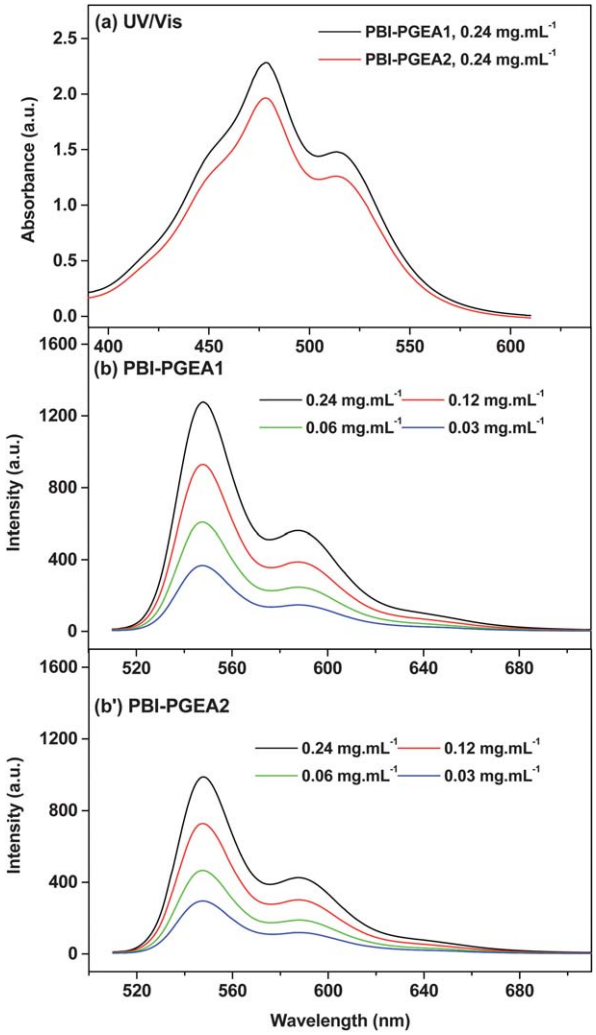

Fig. 3 UV-Vis absorption spectra (a) and fluorescence spectra (b,b′) of aqueous solutions of PBI–PGEA polymers.

Cell labeling

Prior to labeling, C6 and HEK293 cell lines were seeded into the 24 wells at a density of 5×10^4 cells/well and incubated for 24 h at 37 °C under a humidified 5% CO_2 atmosphere in 1 mL Dulbecco's modified eagle media (DMEM) supplemented with 10% fetal bovine serum, 100 units mL^{-1} of penicillin and 100 μg mL^{-1} of streptomycin. The culture media were replaced with fresh culture media containing serial dilutions of PBI–PGEA polymers (0.06–0.24 mg mL^{-1}), and the cells were dyed for 2–10 min. The cells were then washed with phosphate buffered saline (PBS; pH = 7.4) thoroughly to remove loosely attached polymer in the medium. The cells were then fixed in 4% formaldehyde in PBS for 1 h, and dehydration in a series of ethanol aqueous solutions (50–100%) was carried out. The fixed cells were imaged by using a Leica DMIL Fluorescence Microscope with a FITC filter.

Cytotoxicity assay

The cell viability under the cell labeling conditions was evaluated using the MTT assay in HEK293 and C6 cell lines. 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/well for 24 h. The culture media were replaced with fresh culture media containing serial dilutions of polymers (0.06–0.24 mg mL^{-1}), and the cells were incubated for 5–10 min. Then, 10 μL of sterile-filtered MTT stock solution in PBS (5 mg mL^{-1}) was added to each well, reaching a final MTT concentration of 0.5 mg mL^{-1}. After 5 h, the unreacted dye was removed by aspiration. The produced formazan crystals were dissolved in DMSO (100 μL/well). The absorbance was measured using a Bio-Rad Model 680 Microplate Reader (UK) at a wavelength of 570 nm. The cell viability (%) relative to control cells cultured in media without polymers was calculated from $[A]_{test}/[A]_{control} \times 100\%$, where $[A]_{test}$ and $[A]_{control}$ are the absorbance values of the wells (with the polymers) and control wells (without the polymers), respectively. For each sample, the final absorbance was the average of those measured from six wells in parallel.

Characterization of PBI–PGEA–pDNA complexes

The plasmid (encoding *Renilla* luciferase) used in this work was pRL-CMV (Promega Co., Cergy Pontoise, France).[10] The purified pDNA was resuspended in Tris-EDTA (TE) buffer, pH 7.4, and kept in aliquots of 0.5 mg mL^{-1} in concentration. All polymer stock solutions were prepared at a nitrogen concentration of 10 mM in distilled water. Polymers to DNA ratios are expressed as molar ratios of nitrogen (N) in PGEAs to phosphate (P) in DNA (or as N : P ratios). The average mass weight of 325 per phosphate group of DNA was assumed. All polymer–pDNA complexes were formed by mixing equal volumes of polymer and pDNA solutions to achieve the desired N : P ratio. Each mixture was vortexed and incubated for 30 min at room temperature. Each cationic polymer was examined for its ability to bind pDNA through agarose gel electrophoresis using the similar procedures to those described earlier.[10] The particle sizes and zeta potentials of the polymer–pDNA complexes were measured in triplicate using a Zetasizer Nano ZS (Malvern Instruments, Southborough, MA) and procedures similar to those described earlier.[10]

Transfection assay

Transfection assays were performed first using plasmid pRL-CMV as the reporter gene in HEK293 and C6 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 with 500 μL of medium/well and incubated for 24 h. The PBI–PGEA–pDNA complexes (20 μL/well containing 1.0 μg of pDNA) at various N : P ratios were prepared by adding the polymer into the DNA solutions, 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 into the transfection medium and incubated with the cells for 4 h under standard incubator conditions. Then, the medium was replaced with 500 μL of the fresh normal medium. 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 with PBS twice, and lysed in 100 μL of the cell culture lysis reagent (Promega Co., Cergy Pontoise, France).

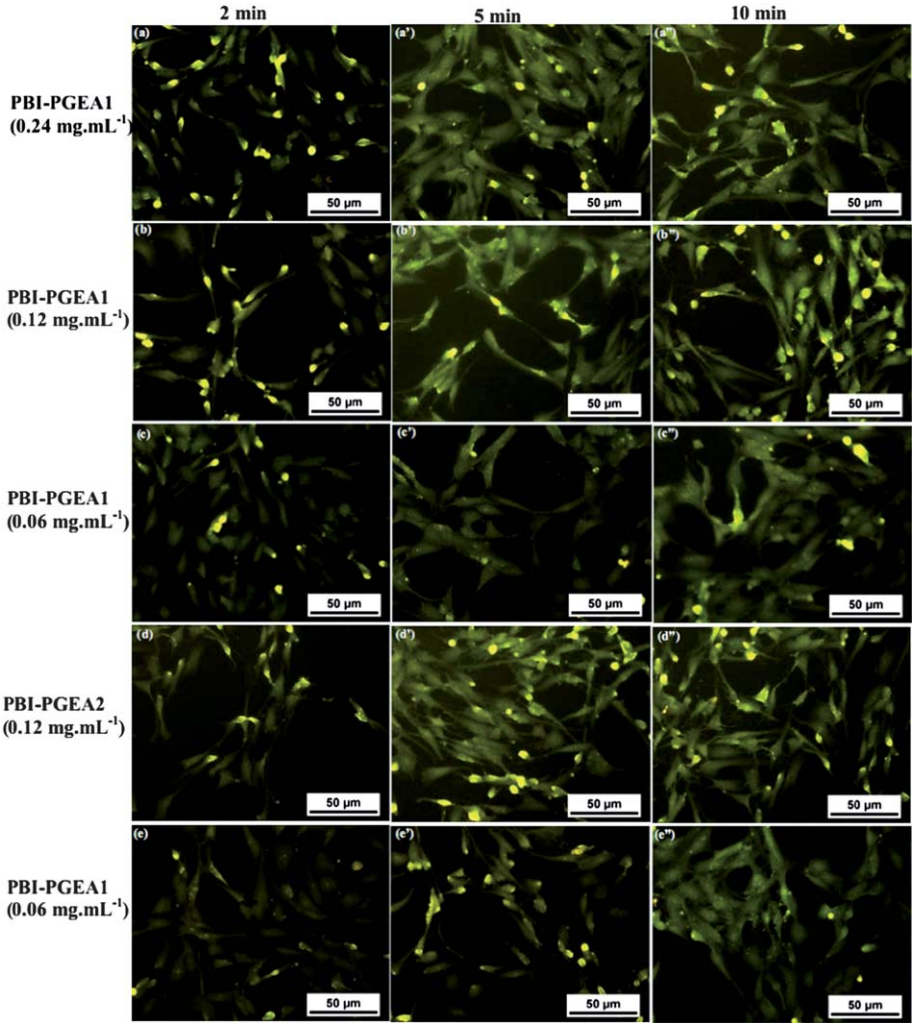

Fig. 4 Fluorescent microscopy images of C6 cells after culturing in the medium containing different concentrations of PBI–PGEA polymers for 2 (a,b,c,d,e), 5 (a′,b′,c′,d′,e′), and 10 (a″,b″,c″,d″,e″) min.

Luciferase gene expression was quantified using a commercial kit (Promega Co., Cergy Pontoise, France) and a luminometer (Berthold Lumat LB 9507, Berthold Technologies GmbH. KG, Bad Wildbad, Germany) using similar procedures to those described earlier.[10] Gene expression results were expressed as relative light units (RLUs) per milligram of cell protein lysate (RLU mg^{-1} protein).

3. Results and discussion

Synthesis of PBI–PGEA

As shown in Fig. 1, the PBI–PGEA conjugate was prepared based on ATRP. ATRP is a well-known facile 'controlled' radical polymerization method, which has been widely used in the biological field.[13] The ATRP was readily initiated from the aromatic ring or imide group of perylene.[3,4,14] The starting bromoisobutyryl-terminated PBI (PBI–Br) initiator was synthesized *via* the direct reaction of hydroxyl groups of PBI–OH with 2-bromoisobutyryl bromide (BIBB). Unlike PBI–OH, the PBI–Br initiator can dissolve in CH_2Cl_2 and THF. The chemical structure of PBI–Br was characterized by ^1H NMR spectroscopy in $CDCl_3$ (Fig. 2(a)). The signals at 8.19 and 8.41 ppm (*a*) were the contribution from the aromatic structure of the PBI initiator. The chemical shifts at 4.46 and 4.34 ppm were mainly attributable to the (*b*) $\underline{CH_2}$–N–C=O and (*c*) $\underline{CH_2}$–O–C=O methylene protons, respectively. The signals at 3.86 and 3.92 ppm were associated with the (*d*) $\underline{CH_2}$–O methylene protons. The chemical shift at $\delta = 1.90$ ppm was associated with the methyl protons (*e*, C(Br)–$\underline{CH_3}$) of the 2-bromoisobutyryl groups. The area ratio of peak *e* and peak *b* indicated that both hydroxyl groups of PBI–OH were successfully converted into the corresponding initiation sites.

Well-defined PBI–PGMA conjugates were subsequently synthesized from PBI–Br (Fig.1). The PBI–PGMA with different lengths of PGMA can be synthesized by varying the ATRP time. In this work, two PBI–PGMA conjugates (PBI–PGMA1 from 12 h of ATRP and PBI–PGMA2 from 24 h of ATRP) were obtained. The corresponding number average molecular weights (M_n) (and polydispersity index (PDI)) were 1.06×10^4 (and 1.2) for PBI–PGMA1 and 1.48×10^4 g mol^{-1} (and 1.3) for PBI–PGMA2. The pendant epoxide groups of PGMA were readily

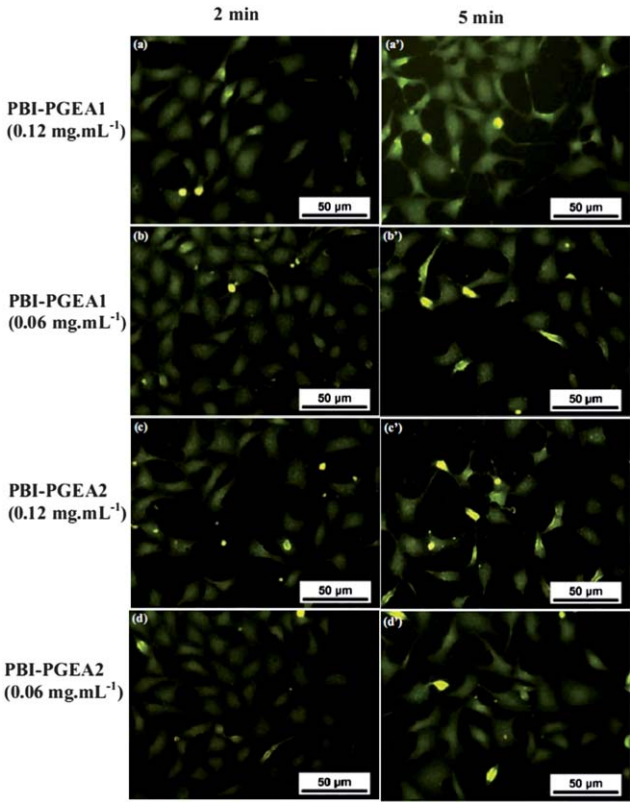

Fig. 5 Fluorescent microscopy images of HEK293 cells after culturing in the media containing different concentrations of PBI–PGEA polymers for 2 (a,b,c,d) and 5 (a′,b′,c′,d′) min.

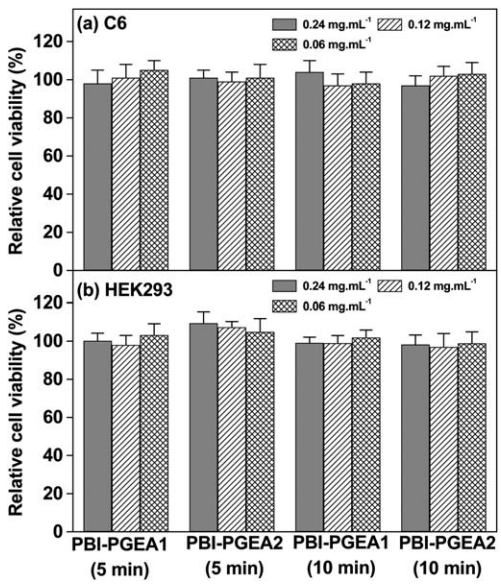

Fig. 6 Cell viability assay in (a) C6 and (b) HEK293 cells after culturing in the media containing different concentrations (0.24–0.12 mg mL^{-1}) of PBI–PGEA polymers for 5 and 10 min. Cell viability was determined by the MTT assay and expressed as a percentage of the control cell culture.

reacted with the amine moieties of ethanolamine (EA).[10,11] The PBI–PGEA conjugates were derived from the corresponding PBI–PGMA counterparts. The representative structures of PBI–

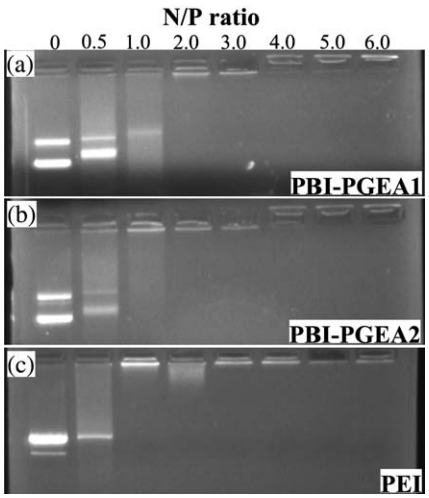

Fig. 7 Electrophoretic mobility of pDNA in the complexes of the cationic polymers ((a) PBI–PGEA1, (b) PBI–PGEA2, and (c) PEI) at various N : P ratios.

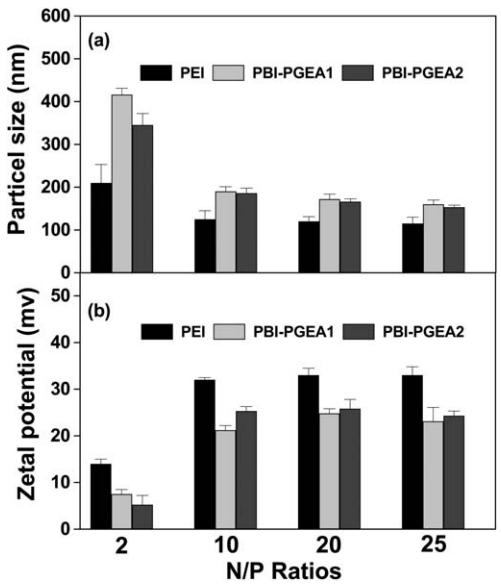

Fig. 8 Particle size (a) and zeta potential (b) of the complexes between the cationic polymers (PEI (25 kDa), PBI–PGEA1, and PBI–PGEA2) and pDNA at various N : P ratios.

PGMA and PBI–PGEA were characterized by ^{1}H NMR spectroscopy as shown in Fig. 2(b) and Fig. 2(c), respectively. For PBI–PGMA, the signals at $\delta = 3.8$ and 4.3 ppm correspond to the methylene protons adjacent to the oxygen moieties of the ester linkages (c', $\underline{CH_2}$–O–C═O). The peaks at $\delta = 3.3$ ppm (d') and $\delta = 2.65$ and 2.87 ppm (d'') can be assigned to the protons of the epoxide ring. The ratio of peak areas of c', d' and d'' was about 2 : 1 : 2, indicating that the epoxy groups in the PGMA remained intact throughout ATRP. After the ring-opening reactions of PGMA with EA, the peaks (d',d'') associated with the epoxide ring had disappeared completely (Fig. 2(c)). The peaks (c', $\underline{CH_2}$–O–C═O) shifted to one position (c'') at $\delta = 3.95$ ppm. The new peaks at $\delta = 3.7$ and $\delta = 2.7$ ppm were mainly attributable to the $\underline{CH}$–OH methylidyne and $\underline{CH_2}$–OH

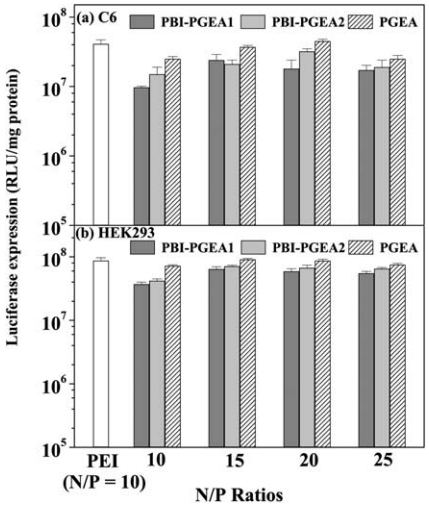

Fig. 9 *In vitro* gene transfection efficiency of the cationic polymers (PBI–PGEA1, PBI–PGEA2, and PGEA)–pDNA complexes at various N : P ratios in comparison with that of PEI (25 kDa) at the optimal N : P ratio of 10 in (a) C6 and (b) HEK293 cells, where the PGEA was derived from the PGMA homopolymer ($M_n = 1.28 \times 10^4$ g mol^{-1}).

methylene protons (*g*) and methylene protons (*f*, NH–$\underline{CH_2}$), respectively. The area ratio (about 4 : 5) of peak *f* and peaks *g*,*c*′′ indicated that almost all oxirane rings of PGMAs were opened by EA under the present reaction conditions.

Spectroscopic properties of PBI–PGEA

Fig. 3 shows the UV-Vis absorption spectra (a) and fluorescence spectra (b,b′) of different PBI–PGEA polymers. All the spectra possessed well-resolved vibronic patterns which are characteristics of monomeric PBIs.[15,16] At the same mass concentration, the spectroscopic intensities of PBI–PGEA2 were slightly lower than those of PBI–PGEA1, which was consistent with the fact that PBI–PGEA2 possessed a relatively low PBI content. For the UV-Vis absorption spectra, the PBI–PGEA conjugates possessed the typical absorption peaks of PBIs at 470 and 520 nm. For the UV-Vis fluorescence spectra, the PBI–PGEA conjugates demonstrated the characteristic fluorescence emission peaks of PBIs at 540 and 580 nm. As shown in Fig. 3(b,b′), the PBI–PGEA conjugates exhibited the concentration-dependent fluorescent behavior. As expected, the fluorescence became stronger, as the polymer concentration increased. In addition, the PBI–PGEA polymers still possessed excellent photostability. After exposure to natural light for one month, the given PBI–PGEA solution did not produce obvious changes in the vibronic patterns and intensities of the UV-Vis absorption and fluorescence spectra.

Cell labeling

For the fluorescent cell labeling application, the water soluble uncharged PBI-based species mainly depend on the cellular uptake process, which generally needs several hours to complete the cell labeling process.[3] The mass content of the hydrophobic PBI molecule in PBI–PGEA was very low (<4%), and the PBI–PGEA readily dissolved in water due to the high water solubility of the PGEA species. With their high fluorescence in water, the present cationic PBI–PGEA conjugates probably can be used as efficient cell labels with a short labeling time. To investigate the possibility of the PBI–PGEA conjugates as rapid cell labeling agents, the fluorescent microscopy images of C6 cells were first obtained after culturing the cells in media containing different concentrations (0.24–0.06 mg mL^{-1}) of PBI–PGEA for 2–10 min (Fig. 4). The significant green–yellow fluorescence derived from the parent PBI parts was observed from the images of the labeled cells. The whole cells were labeled and their morphologies could be clearly seen. At the same polymer concentration, the fluorescence from the cells labeled for 5 and 10 min seems slightly more intense than that from 2 min. No obvious changes were observed among the fluorescence intensities of the cells labeled for 5 and 10 min. About the effects of dye concentrations, at the same labeling time the fluorescence from the cells labeled from 0.12 and 0.24 mg mL^{-1} was slightly more intense than that from 0.06 mg mL^{-1}. There was no obvious difference among the fluorescence intensities of the cells labeled from 0.12 and 0.24 mg mL^{-1}. The above results indicated that the dye concentration and labeling time for the PBI–PGEA conjugates were 0.06–0.12 mg mL^{-1} and 2–5 min, respectively.

To further investigate the applicability of the PBI–PGEA conjugates as rapid cell labeling agents, another type of cell, HEK293, was used also in this work. Fig. 5 shows fluorescent microscopy images of HEK293 cells after culturing the cells in the media containing different concentrations (0.12–0.06 mg mL^{-1}) for 2 and 5 min. The bright green–yellow fluorescence was also observed from the images of the labeled HEK293 cells. The morphologies of the HEK293 cells, different from those of the C6 cells (Fig. 4), were clearly exhibited out (Fig. 5). The above rapid labeling was probably attributed to the fast interaction between the PBI–PGEA conjugates and cells. It is well known that cell membranes are generally negatively charged. It was quite easy for the cationic PBI–PGEA conjugates to complex the negative-charged cells and rapidly cover the cell membranes, making the labeled cells exhibit bright fluorescence. For the cellular uptake-dependent labeling process of the uncharged hydrophilic species-functionalized PBIs, such PBI-based dyes had to be internalized and accumulated within the cytoplasm.[3] The involved hydrophilic species, especially PEG, does not benefit rapid cellular uptake,[9] which would take quite a long time to complete the cell labeling process.

Cytotoxicity is one of the most important factors to be considered in selecting cell-labeling dyes. Our recently studies indicated that PGEA exhibited very low toxicity in different cell lines.[10,11] Fig. 6 shows the cell viabilities of C6 and HEK293 cells under culture conditions the same as those used for cell labeling. Under the mass concentrations (0.24–0.06 mg mL^{-1}) of PBI–PGEA and culture time (5–10 min), no obvious cytotoxicity was observed. The cell viability assay indicated that at the low concentration of PBI–PGEA used in this present work, the above fast labeling process did not induce cytotoxicity, which could avoid possible side-effect to the cells.

Gene transfection

For cellular transfection, DNA has to be condensed by cationic polymers into polymer–plasmid nanoparticles suitable for cellular uptake. The ability of PBI–PGEAs to condense plasmid

DNA (pDNA) into particulate structures was confirmed by agarose gel electrophoresis, particle size and zeta potential measurements. Fig. 7 shows the gel retardation results of cationic PBI–PGEAs–pDNA complexes with increasing N : P ratios in comparison with that of PEI (25 kDa). PBI–PGEA could compact pDNA completely at a N : P ratio of above 2. No obvious difference in their condensation capability was observed. As shown in Fig. 8(a), all the cationic PBI–PGEA can efficiently compact pDNA into small nanoparticles. PBI–PGEA can condense pDNA into nanoparticles of around 200 nm in diameter above the a N : P ratio of 10, which was similar to those of PGEA.[10] Zeta potential is an indicator of surface charges on the polymer–pDNA nanoparticles. The PBI–PGEA–pDNA complexes possessed positively charged surfaces above a N : P ratio of 2 (Fig. 8(b)). A positively charged surface allows electrostatic interaction with anionic cell surfaces and facilitates cellular uptake. In addition, it was found that the PBI–PGEA conjugates possessed similar cytotoxicity to that of PGEA (data not shown here).[10]

The *in vitro* gene transfection efficiency of the cationic PBI–PGEA–pDNA nanoparticles was assessed using luciferase as a gene reporter. Fig. 9 shows the gene transfection efficiency of PBI–PGEA at various N : P ratios in comparison to that of PEI (25 kDa) at its optimal N : P ratio of 10 in C6 and HEK293 cells. The optimal N : P ratios for PBI–PGEA were 15–20, in comparison with the optimal N : P ratio (10)[10] of PEI. The lower optimal N : P ratio for PEI was consistent with its higher DNA condensation capability (Fig. (8)). It was reported that the incorporation of hydrophobic segments into gene carriers could enhance gene transfection efficiency.[17,18] In comparison with the PGEA derived from the PGMA homopolymer ($M_n = 1.28 \times 10^4$ g mol^{-1}[10]) (Fig. 9), the PBI–PGEA1 (derived from the PBI–PGMA with M_n of 1.06×10^4 g mol^{-1}) and PBI–PGEA2 (derived from the PBI–PGMA with M_n of 1.48×10^4 g mol^{-1}) exhibited slightly lower transfection efficiencies in both cell lines at most N : P ratios. The above results indicated that in this present work the hydrophobic PBI species did not enhance the gene transfection efficiency, probably because the incorporated PBI could not facilitate the interaction between the cationic carrier and pDNA. The hydrophobic PBI species did not significantly deteriorate transfection efficiency either. As shown in Fig. 9, the transfection efficiency mediated by the PBI–PGEA conjugates at their optimal N : P ratios was comparable to that mediated by the 'gold-standard' PEI (25 kDa).

4. Conclusions

The well-defined highly fluorescent cationic bifunctional conjugates (PBI–PGEA) were successfully prepared by modifying PBIs with low-toxicity cationic PGEA. The resultant PBI–PGEA conjugates exhibited good water-solubility properties, characteristic spectroscopic pattern of PBIs, and excellent photostability. The present cationic PBI–PGEA conjugates can be used as efficient cell bio-dyes at low concentrations with a short labeling time (2–5 min). Such a fast labeling process did not induce cytotoxicity, avoiding possible side-effects to the cells. In addition, the PBI–PGEA also possessed a good gene transfection efficiency comparable to that mediated by the 'gold-standard' PEI (25 kDa) in different cell lines. The present bifunctional PBI–PGEA conjugate should possess more potential applications in bioimaging and gene delivery.

Acknowledgements

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