Photo-induced release of active plasmid from crosslinked nanoparticles: *o*-nitrobenzyl/methacrylate functionalized polyethyleneimine†

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To facilitate DNA packaging and photolytic release, *o*-nitrobenzyl and methacrylate functionalized PEI (P10A) was synthesized for condensing DNA into nanoparticles as small as 160 nm after radical polymerization with initiator. The gene expression following delivery of uncross-linked P10A/DNA was unchanged by photoirradiation of cells. However, exposure to photo-irradiation caused a 3-fold increase in gene expression in cells transfected with cross-linked P10A/DNA. These polyplexes were designed to mimic viral particles that condense and protect DNA while allowing subsequent triggered release of DNA.

1. Introduction

Non-viral vectors for gene delivery offer several potential advantages over viral vectors such as ease of scalable manufacture and safety.[1,2] Since its first use as a non-viral vector in 1995 by Boussif, Lezoualc'h *et al.*, polyethylenimine (PEI) with densely positively charged cationic amine has remained a commonly used cationic polymer for polyplex formation.[3] Early studies focused on the physical properties of the PEI/plasmid DNA (DNA) polyplexes such as size and zeta potential and transfection efficiency in different cell lines as a function of polymer nitrogen to DNA phosphate ratio (N/P ratio).[4,5]

Chemical modifications of PEI are directed at enhancing transfection efficiency and reducing aggregation, cytotoxicity, and the lack of cell selectivity.[6–10] There have been several attempts at engineering temperature-sensitive, pH-sensitive, or biodegradable PEI for controlled DNA release.[11–14] A polyplex should remain intact in the endosomes and lysosmes to protect the DNA from cellular nucleases, but should degrade or disassemble to facilitate DNA release once the polyplex has reached the cytoplasm. Thus, a fine-tuned balance needs to be achieved between a polyplex that is packaged too loosely, allowing for DNA degradation by cellular nucleases and a polyplex that is packaged too tightly, not allowing for dissociation and eventual transcription of the released plasmid.

Photolabile protecting groups have been used extensively for controlled release, spatial, temporal, concentration dependent, and kinetic studies.[15–17] The advantage of using photolabile protecting groups is that the "caged" compounds are rendered inert until photolysis which should liberate the compound in μsec to msec time frames. Numerous caged compounds for biological use have been synthesized, including, but not limited to, ATP and analogues, alanine, nitric oxide, nitric oxide inhibitors, receptor ligands, DNA, and inositol trisphosphate (IP_3); many are commercially available.[18,19]

The potential to use this technology for gene delivery, however, has not been fully realized. Monroe *et al.* synthesized a caged DNA for photolytically activated gene delivery *via* direct chemical modification of the DNA backbone.[19] They found a significant decrease in the amount of DNA that could be transcribed for the caged DNA compared to both uncaged DNA where the caging group had been photocleaved and native DNA. Additionally, the photolysis of the caged DNA had the potential to cause DNA nicking. However, the method was the first report of successful light activation of DNA and transgene expression within a living cell.

Viral vectors such as adenovirus provide functionality for DNA packaging and protection, cellular uptake and endosome escape, and triggered release of DNA at the nuclear pore. The packaging of DNA and the release of DNA by a single polymeric material presents competing material design goals. We report the synthesis of a crosslinkable PEI that is amenable to photolytic degradation for the controlled intracellular release of plasmid DNA. In this work, we attempted to design a novel photolabile gene carrier. The designed photolabile carrier for gene delivery consist of three functional domains: a cationic domain to electrostatically interact with and condense the DNA to nanoparticle, a cross-linking domain to entrap the DNA within the polyplex, and a photolabile domain to release the DNA with the addition of light of an appropriate wavelength. A photoactivated cross-linked DNA nanoparticle could release DNA nanoparticle and transgene expression for spatial, temporal, and metered dosing.

2. Experimental

2.1 Materials

Commercially available extra pure *o*-nitroacetophenone (Aldrich), benzoyl chloride (Aldrich), acetovanillone (Aldrich), *N*-hydroxysuccinimide (Aldrich), *N*,*N*-dicyclohexylcarbodiimide

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(Aldrich), trimellitic anhydride chloride (Aldrich) polyethyleneimine MW 600 (Aldrich), MW 10000 (Polyscience), and *N*-(3-aminopropyl)methacrylamide hydrochloride (Polyscience) were used as received without further purification.

2.2 Measurements

^{1}H NMR and ^{13}C NMR were recorded with Bruker AC-250 spectrometer, using tetramethylsilane (TMS) as an internal standard. HPLC was performed on HP-series 1050 HPLC system consisting of HAMILTON PRP-1 column and photodiode array detectors. Elution was performed using a linear gradient (30–70%) of acetonitrile in 1% TFA aqueous with a flow rate of 1.0 ~ 4.0 mL min^{-1}. Photoirradiation was carried out with Model B-100A Hi Intensity UV Lamp (UVP Inc) at a distance of 10 cm (emission peak is at 365 nm, 3.5 mW intensity at 30 cm). Particle size was measured at 25 °C with Dynapro Dynamic Light Scattering of Protein solution. Fluorescence was measured with SLM-AMINCO Model 8100 spectrofluorometer.

2.3 Synthesis of *tert*-butyl (4-acetyl-2-methoxyphenoxy)acetate (B1)

Acetovanillone (2 g, 12 mmol) was placed in a flask and dissolved in MeOH (10 mL). NaOH pellets (0.626 g, 14.2 mmol) were added to the solution. The color of the solution turned deep yellow as the NaOH gradually dissolved. The solution was kept at room temperature for 15 h. Methyl bromoacetate (2.59 g, 16.9 mmol) was added and the solution was refluxed for 15 h. After cooling, the MeOH was evaporated and the solid dissolved in EaOAc. The resulting solution was washed with 5% HCl aqueous solution and water several times. The organic phase was dried over anhydrous magnesium sulfate and concentrated by evaporation to give to afford quantitative B1 of light yellow solid. ^{1}H NMR ($CDCl_3$): 7.55–7.51 (m, 2H, C_6H_2), 6.78 (d, J = 8.8 Hz, 1H, C_6H_2), 4.66 (s, 2H, –CH_2–), 3.94 (s, 3H, OCH_3), 2.57 (s, 3H, CH_3), 1.48 (s, 9H, $3CH_3$). ^{13}C NMR ($CDCl_3$): 196.6, 167.1, 149.2, 131.3, 122.7, 111.6, 110.8, 82.7, 66.0, 56.0, 28.0, 26.2. Anal. Calcd for $C_{15}H_{20}O_5$: C; 64.27, H; 7.19. Found: C; 64.65, H; 7.40.

2.4 Synthesis of (4-acetyl-2-methoxy-5-nitrophenoxy)acetic acid (B2)

A solution of B1 (5.8 g, 20.7 mmol) in 15 mL of acetic anhydride was added to a solution 15 mL of 70% HNO_3 and 10 mL of acetic anhydride at 0 °C. The solution was stirred for 2 h and then room temperature for 4 h. The solution was poured into water and chilled to 4 °C overnight. The product was isolated by filtration, and the precipitate was washed with water to give the B2 (4.73 g, 17.6 mmol, 85%) as yellow solid. ^{1}H NMR (CD_3OD): 7.62 (s, 1H, C_6H_2), 7.05 (s, 1H, C_6H_2), 4.83 (s, 2H, –CH_2–), 3.98 (s, 3H, OCH_3), 2.47 (s, 3H, CH_3). ^{13}C NMR (CD_3OD): 202.0, 171.6, 168.5, 155.9, 134.6, 110.8, 66.9, 57.3, 30.4. Anal. Calcd for $C_{11}H_{11}NO_7$: C; 49.08, H; 4.12, N; 5.20. Found: C; 48.72, H; 4.02, N; 5.24.

2.5 Synthesis of *N*-(3-(4-acetyl-2-methoxy-5-nitrophenoxy)acetamide)propyl methacry-lamide (MCO)

A slurry of (4-acetyl-2-methoxy-5-nitrophenoxy) acetic acid (B2) (0.6 g, 2.25 mmol), *N*-hydroxysuccinimide (0.6 g, 5.2 mmol), and *N*,*N*-dicyclohexylcarbodiimide (0.8 g, 3.9 mmol) in CH_2Cl_2 (15 mL) was stirred at room temperature for 1 h under nitrogen to make the activated carboxylic group. *N*-(3-aminopropyl)methacrylamide hydrochloride (0.6 g, 3.4 mmol) was poured into the activated carboxylic solution. Triethylamine (TEA) (0.4 ml, 2.9 mmol) added after 30 min. The solution was stirred for 48 h at room temperature. The solution was filtered and poured into water. The solution was extracted with ethyl acetate and saturated $NaHCO_3$. The combined organic solution dried with anhydrous $MgSO_4$ and concentrated by evaporation. The obtained mixture was dissolved in acetonitrile, and the resulting solution was filtrated. The combined organic solution concentrated by evaporation to give the MCO (0.72 g, 1.8 mmol, 80%) as viscous liquid of slight yellow, which was used at next step without further purification. ^{1}H NMR ($CDCl_3$): 7.68 (s, 1H, C_6H_2), 7.39 (s, 1H, C_6H_2), 7.30 (m, 1H, NH), 6.81(s, 1H, C_6H_2), 6.75 (m, 1H, NH), 5.77 (s, 1H, =CH_2), 5.35 (s, 1H, =CH_2), 4.63 (s, 2H, –CH_2–), 4.01 (s, 3H, OCH_3), 3.44 – 3.34 (m, 4H, NHC$H_2$$CH_2CH_2$NH), 2.51 (s, 3H, CH_3), 1.99 (s, 3H, CH_3C=), 1.74 (m, 2H, NHCH_2C$H_2$$CH_2$NH).

2.6 Synthesis of *N*-(3-(4-(1-hydroxylethyl)-5-nitrophenoxy) acetamide) propyl methacryl-amide (MOH)

To a solution of MCO (0.71 g, 1.8 mmol) in EtOH (10 mL) was added $NaBH_4$ (0.132 g, 3.5 mmol) at 0 °C. The reaction mixture was stirred at room temperature for overnight. After the removal of solvent, the residue was dissolved in ethyl acetate, and the resulting solution was washed with NH_4Cl solution several times. The organic phase was dried over anhydrous $MgSO_4$ and concentrated by evaporation to give yellow oil, which was purified by silica gel column chromatography using a solution of $CHCl_3$ and acetonitrile (v/v = 60/40, R_f 0.45) as an eluent to afford 0.52 g (1.32 mmol, 73.3%) of light yellow oil. ^{1}H NMR ($CDCl_3$): 7.61 (s, 1H, C_6H_2), 7.41 (s, 1H, C_6H_2), 7.23 (m, 1H, NH), 6.67 (m, 1H, NH), 5.76 (s, 1H, =CH_2), 5.55 (m, 1H, HOC(*H*)-), 5.35 (s, 1H, =CH_2), 4.57 (s, 2H, –CH_2–), 4.02 (s, 3H, OCH_3), 3.53 – 3.24 (m, 4H, NHC$H_2$$CH_2CH_2$NH), 1.98 (s, 3H, C$H_3$C=), 1.73 (m, 2H, NHC$H_2CH_2$$CH_2$NH), 1.54 (d, J = 6.2 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$): 168.66, 168.28, 154.21, 145.17, 139.59, 139.48, 139.24, 119.84, 111.31, 109.44, 68.89, 65.43, 56.35, 35.87, 35.76, 29.42, 24.47, 18.47. HRMS Anal. Calcd for $C_{18}H_{25}N_3O_7$ $(M + Na)^+$: 418.1590. Found: 418.1566.

2.7 Synthesis of *N*-(3-(4-(1-(2,3-anhydridehydroxylethyl)-5-nitrophenoxy)acetamide)propyl methacryl amide (AMCO)

To a solution of MOH (0.25 g, 0.63 mmol) and pyridine (0.1 g, 1.3 mmol) in CH_2Cl_2 (10 mL) was added a solution of trimellitic anhydride chloride (0.18 g, 0.85 mmol) in CH_2Cl_2 (5 mL) at 0 °C under an atmosphere of nitrogen. The mixture was stirred at room temperature overnight. The precipitate formed in the reaction mixture was filtered off and washed with CH_2Cl_2. After the removal of CH_2Cl_2, the residue was dissolved in chloroform, and the resulting solution was washed with water several times

and a dilute $NaHCO_3$ aqueous solution. The organic phase was dried over anhydrous $MgSO_4$ and concentrated by evaporation to give light yellow solid (0.32 g, 0.56 mmol, 89%). It was further purified by HPLC using acetonitrile as an eluent. ^{1}H NMR ($CDCl_3$): 8.66 (s, 1H, C_6H_3), 8.56 (d, $J = 7.7$ Hz, 1 H, C_6H_3) 8.13 (d, $J = 8.0$ Hz, 1 H, C_6H_3) 7.67 (s, 1H, C_6H_2), 7.23 (m, 1H, NH), 7.09 (s, 1H, C_6H_2), 6.77 (m, 1H, –OC(H)–), 6.56 (m, 1H, NH), 5.77 (s, 1H, $=CH_2$), 5.35 (s, 1H, $=CH_2$), 4.60 (s, 2H, $–CH_2–$), 3.96 (s, 3H, OCH_3), 3.49 – 3.30 (m, 4H, $NHCH_2CH_2CH_2NH$), 1.98 (s, 3H, $CH_3C=$), 1.84 (d, $J = 6.6$ Hz, 3H, CH_3), 1.74 (m, 2H, $NHCH_2CH_2CH_2NH$). HRMS Anal. Calcd for $C_{27}H_{27}N_3O_{11}$ $(M + Na)^+$: 592.1543. Found: 592.1449.

2.8 Synthesis of PEI with AMCO (P6A and P10A)

To a solution of PEI (MW = 600) (75 mg, 0.125 mmol) in 5 ml H_2O–THF (v/v = 40/60) was added a solution of AMCO (19 mg, 33.4 μmol) in THF (2 mL) at room temperature under an atmosphere of nitrogen. The mixture was stirred at room temperature for 30 min. After evaporation of THF, the residue was dissolved in distillated water. P6A was purified by HPLC using a solution of acetonitrile and 1% TFA as an eluent to afford 58.9 mg (62.7%) of slight light yellow oil. According to similar procedure, a 48.1 mg (82%) of P10A was obtained by using of PEI (MW = 10,000) (51 mg, 0.05 mmol) and AMCO (7.6 mg, 13.4 μmol). The concentration of the photolabile group can be calculated by integration of the ^{1}H NMR spectrum that is by calculating the ratio of the vinyl protons to the characteristic methylene protons of PEI main chain. The concentration values of approximately 20 mol% calculated in this manner are in good agreement with the theoretical values.

2.9 Monitoring complexes formation by gel electrophoresis

P6A/DNA or P10A/DNA complexes, containing 1 μg DNA, with N/P charge ration from 1 to 24 were visualized on 1% agarose gel stained with 0.125 μg ml^{-1} ethidium bromide (EtBr).

2.10 By fluorescence

Complex formation of P6A/DNA or P10A/DNA was measured with a loss of fluorescence intensity by intercalating of EtBr. A 20 μl of EtBr solution (0.625 μg ml^{-1}) was added to 50 μl solution of P6A/DNA or P10A/DNA complexes with N/P charge ratio from 1 to 24. After incubation of 10 min, the solution was measured for the excitation of 510 nm and 590 nm emission with a fluorescence spectrofluorometer.

2.11 Polymerization of P10A/DNA nanoparticles

The complexes were formed by the addition of P10A solution to DNA solution to achieve the 16 or 24 N/P charge ratio in 40 μl of Opti-MEM medium. The ammonium persulfate initiator solution (1 mM) was added to the complexes and then purged with dry nitrogen gas for 10 min. The final concentration of P10A, DNA, and initiator in the polymerization was 2.53 nmol, 3 nmol, and 1 nmol, respectively. The mixture was polymerized at 37 °C for 2 h, and then 160 μl of additional Opti-MEM was added to use for transfection.

2.12 DNase protection

The naked DNA and P10A/DNA complexes were incubated with DNase (Promega, Madison, WI) to confirm the protection of complexes of DNA with P10A with experimental procedure modified from a reported method.[20] DNase (2 U) was added to 100 μl of the corresponding solution of DNA and P10A/DNA complexes, containing 1 μg/100 μl of DNA. The mixture was incubated at 37 °C and aliquots of 20 μl were taken at 10, 20, 40, 60 min, to which 8 μl of 100 mM EDTA were immediately added to inactivate DNase degradation and incubated for 15 min. Then, 10 μl of 2 mg ml^{-1} of heparin was added to the mixture and incubated for 2 h at room temperature. To the time 0 min sample, the same proportions of EDTA and DNase solutions were added, but starting with the EDTA solution to prevent any DNase degradation.

2.13 Photo irradiation

Photolysis was performed at room temperature using Model B-100A Hi Intensity UV Lamp (UVP Inc, Optical density is 3.5 mW cm^{-2} at 30 cm) with distance of 10 cm.

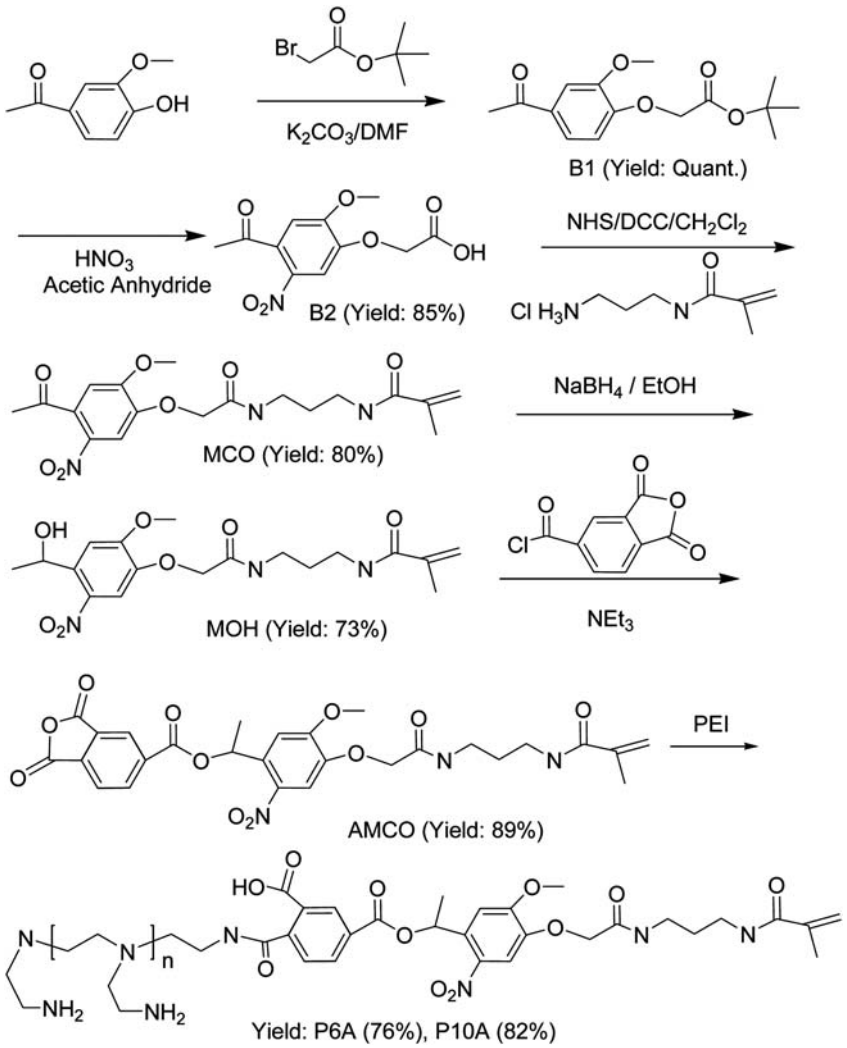
2.14 Cell culture and transfection

The plasmid pEGFP-N3 was obtained from Clontech, amplified in high copy E. Coli, and column purified by the standard procedure of the manufacturer (Qiagen, Germany). The concentration was determined by UV absorbance at 260 nm. COS cells were cultured in Dulbeccos's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (Hyclone), 0.3 mg ml^{-1} L-glutamine (Gibco BRL), and 150 U/ml penicillin (Gibco BRL), and 0.15 mg ml^{-1} streptomycin (Gibco BRL) and maintained at 37 °C in 5% CO_2 humidified atmosphere. Cells were seeded in 24-well plates at 1×10^5 cells/well 24 h prior to transfection. At the time of transfection, cells were at about 60%–70% confluence. P10A/DNA complexes were prepared by the addition of P10A solution to pEGFP plasmid DNA solution to make the required N/P ratios for 1 μg of plasmid DNA in 200 μl of Opti-MEM medium, and mixed by vortexing, and incubated for 20 min at room temperature. Culture medium of cells removed. Then the complexes were added to COS cells and then incubate for 3 h at 37 °C (5% CO_2). In case of photo-irradiation, the well plates exposed to 365 nm at distance of 10 cm for 10 min. The medium was replaced with growth medium to remove the complexes after 4 h and incubated for 24 h. Gene transfection efficiency was measured by analyzing the expression of the EGFP by flow cytometry. All samples were measured in triplicate.

3. Results and discussion

o-Nitrobenzyl group is one of the most useful photolabile compounds available for light triggered investigations of biological processes.[21–23] Moreover, the acrylate group facilitates radical or anion (co)polymerization with acrylate monomer of other series as well as homopolymerization.[24] Therefore, *o*-nitrobenzyl and methacrylate groups were selected for the synthesis of functionalized PEI.

The functional reaction of PEI was carried out according to Scheme 1. The acid group of B2 was activated with



Scheme 1

N-hydroxysuccinimide (NHS) and *N*,*N*′-dicyclohexylcarbodiimide (DCC) at room temperature for 1 h. The addition of *N*-(3-aminopropyl)methacrylamide hydrochloride gave the MCO and dicyclohexylurea which was easily separated by filtration. MOH was obtained by the reduction of acetophenone site of MCO with $NaBH_4$. In subsequent step, the hydroxyl group was reacted with trimellitic anhydride chloride. Finally, reaction with PEI (MW: 600 and 10,000) was carried out to give the functionalized PEI (referred to as: P6A and P10A).

The ^{1}H NMR spectra of the P6A and P10A showed a peak at 6.7 ppm and two separated peaks at 5.5–5.3 ppm, which were attributed to methylidyne proton of *o*-nitrobenzyl group and vinyl protons of methacrylate group, respectively, as well as peaks assignable to PEI (Fig. 1). From NMR, the conjugation of *o*-nitrobenzyl and methacrylate groups on PEI showed almost similar molar ratio with targeted ratio. P6A and P10A showed UV signal around at 270–400 nm due to nitro and aromatic group.

Our photo labile compound contained a carboxylate and a nitrobenzyl group cleavable upon exposure to light in the range of 300 to 400 nm, especially 365 nm. Firstly, the decomposition of P6A alone was carried out in distilled water and monitored for the cleavage under the irradiation (365 nm) using HPLC as shown in Fig. 2. The carboxylate functional group was cleaved by internal reaction with the nitrobenzyl functional group, thereby separating the polyamine ligand from the cross-linkable moiety. The photodecomposition reaction of P6A was shown in Scheme 2. The intensity of signal A decreased with an increase of the irradiation time, while the signal B increased. Moreover, signal A slightly shifted to the region of fast retention time, indicating change of polarity of decomposed PEI by irradiation. The signal B in UV detector showed high intensity and reached maximum value after 4 min. The UV spectra of signal B showed an absorbance around 300 nm due to nitrosoacetophenone.

The formation of P6A/DNA and P10A/DNA nanoparticles was performed by the adding of the P6A and P10A solution to the DNA solution to make desired N/P charge ratios. Gel electrophoresis showed the charge ratio where DNA completely binds with P6A and P10A. DNA forms the nanoparticle complex at N/P ratio of 16 with P6A and 8 with P10A (Fig. 3).

Fig. 4 shows the fluorescence change of P6A/DNA and P10A/DNA nanoparticles for various N/P charge ratios. The

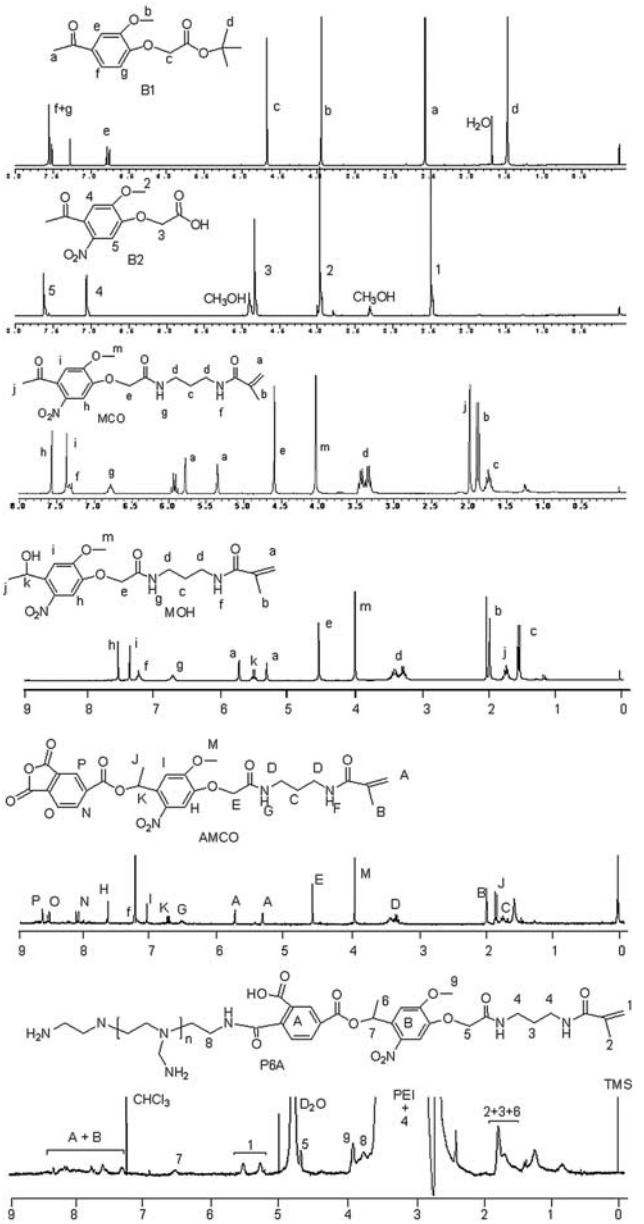


Fig. 1 ^{1}H NMR spectra of B1, B2, MCO, MOH, AMCO, and P6A.

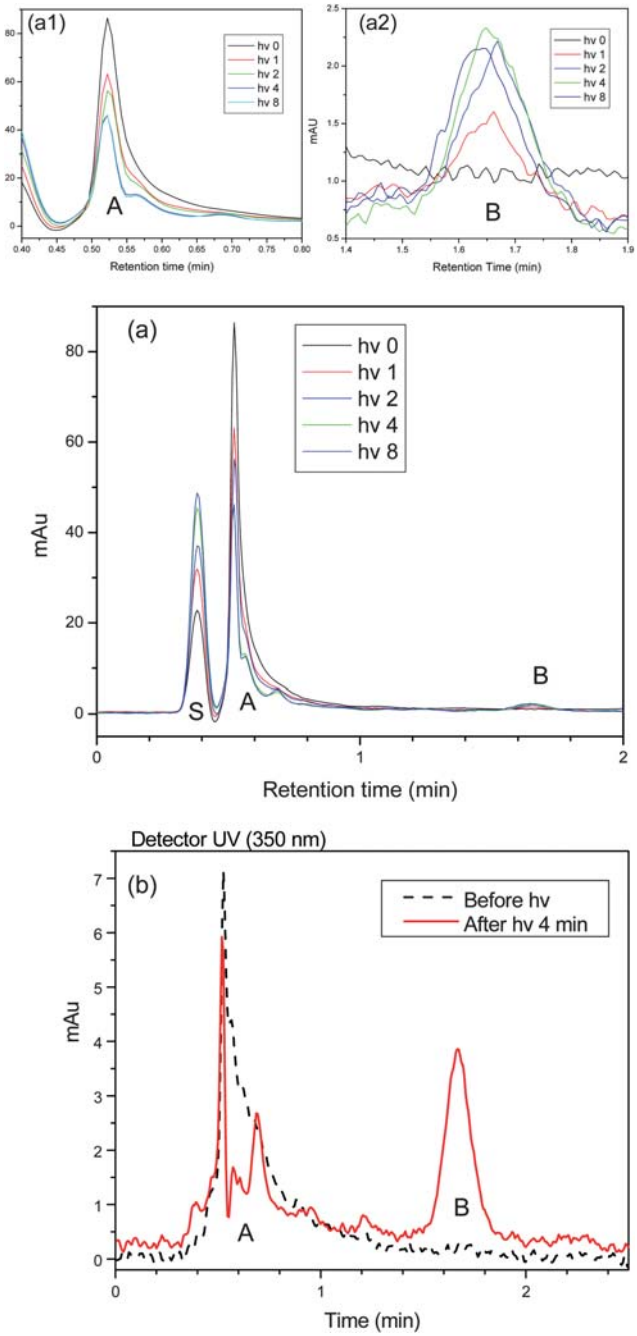
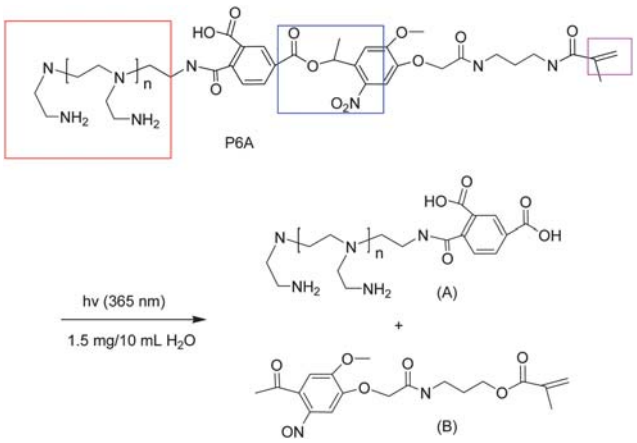


Fig. 2 HPLC charts were measured by (a) RI and (b) UV detector of functionalized PEI (P6A) according to photo irradiation at 365 nm for 0–8 min. (a1) and (a2) represent chart at enlarge scale of peak A and B. S is due to the solvent peak.

fluorescence reached on minimum at N/P charge ratio of 24 for P6A and 8 for P10A, indicating that EtBr could not intercalate with DNA further because the DNA was completely condensed with P6A and P10A. The radius of the nanoparticles was dependent upon the N/P ratio used. Moreover, particle size showed diameters of about 40–80 nm in case of P10A. In contrast, P6A of low molecular weight PEI showed slightly larger diameters around 100–160 nm. These results indicated that DNA condensed more sufficiently with P10A of high molecular weight.

To evaluate the protective effect of the complex, naked DNA and DNA/P10A nanoparticles were incubated with DNase (Fig. 5). Degradation was monitored by agarose gel electrophoresis. The DNA was competed off the polymer using the anionic displacer, heparin. The condensation of DNA with P10A protected DNA from DNase even after 60 min, whereas free DNA was rapidly degraded by added DNase.

The polymerization of P10A alone was carried out with ammonium persulfate (AS) as an initiator at 37 °C for 2 h. In monitoring by HPLC, the signal slightly shifted to a region of slow retention time, indicating polarity change by polymerization. Polymerization of the P10A/DNA nanoparticles with N/P charge ratio of 16 and 24 was then carried out. Particle size of P10A/DNA nanoparticles increased slightly from 40–80 nm to 60–120 nm after polymerization (Fig. 6). This might be due to the inter-polymerization between methacrylate groups on



Scheme 2

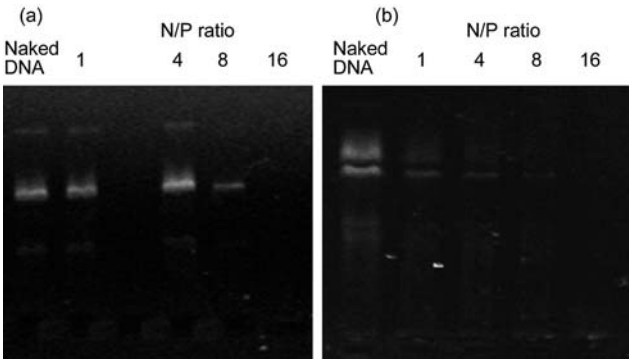


Fig. 3 Gel electrophoresis of (a) P6A/DNA and (b) P10A/DNA complexes with different N/P charge ratios. P6A/DNA or P10A/DNA complexes, containing 1 μg DNA, with N/P charge ration from 1 to 24 are visualized on 1% agarose gel stained with 0.125 μg ml^{-1} ethidium bromide (EtBr).

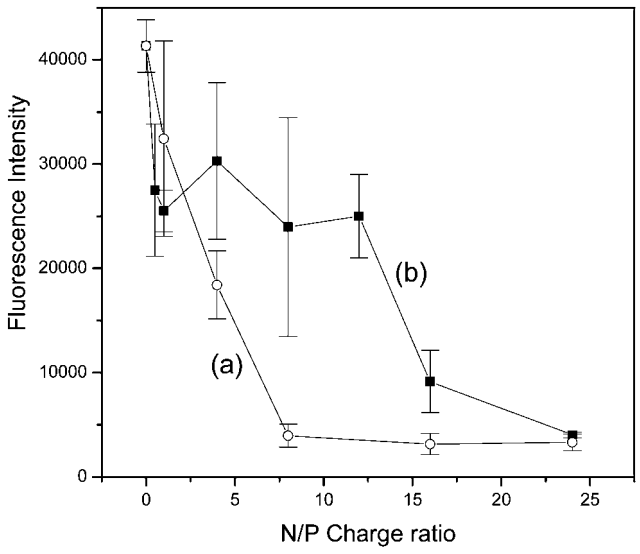


Fig. 4 Fluorescence change of (a) DNA/P10A and (b) DNA/P6A with different N/P charge ratios. Complex formation of DNA is measured with a loss of fluorescence intensity by intercalating of EtBr. EtBr solution alone showed fluorescence intensity of 400–600.

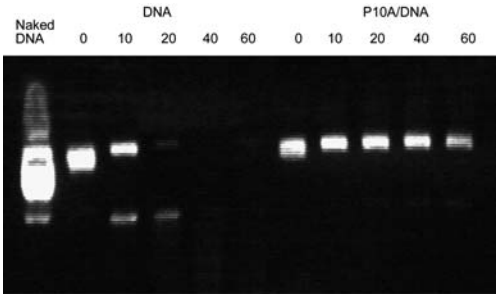


Fig. 5 Gel electrophoresis exposed to DNase for free DNA and P10A/DNA for 60 min.

P10A/DNA nanoparticles. P10A/DNA nanoparticles after polymerization had limited mobility during gel electrophoresis (Fig. 7). This suggested that the P10A/DNA nanoparticles formed was coated by the polymerization of methacrylate group. DNA cross-linked within the nanoparticle was also protected against DNase.

To examine transfection efficiency, several samples were prepared and transfected into dividing COS cells. AS initiator was used to form cross-linked P10A/DNA nanoparticle with N/P charge ratio of 16. Post-transfection photo-irradiation was then tested for release of DNA intracellularly. Cell viability and growth were unaffected by the 10 min light exposure at 365 nm. Enhanced green fluorescence protein (EGFP) was monitored by flow cytometry (Fig. 8). The histograms in flow cytometry show the count of cells in each sample that showed expression of the transfected DNA. The control sample shows a control DNA, transfected into dividing COS cells, which produced little fluorescence as shown in Table 1. Delivery of EGFP plasmid with uncross-linked P10A, which was not regulated by light, resulted in 6.3% transfection. Delivery of EGFP plasmid with uncross-linked P10A, which was exposed to 365 nm light showed 5.7% transfection. This demonstrates that the uncross-linked P10A had no effect even though the P10A can be photocleaved. Delivery of EGFP plasmid with cross-linked P10A without photoactivation resulted in 5.7% transfection. In contrast, the

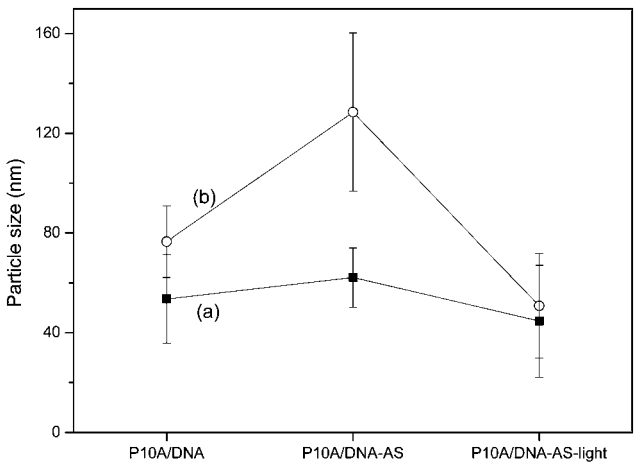


Fig. 6 Particle size before and after polymerization, and after photo irradiation for P10A/DNA complexes with N/P charge ratios (a) 16 and (b) 24.

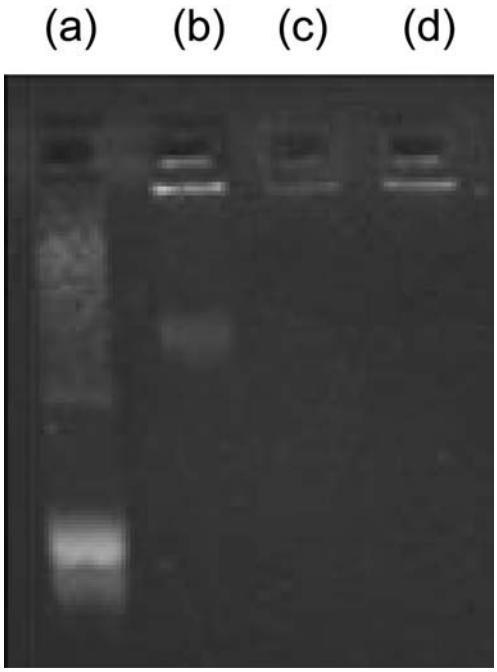


Fig. 7 Gel electrophoresis of (a) naked DNA, (b) before polymerization of P10A/DNA (N/P charge ratio 16), and (c) without and (d) with photoirradiation (365 nm) for 10 min after polymerization with ammonium persulfate.

amount of transfection was dramatically increased from 5.7% to 18.4% when cells transfected with cross-linked P10A/DNA were exposed to 365 nm light. This experiment demonstrated that DNA was caged by the cross-linked P10A and that gene expression increased markedly when the *o*-nitrobenzyl in P10A was broken down with light.

The present study sought to demonstrate the phototriggered release of DNA from cationic polymers. In comparison with our prior studies,[2] the resulting transfection efficiency after photolytic release is comparable, but not exceeding, that obtained with lipofectamine. Future improvements of this approach will seek to increase DNA caging prior to light exposure and to increase the functionally of DNA after light exposure.

Table 1 Gene expression efficiency

Sample	% FI > 100[a]	Mean FI
Control	0.01%	55
Uncross-linked P10A/DNA	6.3%	3266
Uncross-linked P10A/DNA + light	5.7%	3206
Cross-linked P10A/DNA	5.7%	3187
Cross-linked P10A/DNA + light	18.4%	3274

[a] The percent transfection is defined as fluorescence intensity (FI) of greater than 100 (background cellular fluorescence < 10). FI incubated with P10A only showed <0.01%.

4. Conclusions

The present work provides a newly designed photolabile gene carrier that is cationic, cross-linkable, and photolytic, permitting the light-triggered, controlled release of a plasmid. In addition, this methodology does not seek to directly alter the DNA backbone with a photolabile moiety. The photolabile gene carrier allowed a 3-fold increase in transgene expression following light exposure. Moreover, the nanoparticles yielded a small packing size, DNA protection, and reduced DNA leakage until photo-triggering. Accordingly, these materials are directed at providing timed delivery of DNA without endosomal degradation, potentially bypassing the deleterious endosomal toll-like receptor response. Consequently, such photo-regulated, cross-linkable reagents offer new options in the useful generation of stable DNA nanoparticles for temporally-controlled, spatial-addressed,

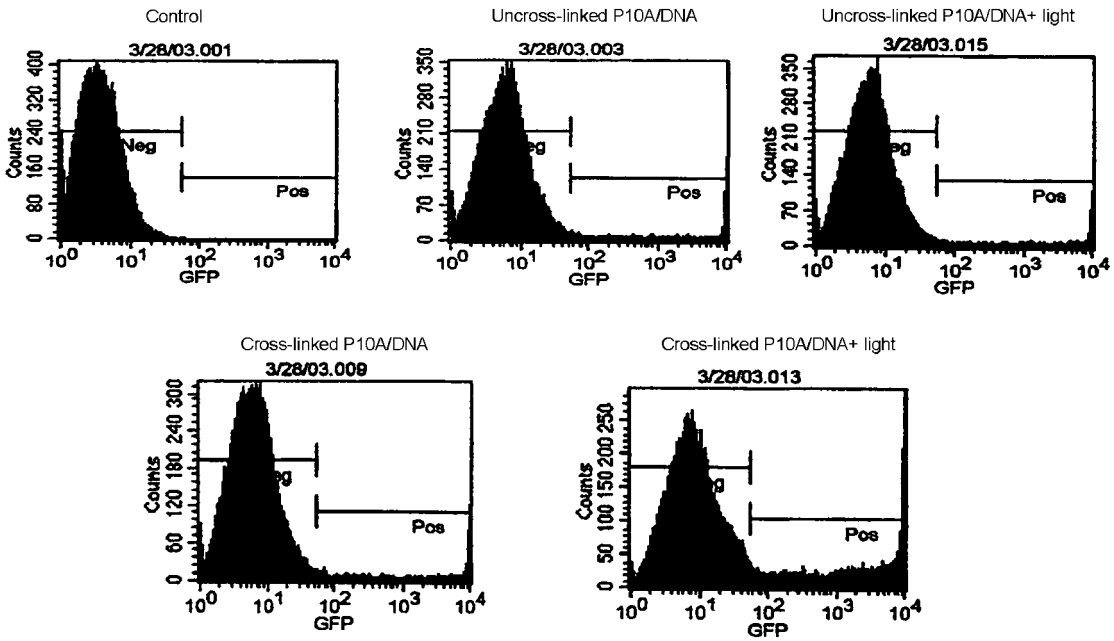


Fig. 8 Histograms for the expression of enhance green fluorescence protein transfected to dividing COS cells.

and metered dosing of DNA for gene transfer. Even though the material in this work acts as a photolabile gene carrier, a special photo-device for *in vivo* working of this material due to the penetration depth of 365 nm light (<0.1mm into the tissue) and an additional procedure for residue initiators might be necessary for the future work.

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