

Copyright 2013 Xiaojian Deng

PHOTO-RESPONSIVE DEGRADABLE POLY (BETA-AMINO ESTER) TOWARD
NON-VIRAL GENE DELIVERY

BY

XIAOJIAN DENG

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Materials Science and Engineering
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2013

Urbana, Illinois

Advisor:

Professor Jianjun Cheng

ABSTRACT

Among synthetic cationic polymers, poly(beta-amino ester) (PBAE) is one of ideal candidates for non-viral gene therapy due to its desired gene delivery efficiency, biocompatibility, and biodegradability. In order to further improve the gene delivery efficiency of PBAE by facilitating the intracellular DNA dissociation, we herein report the design and development of a photo-responsive PBAE as a novel gene delivery vector which undergoes polymer degradation and promoted DNA release in response to external UV irradiation. Photo-responsive PBAE was synthesized by Michael addition of amines to (2-nitro-1,3-phenylene)bis(methylene) diacrylate (NPBMD) as a UV-responsive segment. Non-responsive PBAE (control polymer) was also synthesized when a UV-nonresponsive monomer 1,3-phenylenebis(methylene) diacrylate (PBMD) was used. By changing the monomer type and diacrylate/amine ratio, a library of UV-responsive PBAEs were obtained. Upon a screening process on the transfection efficiencies, A1-13700 was identified to be the top-performing candidate and thereafter subjected to the assessment of UV-responsive gene delivery properties. Gel permeation chromatography (GPC) and UV-vis analyses confirmed that UV irradiation triggered degradation of the responsive polymer but not the control polymer. The cationic PBAE condenses DNA into 100-nm complexes due to electrostatic interactions, and thus facilitated the cellular internalization via caveolae-mediated endocytosis. Upon UV-irradiation, particle size of the polymer/DNA complexes was augmented and DNA release was promoted as evidenced by an ethidium bromide exclusion assay and a gel retardation assay. As a result of the facilitated intracellular DNA release, UV irradiation post-transfection led to an up-to-2-fold improvement in terms of gene transfection efficiency in HeLa, COS-7, 3T3-L1 cells. Control polymers exhibited unappreciable alteration in the above assessments, further substantiating the trigger-responsive performance of PBAE towards UV light. The cytotoxicity of the polymer was slightly reduced upon UV irradiation, which suggested the degradation of cationic polymer induced

cytotoxicity upon polymer degradation. This study thus provides an effective modulation over the gene transfection process using external stimuli, and helps overcome the intracellular barriers against non-viral vector mediated gene transfer.

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to Professor Jianjun Cheng who provides strong mentoring and support to this project. Without his generous guidance, it is impossible to finish this thesis. At the same time, I also want to thank Dr. Lichen Yin who put a lot of efforts in the preparation of this thesis. Dr. Zhang Yanfeng also helped a lot with the chemical synthesis and analysis. Finally, thanks to my family who give me the strength and confidence all the time.

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION.....	1
CHAPTER 2: EXPERIMENTAL SECTIONS.....	3
CHAPTER 3: RESULTS AND DISCUSSIONS.....	8
CHAPTER 4: SUMMARY.....	14
FIGURES AND TABLES.....	15
REFERENCES.....	27

CHAPTER 1

INTRODUCTION

The identification of an efficient and safe gene carrier has always been a huge challenge to the clinical gene therapy for various human diseases such as cancer [1], SCID [2], and Parkinson's disease [3]. Viral vectors, although highly efficient in terms of gene transfection, suffer from immunogenicity, low loading capacity, and challenge of scale-up [4]. As an alternative to viral vectors, non-viral vectors with improved biocompatibility and reduced immunogenicity have been developed. Commonly used non-viral vectors include liposomes, cationic polymer based polyplexes, cationic lipid based lipoplexes, and cell penetrating peptides (CPPs) [5, 6].

Polycations with high charge density and membrane activity have been widely applied as non-viral vectors which are effective in condensing the negatively charged nucleic acids and facilitating the cellular internalization. However, the high positive charge density of the polycations often cause appreciable damage to the host cells and restricts the intracellular gene release due to excessive DNA binding affinity. There have been reports of modifying polycations with various charge-reducing moieties—including saccharides, hydrocarbons, and poly(ethylene glycol) (PEG)—to reduce their toxicities. While the modified polycations benefit from improved safety profiles, they typically suffer from reduced gene delivery capabilities. Given the drawbacks of these materials, it is of great interest to design smart non-viral vectors that can realize the full transfection capacity of highly charged polycations while can be triggered to a DNA-repelling state post-transfection to facilitate intracellular cargo release and effective gene transcription. To overcome the slow release of DNA from the polymer/DNA complex, some stimuli-responsive materials have been developed, most of which rely on the acidic environment in the endosomes and redox potential in the cytoplasm. These strategies, although promising, cannot specifically control the DNA release in the target cells because the degradation kinetics is highly affected by the intracellular environment.

Poly(beta-amino ester) firstly developed by the Langer group [7] is one of the most promising transfection reagents ever developed. As compared to polyethelenimine (PEI) as one of the most widely used transfection agent, poly(beta-amino ester) shows comparable or even higher transfection efficiencies with much lower toxicity. Because the polymer is constructed via ester bond linkages, it exhibits desired degradability that would raise minimal safety issues when applied in vitro and in vivo. However, with its high cationic charges, it also shares the same problem as other polycations. Additionally, as the degradation of the ester bond is affected by the polymer structure and the cellular environment, the release of the DNA from the complex is hard to control [8]. As such, it would be interesting to incorporate the ability to modify the gene release profiles post-transfection using a controllable approach.

Herein, we report the design and development of UV-responsive poly(beta-amino ester) system for the non-viral gene delivery. Stimuli responding polymers have been widely studied and applied in the field of tissue engineering [9], smart sensor [10], and drug delivery [11]. Many of these polymers have a stimuli-responsive functional group at the end of the polymeric main chain [12]. When treated with the external stimuli, the responsive group will be removed and the polymer chain becomes unstable to initiate a step-by-step degradation as a consequence. In contrast to such strategy, we propose here to include the responsive moieties to the backbone as a spacer between individual repeating units, which will thus make the polymer more sensitive to the external stimulus. The fast and complete degradation of the polymer into small fragments is expected to facilitate the complete release of DNA intracellularly and reduce the cytotoxicity upon UV irradiation post-transfection. A typical Michael-Addition was adopted to synthesize PBAE [13], which allows easy modulation of polymer molecular weights by changing the diacrylate to amine ratio. Various types of amine-containing monomers were used to construct the PBAEs, which allows screening and identification of star materials with distinguished gene transfection efficiencies.

CHAPTER 2

EXPERIMENTAL SECTIONS

Materials

N,N'-dimethylethylenediamine (99%), 5-amino-1-pentanal (95%), 6-amino-1-hexanol (97%), bis-tris-propane (>99.0%), N,N'-bis(2-hydroxyethyl)-ethylenediamine (97%), N,N'-dibenzylethylenediamine (97%), N,N-bis(2-hydroxyethyl)-ethylenediamine (97%), cyclooctylamine (97%), 1-(2-aminoethyl)pyrrolidine (98%), serinol (98%), histamine (>97.0%), 1-(3-aminopropyl)-imidazole (98%), 1-(2-aminoethyl)piperidine (98%), 4-amino-1-butanol (98%), 1,3-dimethyl-2-nitrobenzene (99%), borane tetrahydrofuran complex solution, potassium permanganate (99%) and acryloyl chloride (97%) were purchased from Sigma-Aldrich and used as received. 1,3-Bis(4-piperidinyl)propane was obtained from Alfa Aesar and (+)-3-amino-1,2-propanediol (>98%) was purchased from Fluka. All the solvents were bought from Fisher and used as receive.

Monomer Synthesis

All amines were purchased as described above. 2-Nitro-1,3-benzenedimethanol was synthesized from 1,3-dimethyl-2-nitrobenzene, following similar procedures reported in the previous literature [6]. (2-nitro-1,3-phenylene)bis(methylene) diacrylate was synthesized as following. TEA (1 M, 200 mL) was added dropwise into a solution of 1,3-dimethyl-2-nitrobenzene (8.0 g, 38 mmol) dissolved in anhydrous DCM (50 mL) with the protection of N₂ for 1 h. Acryloyl chloride was then slowly added into the reaction mixture by syringe. After 18 hours, the mixture was filtered and the filtrate was evaporated with a rotary evaporator. The residue was redissolved in ethyl acetate and washed with saturated NaCl solution (3×100 mL). The organic layer was dried over anhydrous MgSO₄ overnight before the solvent was removed on a rotary evaporator. The resulting yellow solid was further purified by silica gel chromatography (hexane:ethyl acetate = 1:1). White crystals were obtained. The monomer structure was confirmed by ¹H NMR spectroscopy and further stored in

dark (Scheme 2) (Fig.1).

Polymer Synthesis

The synthesis of polymer was based on the paper published by Langer Group. The approach used in this study prevents the formation of byproducts and makes the purification easier. A library of PBAEs was synthesized and screened using Diacrylate monomers (A and B) and amine monomers (1-16). In attempt to further understand the effects of amine structure, amines with different function groups were used in the study. On the other hand, (2-nitro-1,3-phenylene)bis(methylene) diacrylate was used to react with the amines, yielding the poly(beta-amino ester), whose structure is UV sensitive. 1,3-phenylenebis(methylene) diacrylate was employed to obtain the control polymers.

We selected methylene chloride as the general solvent in the polymerization following the previous papers as it facilitates the yield of the polymers with high molecular weight. For some amines such as 2-(1H-imidazol-4-yl)ethanamine and 3-(1H-imidazol-1-yl)propan-1-amine that could not dissolve in methylene chloride, DMSO was chosen as the solvent. The polymerization usually took four days at 60°C to accommodate different amine structures. Different diacrylate/amine weight ratios (1/1.3-1/1.05) were applied to obtain the polymers with different molecular weight. After four days, methylene chloride was removed by high vacuum and polymers were further purified by precipitation twice in ether to get a gummy solid, or a viscous liquid. In the case of DMSO, pure products could be obtained by direct precipitation in ether for several times. The resultant polymers were subsequently characterized by DMF GPC.

The Degradation of PBAE under UV Irradiation

A1-13700 was dissolved in DMF at the concentration of 10mg/ml, followed by the exposure to UV irradiation (20mW/cm²) for 0min, 1min, 3min, 5min, 10min, 20min, 30min, 60min, respectively. The resultant solutions were further characterized by DMF GPC.

Preparation of PBAE/pDNA Polyplexes

Polymer stock solution was prepared at the concentration of 100mg/mL in DMSO. The stock solution was diluted with different volumes of 25 mM sodium acetate buffer (pH 5.2) to obtain polymer solutions with various concentrations. The eGFP plasmid DNA was dissolved in DI water at 0.1 μ g/ μ L. The polymer/DNA complexes were formed by adding 15 μ L DNA solution into 60 μ L polymer solution. The mixture was vortexed for 30s and further incubated at 37°C for 20 min to allow complex formation. The resultant mixtures were characterized by dynamic light scattering (DLS).

Cell Culture

HeLa, COS-7 and 3T3-L1 cells were purchased from ATCC (Manassas, VA, U.S.A.). Cells were cultured in Dulbecco's Modified Eagle Medium (Sigma-Aldrich, St. Louis, MO) with 10% fetal bovine serum, 100 units/mL penicillin, 100 μ g/mL streptomycin, at 37°C under 5% CO₂.

***In vitro* Transfection**

In vitro transfection was conducted by using eGFP. HeLa, COS-7 and 3T3-L1 cells were seeded in 24-well plates at 15000 cells/well and incubated for 24 h. The medium was replaced by Opti-MEM medium, into which DNA/polymer complexes (50 μ L) were added. After incubation for 4 h, the medium was replaced by serum-containing DMEM and cells were further incubated for 48 h. The cells were harvested and analyzed by flow cytometry. Cells treated with Lipofectamine/DNA complex and naked DNA were included as positive and negative control, respectively. The weight ratio of Lipofectamine/DNA was 1/1. Cells were exposed to UV irradiation after incubation with DNA/polymer complexes for 4 h to facilitate the release of DNA from the polymer/DNA complex.

Cell Viability

The cells were seeded in 96-well plates at 5000 cells/well and incubated for 24 h.

The medium was replaced by Opti-MEM medium, into which DNA/polymer complexes were added (12.5 μ L) at 0.1 μ g DNA/well. After incubation for 4 h, the medium was replaced by serum-containing DMEM and cells were further incubated for 20 h. Cell viability was then evaluated by the MTT assay. MTT Reagent (10 μ L) was added into each well and the cells were incubated for 4 h. Supernatant was removed from each well and 100 μ L DMSO was then added. The plates were put on a shaker in dark for 2 h. The optical absorbance was measured by plate reader at the wavelength of 570 nm. Cells without complex treatment served as the control and results were presented as percentage viability of control cells. Cytotoxicity induced by UV irradiation was measured without the addition of DNA/polymer complexes.

Intracellular Uptake

The cells were seeded in 96-well plates at 5000 cells/well and incubated for 24 h. DNA solution was diluted to 0.1 μ g/ μ L and labeled with YOYO-1. The medium was replaced by Opti-MEM medium, into which DNA/polymer complexes were added (25 μ L) at 0.2 μ g YOYO-1-DNA/well. After incubation for 1 h, 2 h, and 4 h, respectively, cells were washed by PBS for three times and thereafter lysed with 100 μ L of RIPA lysis buffer. The fluorescence of YOYO-1-DNA content was measured by plate reader and the protein content was determined by BSA kit. Uptake level was presented as ng YOYO-1-DNA associated with 1 mg of cellular protein. In order to visualize the intracellular distribution of YOYO-1-DNA/polymer complexes, cells were incubated with complexes in serum-free DMEM (6-well plate) for 1 h, 2 h and 4 h, respectively. Cells were then stained with DAPI according to the manufacturer's protocol and were visualized by confocal laser scanning microscopy (CLSM, LSM 700, Zeiss, Germany).

Gel Retardation

Ethidium bromide was added to plasmid DNA solution and further mixed with different volumes of PBAE solution to obtain the complexes at different polyme/DNA weight ratios. The complexes were incubated at 37 $^{\circ}$ C for 30 min before they were

subjected to electrophoresis in 1% agarose gel (1 X TAE, 65 V) at 100 mV. After 30 min, the gel was rinsed with DI water to remove excess ethidium bromide. DNA printing was visualized with UV transilluminator at 302 nm.

EB Exclusion

EB solution was mixed with DNA at the DNA/EB ratio of 10:1 (w/w) and incubated at room temperature for 30 min. Polymers were then added to the mixture at various weight ratios (polymer to DNA). A pure EB solution and the DNA/EB solution without any cationic polymer were used as negative and positive controls, respectively. EB exclusion efficiency (% DNA condensed) was defined as:

$$\text{EB exclusion efficiency (\%)} = \left(1 - \frac{F - F_{EB}}{F_0 - F_{EB}} \right) \times 100$$

where F_{EB} , F , and F_0 denote the fluorescence intensity of pure EB solution, DNA/EB solution with polymer, and DNA/EB solution without any polymer, respectively. For the EB exclusion under UV irradiation, the complexes were exposed to UV irradiation (20 mW/cm²) for different time before measurement.

CHAPTER 3

RESULTS AND DISCUSSIONS

Polymer Synthesis

In order to obtain the best-performing material in terms of transfection, a library of PBAEs was synthesized and screened (Scheme 1). Most of the synthesized PBAEs in this study were yellowish viscous liquid and could not dissolve in the water at the physiological pH. When the pH was reduced to 5.2 (25 mM sodium acetate buffer), PBAE became water soluble due to the protonation of amine groups, resulting in clear solution. Based on the GPC measurement, it could be concluded that molecular weight varies relative to the diacrylate/amine weight ratio. Theoretically, the highest molecular weight occurs when the diacrylate/amine ratio is 1/1. However, according to the previous paper, amine terminated PBAEs show better transfection efficiency. Therefore, diacrylate/amine mole ratios from 1/1.3 to 1/1.05 were employed in this study. The molecular weight varied from 3000 to 14000 Da based on the DMF GPC results. All the PBAEs were preserved in dark at -20°C soon after synthesis, in order to decrease the hydrolysis of ester bonds in the backbone of polymers.

The Degradation of PBAE under UV Irradiation

The degradation of PBAE upon UV irradiation was an important issue in this study. UV irradiation was fixed at 20mW/cm². Indicated by the previous work of Zhao Group [6], nitrobenzene group could transfer to nitrosobenzene group upon UV irradiation. Meanwhile, the ester bond next to the benzene would be cleaved, resulting in the formation of carboxylic acid and aldehyde groups. A1-13700 and B1-11500 were chosen for the degradation study, which represented the UV responsive and non-responsive PBAEs, respectively. Both solutions were initially clear and colorless. However, after exposure to UV irradiation for 3 min, A1-13700 solution became yellowish while B1-11500 solution remained colorless (Fig. 6). As the exposure time was increased, the color of A1 solution appeared darker. GPC curves displayed that

the molecular weight of A1-13700 decreased from 13700 to 3200 Da after 30 min UV irradiation while B1 maintained the same molecular weight (Fig. 3). A1-13700 and B1-11500 solutions were further analyzed by UV-vis spectrometer. A1-13700 and B1-11500 were dissolved in THF at 0.06mg/mL, followed by the exposure to UV irradiation as described above. A1-13700 immediately showed a strong absorption at the wavelength of 280 nm after exposure to UV irradiation for only 30 s, indicating the formation of nitrosobenzene in the solution (Fig. 4). The absorbance increased as the exposure time was elongated. Meanwhile, the absorbance of B1-11500 remained the same, which demonstrated that B1-11500 did not degrade upon the UV irradiation (Fig. 5).

Characterization of PBAE/DNA Complexes

Complexes were formed at various A1-13700/DNA weight ratios (Fig. 9), the particle size and zeta potential of which were measured by DLS. Particle size became larger with A1-13700/DNA weight ratio increasing. When A1-13700/DNA weight ratio reached 1.5, the largest particle size was observed, which suggested the neutralization of charges. With higher weight ratio, the particle size became smaller. When A1-13700/DNA weight ratio was higher than 20:1, the particle size maintained around 120-180 nm, which was ideal for the internalization of complexes by cells. When particles were exposed to UV irradiation, an interesting phenomenon was observed that particle size dramatically became larger against the irradiation time. After 6 min UV irradiation, the particle size reached about 2000 nm (Fig. 6). It was initially proposed that the particle size should decrease as the polymer degraded. The increase of particle size was contributed to the destabilization of complexes induced by the polymer degradation. Zeta potential was negative when A1-13700/DNA weight ratio was below 2/1. As the weight ratio was above 5/1, the zeta potential maintained at about 30 mV, which would facilitate the interaction between complexes and cell membranes.

The binding ability of PBAE to DNA was characterized via gel retardation. The results suggested DNA could be effectively condensed when A1-13700/DNA weight

ratio was higher than 5/1 (Fig. 7). In order to quantitatively study the binding ability, EB exclusion was also performed. It was indicated that low A1-13700/DNA weight ratio led to poor binding. However, nearly 97% DNA was condensed in the case of high A1-13700/DNA weight ratio (above 5/1).

Cell Uptake

After condensation of DNA by the cationic PBAE, we then explored whether it can promote the cellular internalization of DNA. As shown in Fig. 14, A1-13700 remarkably increased the cellular uptake level of YOYO-1-DNA, and an increase in the A1-13700/DNA weight ratio up to 50 caused significant elevation in the cellular uptake level. When the A1-13700/DNA was further increased, the uptake level slightly decreased rather than kept increasing, which could be presumably attributed to the excessive amount of PBAE that did not tightly associate with the DNA and thus competitively occupied cell membranes to inhibit polyplex binding. When the incubation time was prolonged from 0.5 h to 4 h, the cellular uptake level was notably improved. Confocal laser scanning microscopy (CLSM) observation further revealed that upon 4-h incubation at 37 °C, YOYO-1-DNA could be extensively internalized and transport to the cytoplasm as well as the nuclei, which could thus allow the initiation of effective DNA transcription (Fig. 15). By performing the cell uptake study at low temperature (4 °C) or in the presence of various endocytic inhibitors, we further probed the intracellular kinetics of polyplexes. As illustrated in Fig. 16, the cell uptake level was significantly inhibited by genistein and m β CD, inhibitors of caveolae-mediated endocytosis. Wortmannin that inhibited macropinocytosis and chlorpromazine that inhibited clathrin-mediated endocytosis showed slight or unappreciable inhibitory effect, indicating that the cellular internalization was not strongly related to these two pathways. Lowering the temperature also led to a marked decrease in the cell uptake level, which accorded well with the energy-dependent endocytic mechanism of the polyplexes.

DNA Release in Response to UV Irradiation

The UV-triggered DNA release from polyplexes was evaluated qualitatively using a gel retardation assay and quantitatively using an EB exclusion assay. As shown in Fig. 7, both responsive A1-13700 and non-responsive B1-11500 condensed DNA well and restricted DNA migration in the loading well after gel electrophoresis. Upon UV irradiation for 5 min, some of the DNA was released from the A1-13700/DNA complexes, as evidenced by the migrated DNA band that accorded well with the naked DNA. In comparison, no DNA release was noted for the non-responsive B1-11500/DNA complexes, which further substantiated that DNA release from the A1-13700/DNA complexes was indeed attributed to the UV-triggered polymer degradation. Such observation was further supported by the EB exclusion assay, which revealed that UV-irradiation could accelerate the DNA release from complexes, achieving ~90% of DNA release rate compared with only 30% DNA release for the non-irradiated complexes (Fig. 10, 11).

Transfection in Response to UV Irradiation

Because UV irradiation may cause potential cytotoxicity and mutation in mammalian cells, we first evaluated the UV-induced cytotoxicity in the three tested cell types to provide a proper guidance for transfection usage. Our preliminary study demonstrated that cell viability was not notably compromised upon UV treatment for no longer than 10 min, while a further prolonging of the irradiation time caused appreciable toxicity to cells. As such, the UV irradiation time was fixed to 10 min for all the transfection and cytotoxicity studies.

Prior to the assessment of UV-responsive gene transfection performance, we first screened the polymer library, aiming to identify the top-performing candidate with proper molecular weight and amine type for down-stream assessments. As shown in Fig. 1, we noticed that PBAEs showed different transfecting abilities in response to their different structures and molecular weights. Most of the high transfecting PBAEs shared the similar structures--either having the mono alcohol and amine on both sides of a linear monomer (A1-A3) or possessing an imidazole group (A4-A5) that displays desired buffering ability. Additionally, PBAEs containing hydrophobic side chains

such as A8 and A11 showed low transfecting abilities. The molecular weight also plays an important role in the transfection process. A1 with low molecular weight of about 3000 Da showed poor transfection efficiency, which was due to the insufficient binding with DNA and the weak capability to mediate cellular uptake. For PBAEs with molecular weights of about 6000 Da, most of the polymers also gave the transfection efficiency lower than 20%. For instance, A1-5100 gave the transfection efficiency of 11% while A1-8900 displayed transfection efficiency of 25%. In comparison, when A1-13700 with higher molecular weight was used, a significantly higher transfection efficiency of 42% was obtained. These results thus pointed out the importance of polymer molecular weight in terms of gene transfection, because higher molecular weight polymers usually possess stronger DNA condensation capacity to yield better transfection ability. One interesting finding was that A4 and A5, although possessing low molecular weight of 3000 Da, could still lead to a transfection efficiency of over 10% in comparison to the inability of A1-A3 at the same molecular weight. It thus substantiated the importance of imidazole groups in mediating effective endosomal/lysosomal escape via a “proton-sponge” effect.

As we have determined that UV irradiation could facilitate the release of DNA from the complexes, we then investigated whether such mechanism could ultimately lead to improved transfection upon UV irradiation. Eight PBAEs with higher transfection efficiencies than LipofectamineTM 2000 (Lipo2000) were chosen for such assessments (Fig. 17). Cells were incubated with the complexes for 4 h to allow effective cellular internalization, and UV irradiation was performed post-transfection with an attempt to facilitating cytoplasmic DNA release. In our preliminary studies, we showed that increasing the irradiation time from 0.5-10 min correlated to an increase in the transfection efficiencies (data not shown), which correlated well with their degradation kinetics. When the irradiation time was fixed at 10 min, all the eight tested polyplexes revealed a 1.2-1.5-fold increment in the transfection efficiencies in HeLa cells. Such results were also confirmed by fluorescent images where an increased amount of GFP-positive cells were noted. Non-responsive B1-11500 polyplexes and Lipo2000 showed a slight decrease in their transfection capabilities,

which could be attributed to the slight loss of cell functions induced by UV irradiation.

Cell Viability

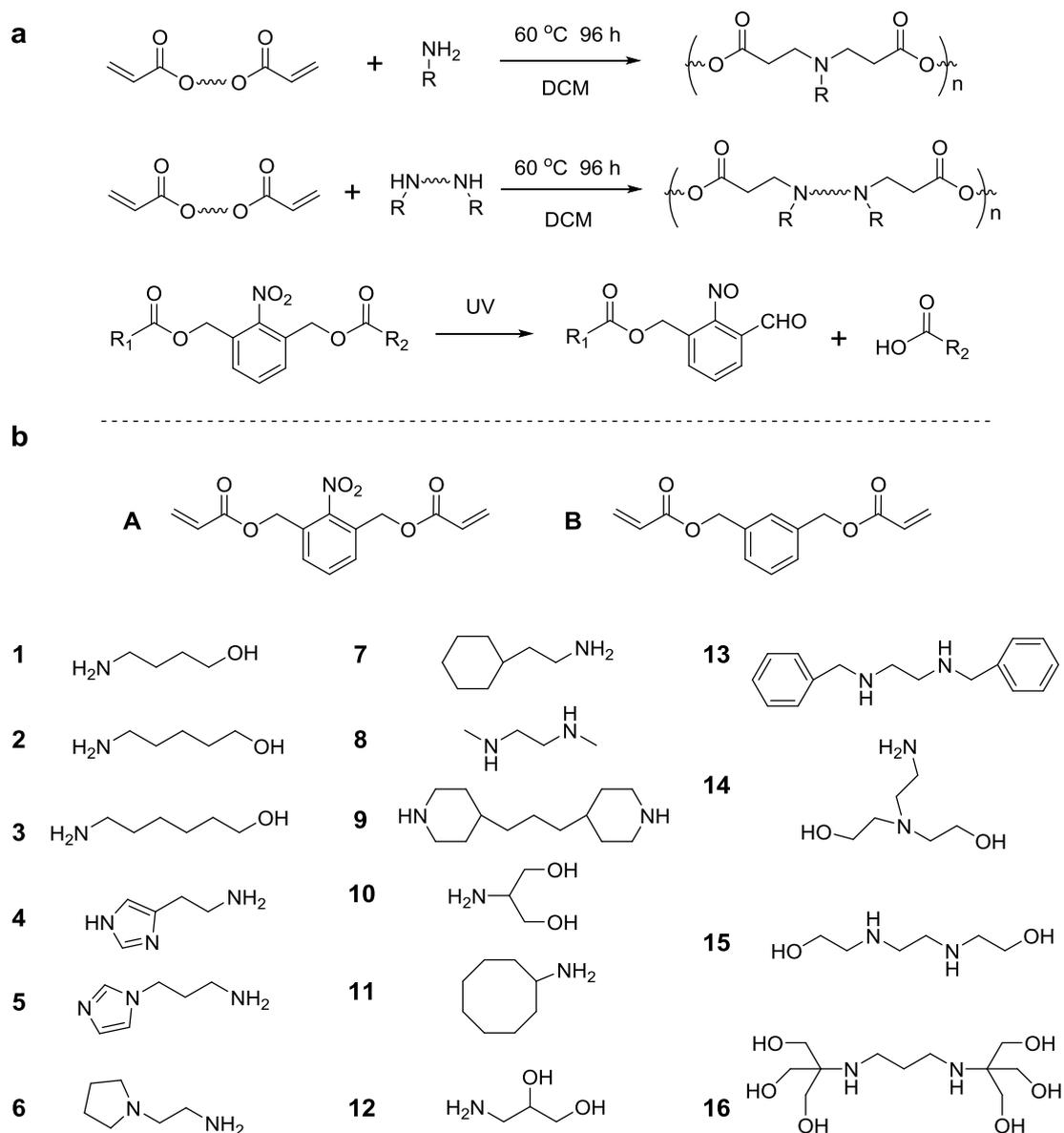
The cytotoxicity of PBAEs in response to UV irradiation was evaluated by a MTT assay wherein non-irradiated or irradiated polyplexes were added to cells before a further incubation for 24 h. As shown in Fig. 20, an increase in the cytotoxicity was noted when the polymer/DNA weight ratio increased, which was due to the excessive amount of free polymer that exerted appreciable damage to the cells. At the transfection condition (polymer/DNA ratio of 50/1), ~80% of the cells remained alive. In comparison to these non-irradiated polyplexes, irradiated polyplexes showed significantly lower cytotoxicity, and an increase in the irradiation time further correlated to higher cell tolerance of the polyplexes. This trend accorded well with the degradation profiles of the PBAE, wherein longer irradiation time led to more complete degradation with smaller degraded segments. These small pieces would exert lower cytotoxicity which could be attributed to the restricted binding sites with cell membranes on each individual polymer chain. Such results also correlated well with previous studies where high molecular weight polymers often induce higher cytotoxicity than lower molecular weight counterparts. In agreement with the degradation profiles, the non-responsive PBAE demonstrated unappreciable alteration in cytotoxicity due to maintenance of its polymer structure after UV irradiation.

CHAPTER 4

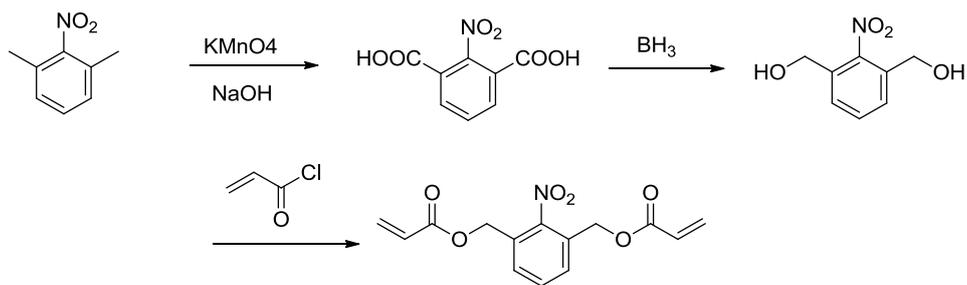
SUMMARY

In this study, a new kind of PBAE which was designed contained a UV responsive function group in the main chain which can break the ester bond under the UV irradiation quickly resulting degrade the PBAE into the small polymer or small molecular. This smart degradable polymer was used to develop a gene delivery system which can release DNA quickly upon the stimulus. By this strategy, transfection can be improved as DNA can be controlled release and cytotoxicity can be reduced as polymer can degrade into small molecules.

FIGURES AND TABLES



Scheme 1. a) Synthesis of poly(beta-amino ester) (PBAE). b) Structures of diacrylates (A and B) and amines (1-16) used for the synthesis



Scheme 2. Synthesis of monomer for poly (beta-amino ester)

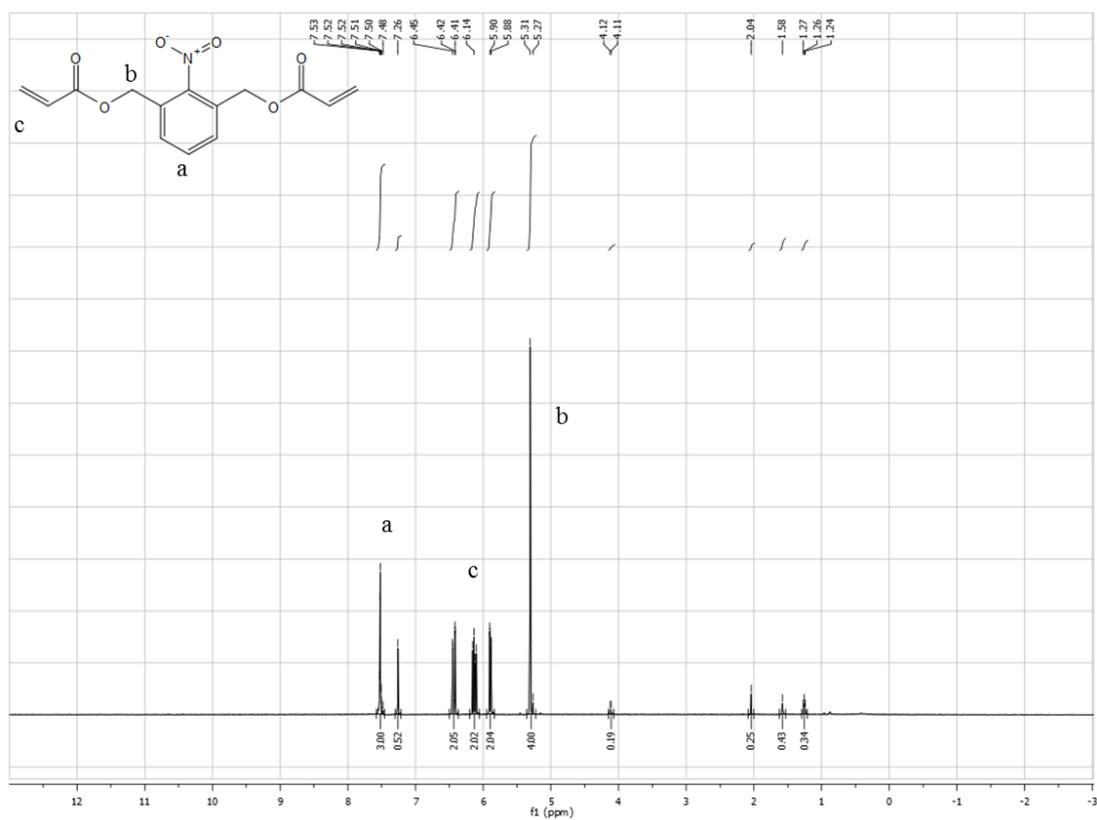


Figure 1. $^1\text{H-NMR}$ spectrum of (2-nitro-1,3-phenylene)bis(methylene) diacrylate (NPBMD) in CDCl_3 .

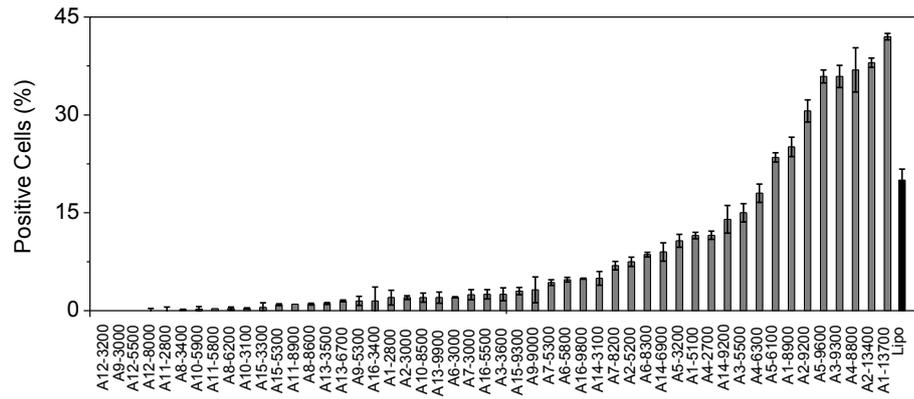


Figure 2. *In vitro* transfection efficiency of PBAE/pEGFP complexes in HeLa cells. Lipo2000 was used as a control (n = 3).

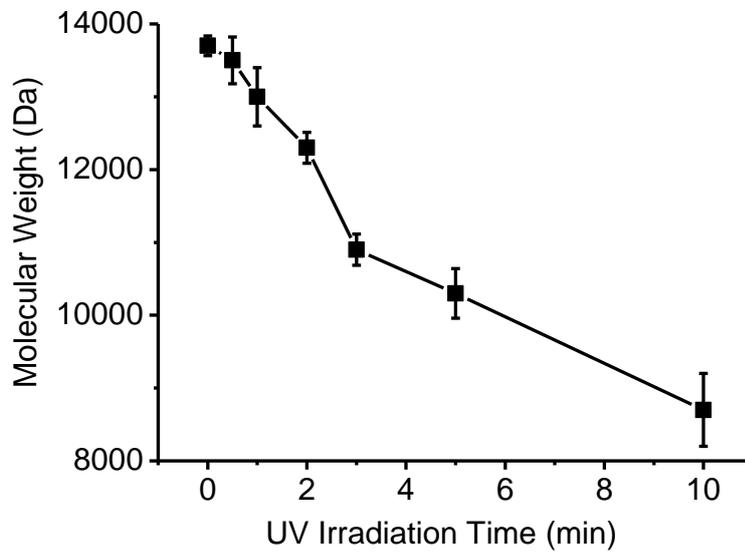


Figure 3. Molecular weight of A1-13700 upon UV irradiation (n = 3).

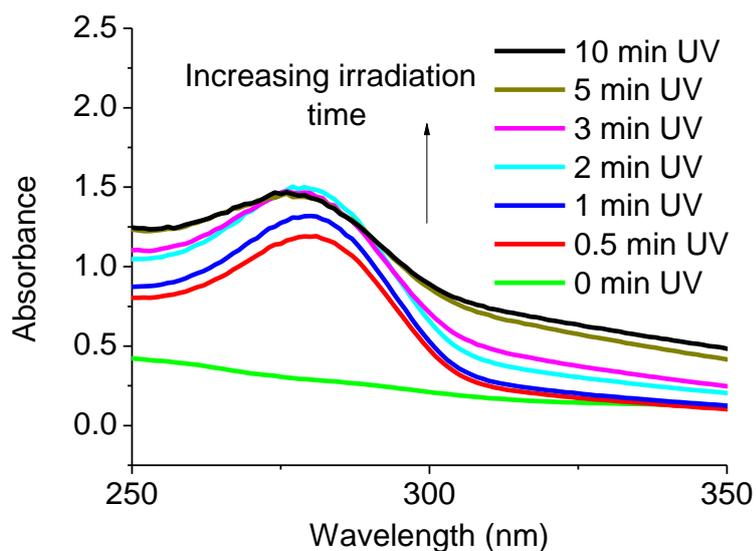


Figure 4. UV-vis spectrum of A1-13700 upon different UV irradiation time.

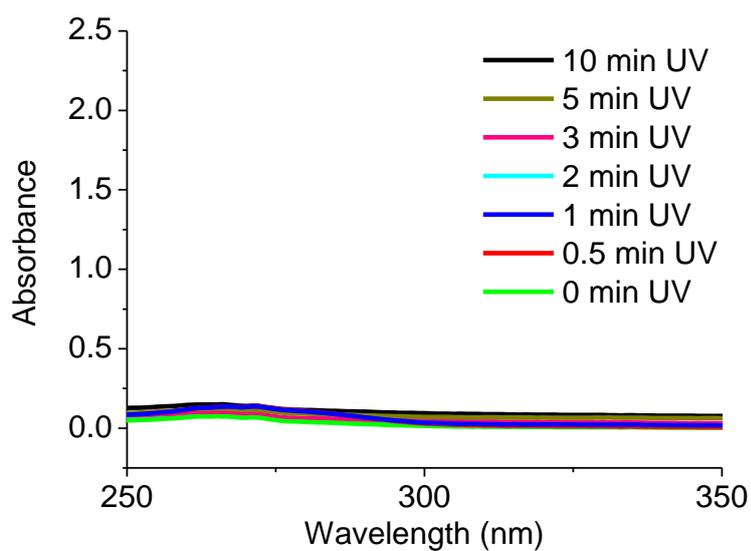


Figure 5. UV-vis spectrum of B1-11500 upon different UV irradiation time.

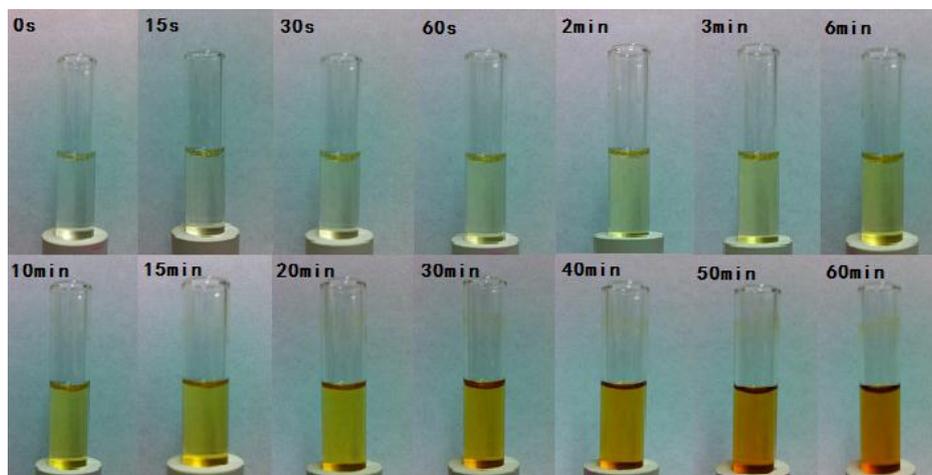


Figure 6. The solution of A1-13700 (10mg/ml in DMF) under UV irradiation ($40\text{mW}/\text{cm}^2$). The nitro group turned to nitroso group, so the color of the solution changed.

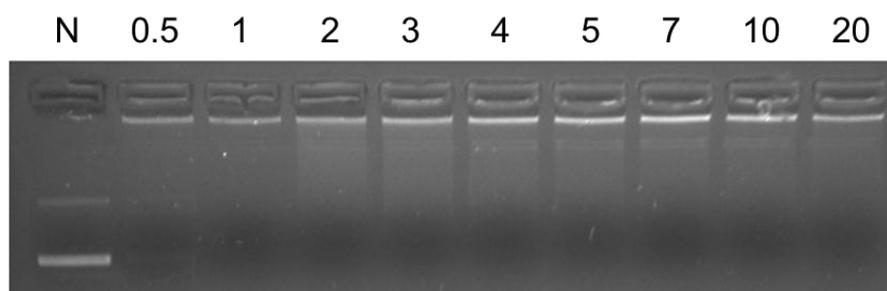


Figure 7. DNA condensation by A1-13700 at different polymer/DNA weight ratios evaluated by gel retardation assay. N represents naked plasmid DNA. The numbers above the gel correspond to the polymer/DNA weight ratios tested.

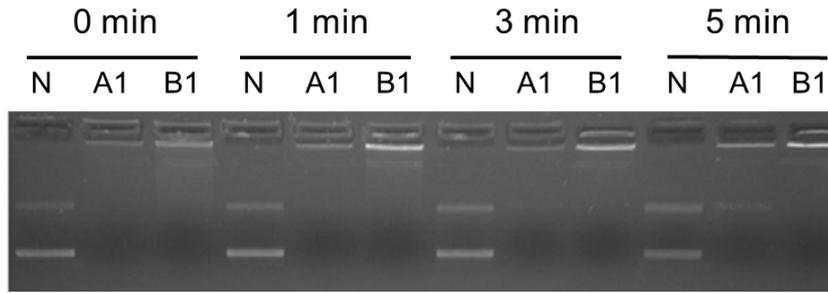


Figure 8. Study of DNA release evaluated by gel retardation assay. N represents naked plasmid DNA. A1 represents A1-13700. B1 represents B1-11500. The numbers above the lanes correspond to the UV irradiation time.

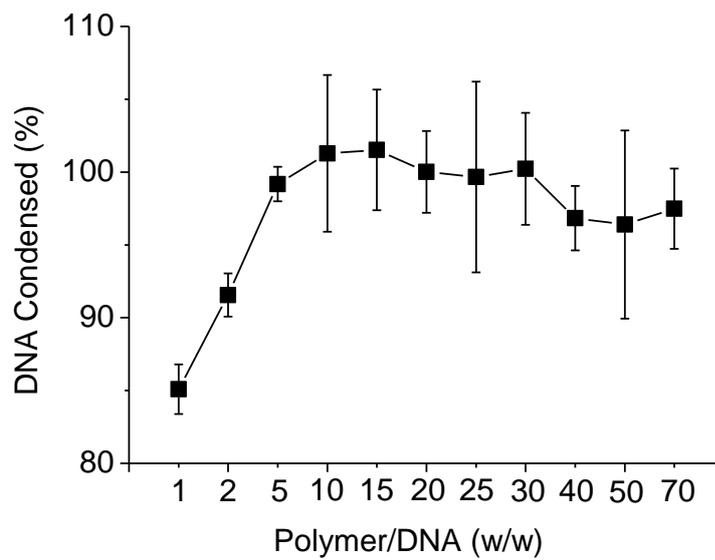


Figure 9. DNA condensation by A1-13700 at different polymer/DNA weight ratios evaluated by EB exclusion assay (n = 3).

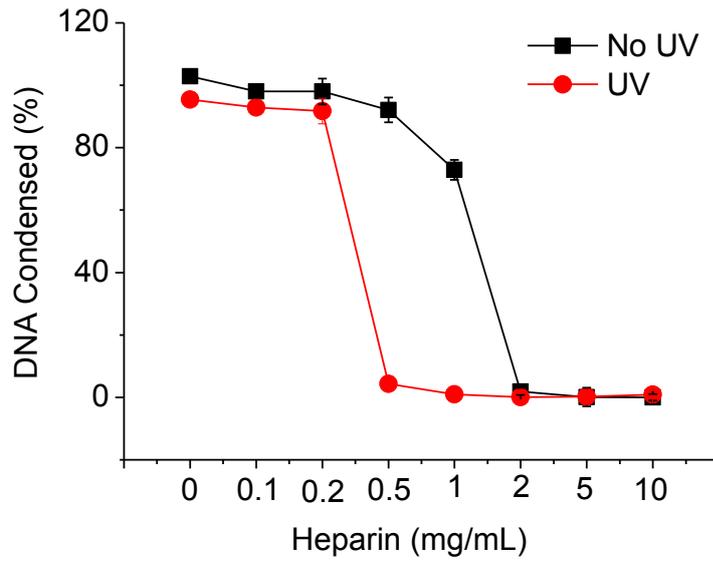


Figure 10. Study of DNA release from A1-13700/DNA complexes in the presence of heparin upon UV irradiation evaluated by EB exclusion (n = 3).

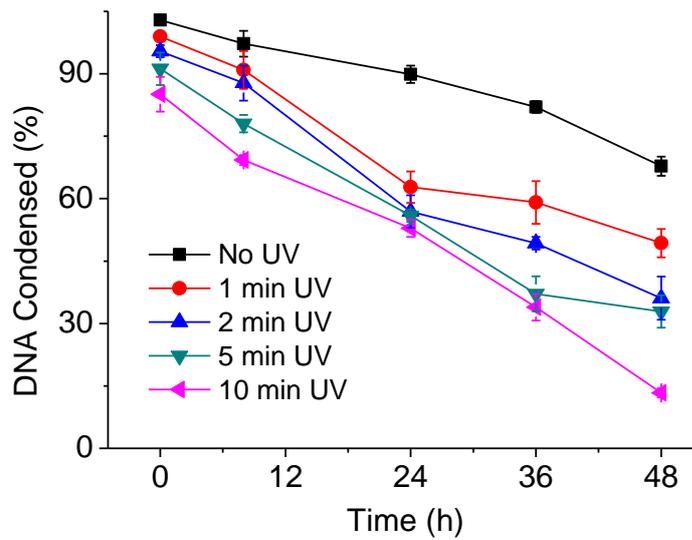


Figure 11. Study of DNA release from A1-13700/DNA complexes after two-day incubation upon different UV irradiation time (n = 3).

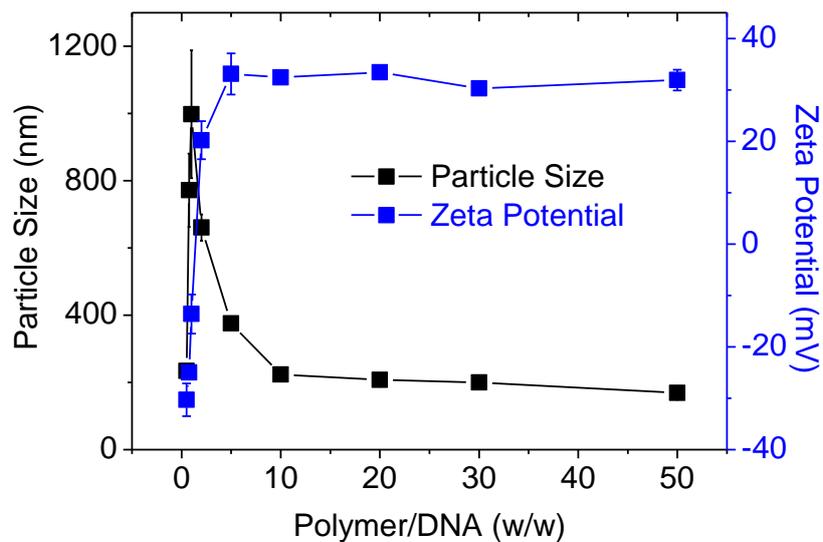


Figure 12. Particle size and zeta potential of A1-13700/DNA complexes.

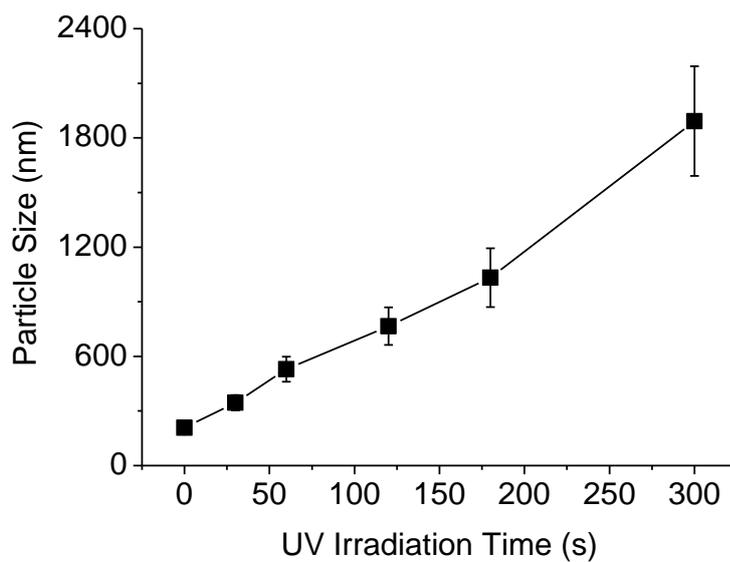


Figure 13. Particle size of A1-13700/DNA complexes upon UV irradiation (n = 3).

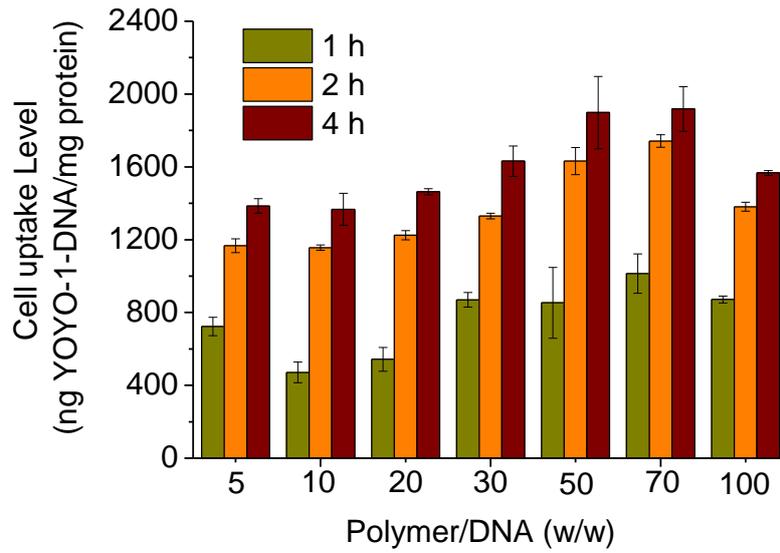


Figure 14. A1-13700 mediate efficient intracellular delivery of DNA in HeLa cells following different incubation time at various polymer/DNA weight ratios (n = 3).

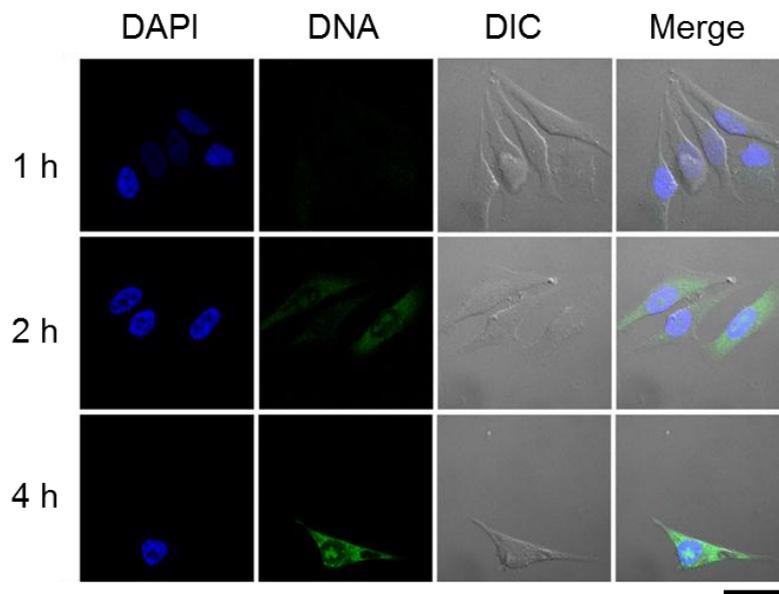


Figure 15. CLSM images showing the cellular internalization of A1-13700/DNA complex in HeLa cells following different incubation time (bar = 20 μ m).

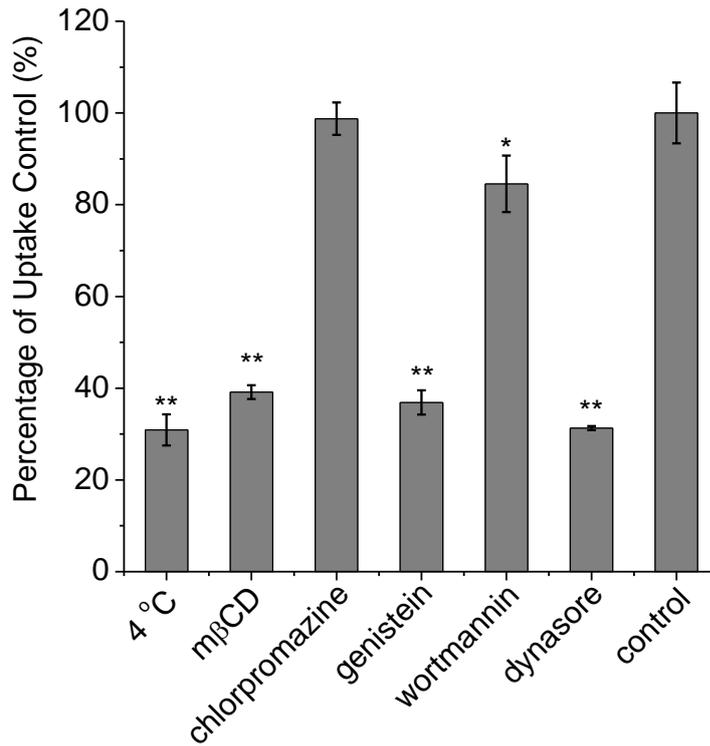


Figure 16. Uptake level of A1-13700/DNA complex in HeLa cells at 4 °C or in the presence of various endocytic inhibitors (n = 3).

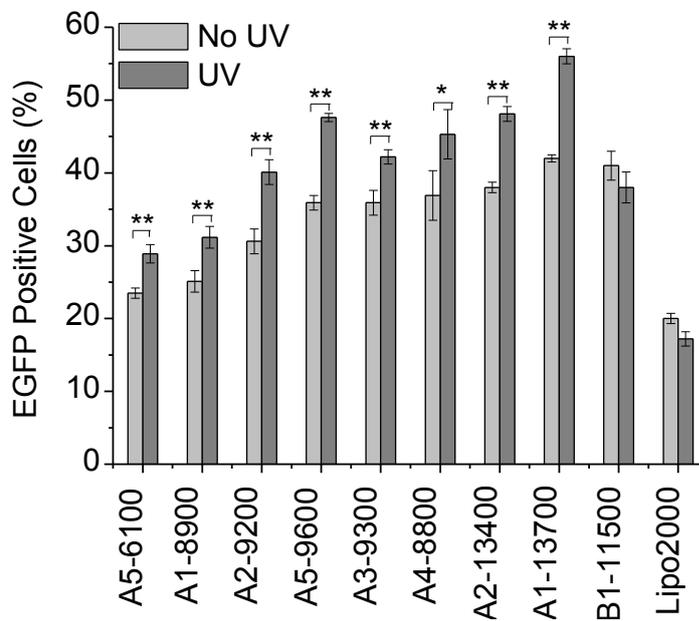


Figure 17. PBAEs mediate efficient gene transfection upon UV irradiation (n = 3).

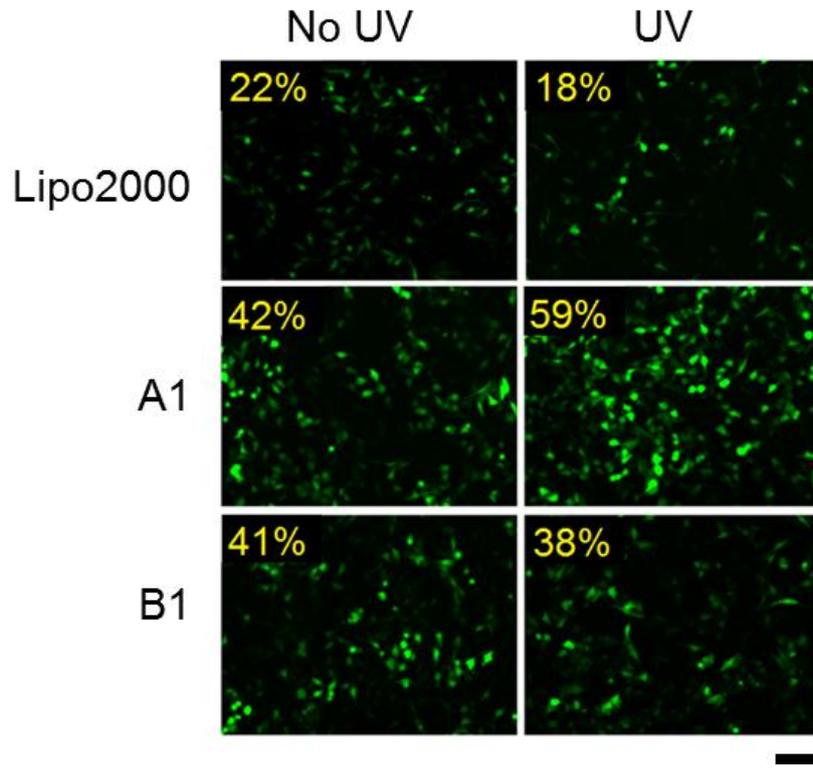


Figure 18. Fluorescence images of HeLa cells transfected by A1-13700/DNA and B1-11500/DNA complexes before and after UV irradiation (2 min) (bar = 50 μ m).

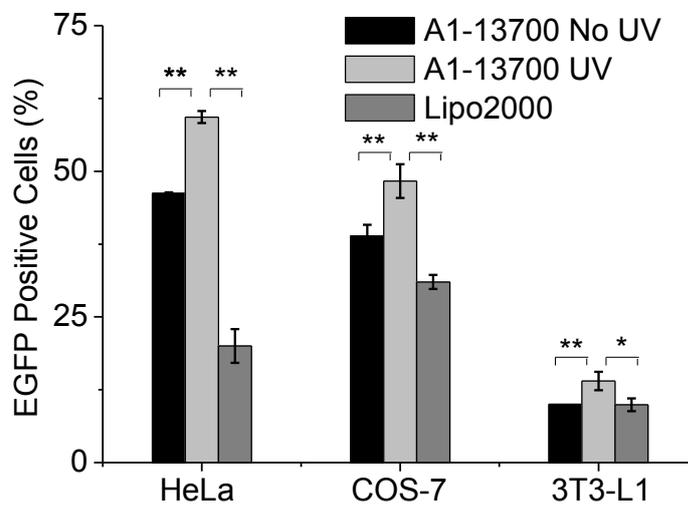


Figure 19. A1-13700 mediate efficient gene transfection upon UV irradiation in HeLa, COS-7 and 3T3-L1 cell lines compared with lipo2000 (n = 3).

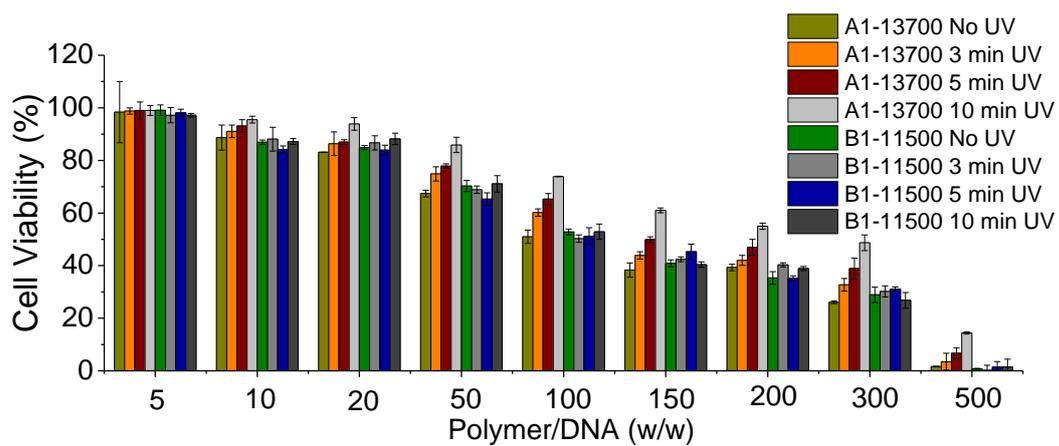


Figure 20. *In vitro* cytotoxicity of PBAEs/DNA complexes in HeLa cells upon different UV irradiation time following 24 h incubation (n = 3).

REFERENCES

- [1] Lynn DM, Langer R. Degradable Poly(β -amino esters) Synthesis, Characterization, and Self-Assembly with Plasmid DNA. *J Am Chem Soc.* 2000;122.
- [2] Han D, Tong X, Zhao Y. Fast Photodegradable Block Copolymer Micelles for Burst Release. *Macromolecules.* 2011;44:437-9.
- [3] Anderson DG, Lynn DM, Langer R. Semi-automated synthesis and screening of a large library of degradable cationic polymers for gene delivery. *Angewandte Chemie.* 2003;42:3153-8.
- [4] Schwartz J, Zhang S. Peptide-mediated cellular delivery. *Current Opinion in Molecular Therapeutics.* 2000;2.
- [5] Wang G, Tong X, Zhao Y. Preparation of Azobenzene-Containing Amphiphilic Diblock Copolymers for Light-Responsive Micellar Aggregates. *Macromolecules.* 2004;37.
- [6] Zhao Y. Photocontrollable block copolymer micelles: what can we control? *Journal of Materials Chemistry.* 2009;19:4887.
- [7] Lynn DM, Langer R. Degradable poly(beta-amino esters): Synthesis, characterization, and self-assembly with plasmid DNA. *J Am Chem Soc.* 2000;122:10761-8.
- [8] Goodwin AP, Mynar JL, Ma Y, Fleming JR, Frechet MJ. Synthetic Micelle Sensitive to IR Light via a Two-Photon Process. *J Am Chem Soc.* 2005;127.
- [9] Wang Y, Han P, Xu H, Wang Z, Zhang X, Kabanov AV. Photocontrolled Self-Assembly and Disassembly of Block Ionomer Complex. *Langmuir.* 2010;26.
- [10] Su W, Luo Y, Yan Q, Wu S, Han K, Zhang Q, et al. Photoinduced Fusion of Micro-Vesicles Self-Assembled from Azobenzene-Containing Amphiphilic Diblock Copolymers. *Macromolecular rapid communications.* 2007;28:1251-6.
- [11] Barrio JD, Oriol L, Sanchez C, Serrano JL, Cicco AD, Keller P, et al. Self-Assembly of Linear-Dendritic Diblock Copolymers From Nanofibers to Polymersomes. *J AM CHEM SOC.* 2010;132.

[12] Zou J, Guan B, Liao X, Jiang M, Tao F. Dual Reversible Self-Assembly of PNIPAM-Based Amphiphiles Formed by Inclusion Complexation. *Macromolecules*. 2009;42:7465-73.

[13] Katz JS, Zhong S, Ricart BJ, Pochan DJ, Hammer DA, Burdick JA. Modular Synthesis of Biodegradable Diblock Copolymers for Designing Functional Polymersomes. *J Am Chem Soc*. 2010;132.