Dual Stimuli-Responsive Poly(β-amino ester) Nanoparticles for On-Demand Burst Release[a]

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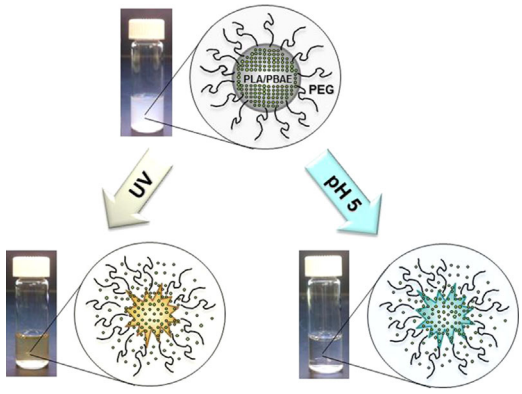
We designed poly(β-amino esters) (PBAEs) bearing both UV light- and pH-sensitive groups and used PBAEs to prepare nanoparticles (NPs) that can be utilized for on-demand burst release of guest molecules in response to multiple triggers. Due to the presence of the photo-cleavable group in each repeating unit of PBAE, rapid release of encapsulated model drug could be achieved even with exposures to low intensity UV ($10\,mW \cdot cm^{-2}$). Especially, the burst release was further accelerated by additional UV treatments in the acidic condition showing the combinatory effect of dual stimuli. We believe these PBAE-based NPs can potentially be used to design intelligent controlled release device and nanomedicines.



1. Introduction

Polymeric nanomedicine is an emerging field that utilizes polymeric nanotechnology to improve or enable the use of therapeutic and diagnostic agents for disease treatment.[1,2] Compared to conventional small molecule-based medicine, it has a number of potential advantages including long blood circulation times, high tissue penetration and retention, and enhanced cellular uptake.[3] When used as therapeutics, it is expected to exhibit improved therapeutic efficacy and reduced side effects. Therapeutic nanomedicine, however, has various challenges that remain to be overcome. One sure specific challenge is the active control over drug release, which may make it difficult to achieve the intended therapeutic efficacy.[4] In many polymeric emulsion particles, drug release rate is primarily determined by the hydrolysis rate of the polymeric materials and, therefore, may not be actively modulated.[5–7] In addition, many therapeutic polymeric delivery nanoparticles (NPs) are self-assembled systems, and such systems may suffer from dynamic instability upon dilution, which result in undesired dose dumping once administered.[8,9] Although it is possible to prepare polymeric NPs with sufficient particle stability and controlled release profiles based on conventional degradable polymers, stimuli-responsive materials should provide some unprecedented control of drug release, such as controlled burst drug release, when used in the formulation of polymeric nanomedicine.[10–12]

In the past decade, several groups showed that programmable drug delivery can be achievable by using poly(β-amino esters) (PBAEs) in the preparation of NPs.[13,14] PBAEs are biodegradable polymers that can be easily synthesized by the Michael-type addition polymerization of amines and diacrylate esters.[15] The polymers are known to have low toxicity and can be degraded into nontoxic small molecules.[16] PBAEs contain tertiary amines that can be protonated at around physiological pH.[17] This unique property provides PBAE-containing systems with enhanced

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[a]**Supporting Information** is available from the Wiley Online Library or from the author.

DOI: 10.1002/mabi.201500111

utility for cancer chemotherapies. By protonating the amines, hydrophobic PBAEs become water soluble, and the NPs derived from PBAEs can, thus, be destabilized to release cargos in acidic environment, e.g., tumor tissue. PBAEs have been reported to construct both polymeric transfection vectors and anticancer drug carriers taking advantage of such pH-responsiveness. For example, Little et al. reported microparticles composed of PLGA and PBAE (5–50%) for gene delivery.[6] When PBAE was added, the microparticle showed an increase of 3–5 orders of magnitude in transfection efficiency along with a distinct reduction in the average growth rate of tumors in mice. NPs formed by Pluronic and PBAE were reported as a pH-sensitive biodegradable system for paclitaxel delivery.[18–21] More than twofold higher concentration of paclitaxel was found in the tumor 1 h post-administration as compared to a Pluronic and PCL-blended NP.[19]

As we discussed, PBAEs originally have amines that are ionizable in acidic conditions, switching the hydrophilic/hydrophobic properties of the entire polymer chain. PBAEs are particularly attractive not only because of their pH-sensitivity but also due to the fact that they could be engineered to possess dual or multiple stimuli-sensitivities.[22] A wide variety of stimuli-responsive amines or diacrylate esters can be easily introduced to the PBAE backbone by a simple addition polymerization, rendering the physiochemical properties of PBAE-containing NPs highly tunable. Utilizing the multi-stimuli sensitivity of PBAEs, NPs fabricated with these polymers can be programmed to release actives after arrival at the site of action "on-demand," where the internal stimuli (e.g., pH, temperature, redox, etc.) will trigger disassembly of the PBAE NPs (environment-controlled delivery). When the local environment of the desired targets does not present a discretely differentiable stimuli condition from the non-target surrounding tissues, external stimuli such as light, ultrasound, and magnetism can also be employed to induce a local release of actives (remote-controlled delivery). More interestingly, combination of the two delivery strategies (environment-controlled delivery and remote-controlled delivery) will further enhance PBAE's responsivity and disassembly, which would establish PBAE-based NPs as an efficient and site-specific platform for drug delivery (combinatory controlled delivery).

In this study, we hypothesized that the incorporation of photo- and pH-sensitive PBAE in poly(ethylene glycol)-block-poly(d,l-lactide) (PEG-PLA) NPs would facilitate a dual stimuli-responsivity, as demonstrated by the UV-triggered degradation and pH-triggered solubility change of PBAE illustrated in Figure 1. In order to utilize the combination degradation pathways, photocleavage of nitrobenzyl groups by UV irradiation and pH-mediated protonation and solubilization of PBAE in water, for drug delivery platform design, NPs based on PBAE and PEG-PLA mixed at different ratios were prepared by nano-precipitation of polymer DMF solution into DI water. The effect of UV treatment or pH change on the size and morphology of NPs was investigated by dynamic light scattering (DLS) and scanning electron microscopy (SEM), respectively. Nile red (NR) fluorescent dye was used as a model payload for comparing the kinetics of single and dual stimuli-mediated release from NR-loaded PBAE NPs (NR-NPs).

2. Materials and Methods

2.1. Materials

d,l-Lactide (LA) was purchased from TCI America (Portland, OR, USA). It was recrystallized three times in toluene and stored at −20 °C. Monomethoxy poly(ethylene glycol) with a molecular weight (MW) of 5 000 g · mol^{-1} (PEG, Polyscience, Warrington, PA, USA) was dried by dissolution in anhydrous toluene followed by azeotropic distillation under N_2. Stannous octoate ($Sn(Oct)_2$), 4-amino-1-butanol, 1,3-dimethyl-2-nitrobenzene, borane tetrahydrofuran complex solution, potassium permanganate, and acryloyl chloride were purchased from Sigma–Aldrich (St. Louis, MO, USA) and used as received. NR was supplied from Acros (Fisher Scientific USA, Pittsburgh, PA, USA). Deionized water (DI water) was obtained from a Milli-Q water purification system (Millipore, Billerica, MA, USA), and phosphate-buffered saline (PBS, 10 mM, pH 7.4, Cellgro, Manassas, VA, USA) was used for release experiments and cell studies. HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was

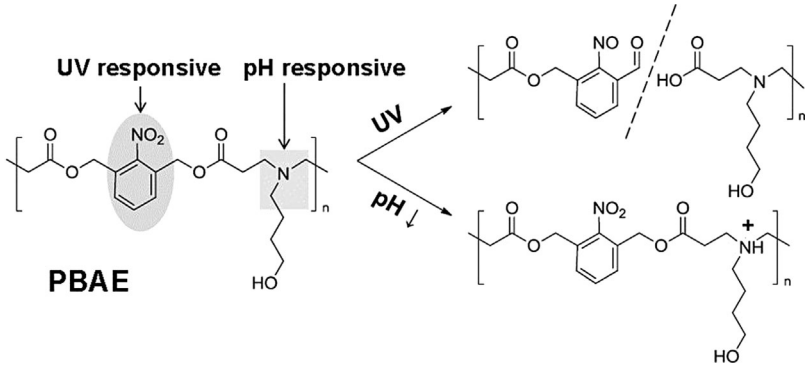

Figure 1. Dual responsivity of PBAE to UV and pH.

purchased from Invitrogen (Carlsbad, CA, USA) for cytotoxicity study.

2.2. Synthesis of Polymers

UV-responsive diacrylate was synthesized by reacting 2-nitro-benzene-1,3-dimethanol with acryloyl chloride. The 2-nitro-benzene-1,3-dimethanol was prepared using 2-nitro-1,3-benzenedicarboxylic acid and borane-tetrahydrofuran as previously reported.[23] The 2-nitro-1,3-benzenedicarboxylic acid was synthesized from 1,3-dimethyl-2-nitrobenzene. Briefly, 1,3-dimethyl-2-nitrobenzene (15.8 g, 0.105 mol) was added in an aqueous solution of NaOH (800 mL, 0.2 M) at 95 °C. The reaction mixture was refluxed after $KMnO_4$ (66 g, 0.418 mol) was added to yield 2-nitro-1,3-benzenedicarboxylic acid. To prepare 2-nitro-benzene-1,3-dimethanol, a solution of 2-nitro-1,3-benzenedicarboxylic acid (8.0 g, 38 mmol) and borane-tetrahydrofuran (200 mL, 1.0 M) were dissolved in anhydrous THF (50 mL), and stirred for 48 h. TEA (20 mL, 0.2 mol) was added in the anhydrous DCM solution (50 mL) of 2-nitro-benzene-1,3-dimethanol (15.0 g, 82 mmol) under N_2 atmosphere. Acryloyl chloride (18.1 g, 20 mmol) was then slowly added and the mixture was stirred for 18 h. The resulting product was dissolved in ethyl acetate and washed with saturated NaCl solution (3×100 mL). The product was further purified by silica gel chromatography (hexane: ethyl acetate = 1:1) to obtain (2-nitro-1,3-phenylene) bis-(methylene) diacrylate.

PBAE was synthesized using a Michael-type step polymerization as previously reported (Figure S1a).[16,24] Briefly, photosensitive (2-nitro-1,3-phenylene) bis(methylene) diacrylate (2.91 g, 10 mmol) and aminobutanol (1.34 g, 10.5 mmol) were dissolved in DCM and polymerized at 60 °C for 4 d. After the reaction, the solution was cooled down to room temperature and precipitated in diethyl ether or hexanes to get the polymer in a gummy solid or a viscous liquid. The chemical structure of polymer was determined by ^{1}H NMR (VARIAN UNITY 500 MHz, Varian Inc., Palo Alto, CA, USA). ^{1}H NMR (DMSO-d_6): δ 7.52 (ArH), 4.52 (Ar–CH_2), 3.93 (CH_2–OH), 2.62 (N–CH_2), 2.31 (CO–CH_2), 1.45 (CH_2–CH_2–CH_2–CH_2) (Figure S2a).

PEG-PLA copolymer was synthesized by ring-opening polymerization of LA using $Sn(Oct)_2$ and PEG (Figure S1b).[25] In a typical experiment, PEG (1.00 g, 0.2 mmol), LA (0.80 g, 5.6 mmol), $Sn(Oct)_2$ (0.08 g, 0.2 mmol), and toluene (60 mL) were charged in a reaction vessel. The vessel was closed with a stopper and immersed in an oil bath, thermostated at 110 °C. The polymerization was allowed to proceed for 26 h under stirring. The solvent was evaporated and the copolymer was dissolved in dichloromethane (DCM) to be subjected to precipitation in diethyl ether. ^{1}H NMR ($CDCl_3$): δ 5.18 (CH–CH_3), 3.62 (CH_2–CH_2–O), 1.57 (CH–CH_3) (Figure S2b).

2.3. Gel Permeation Chromatography (GPC) Analysis

Photolysis of PBAE (10 mg · mL^{-1} in DMF) was evaluated by GPC depending on UV irradiation time (350 nm, 20 mW · cm^{-2}). The GPC was equipped with an isocratic pump (Model 1100, Agilent Technologies, Santa Clara, CA, USA), a DAWN HELEOS 18-angle laser light scattering at 658 nm (Wyatt Technology, Santa Barbara, CA, USA), and an Optilab rEX refractive index detector (Wyatt Technology). Separations were performed on serially connected size exclusion columns (100, 500, 103, and 104 Å Phenogel columns, 5 μm, 300×7.8 mm, Phenomenex, Torrance, CA, USA) at 60 °C with DMF containing 0.1 M LiBr as a mobile phase.

2.4. Titration of PBAE for pKa

The pKa of PBAE was determined by recording the pH change during the titration of a concentrated acidic polymer solution with NaOH solution (0.1 M). PBAE (10 mg) was dissolved in NaCl solution (10 mL, 150 mM), and then, the pH was adjusted to 2 with a few drops of HCl (1.0 M). The NaOH solution was added slowly and pH was recorded as a function of the volume of added sodium hydroxide solution. The pKa was calculated from the averaged inflection of the titration curves as previously reported.[26]

2.5. Preparation of NPs

NPs based on PBAE and PEG-PLA were prepared by the nanoprecipitation method.[27] In brief, PBAE and PEG-PLA were dissolved in DMF (10 mg · mL^{-1}), and the two DMF solutions were mixed at the volume ratio of 100:0, 90:10, 75:25, 50:50, 25:75, and 0:100 (PBAE:PEG-PLA). The DMF mixture solutions (100 μL) were dropped into DI water (1 mL) and gently stirred for 10 min. The organic solvent was removed by centrifugation at 3 000 rpm for 10 min using a centrifugal filter (MWCO 10 000 g · mol^{-1}), yielding a turbid NP suspension. The NPs are denoted as NP# (NP1, NP2, NP3, NP4, NP5, and NP6 for PBAE:PEG-PLA ratio of 100:0, 90:10, 75:25, 50:50, 25:75, and 0:100, respectively).

2.6. DLS and Zeta-Potential of NPs

The NP dispersions were filtrated through a 0.45 μm Millipore filter into cuvettes and applied for Zetasizer (Malvern, Westborough, MA, USA) to determine the size and PDI. The light scattering was measured by back scattering at a detection angle of 173° at the wavelength of 532 nm, and the hydrodynamic radius was calculated using the Stokes–Einstein equation. The effect of UV or pH on the size of NPs was investigated using NP3s (PBAE:PEG-PLA = 75:25). The NP3s were prepared in DI water and diluted twice with buffer solutions (20 mM) at pH 4 (citrate), 5 (citrate), 6 (MES),

and 7.4 (PBS) and incubated at 37 °C. To validate UV sensitivity of the system, the NP3s in DI water was irradiated with UV (10 mW · cm^{-2}). The size, PDI, and Kcps of the samples were monitored by Zetasizer as a function of time. Surface charge of the NPs depending on the pH for the NP3s was determined from pH 8.5 to 5.5 by MPT2 autotitrator (Malvern, Westborough, MA, USA) using NaCl and HCl aqueous solutions (0.25 M).

2.7. SEM Upon UV Irradiation

Hitachi S-4800 high resolution SEM (Hitachi High Technologies Inc., Tokyo, Japan) was performed to elucidate the morphology of NP3s depending on UV irradiation time. A dispersion of NP3s in DI water (2 μL) was placed on the wafer substrate and dried overnight at room temperature after treatment with UV (10 mW · cm^{-2}) for 0, 0.5, 2, and 3 h. The sample was mounted on the aluminum sample holder and sputter-coated with a gold–palladium alloy. The NPs were observed with an accelerating voltage of 15 kV at a working distance of 4 mm. NPs composed of only PEG-PLA (NP6) were also treated by UV and compared with NP3s.

2.8. Fluorescence Spectroscopy of Nile Red-Loaded NPs

NR was employed as a photostable model drug and loaded into four different NPs (NP3, NP4, NP5, and NP6) by adding NR in the polymer solutions in DMF (1 mg · mL^{-1}). The DMF solutions (100 μL) were dropped into DI water (1 mL). The DMF was removed by centrifugation and the NP dispersion was filtrated by using a syringe filter. The NPs were diluted twice with PBS (×2, pH 7.4). Initial fluorescence intensity was very similar between the particles (ex 550 nm, em 615 nm). The particles were treated with UV light at pH 7.4 (10 mW · cm^{-2}) or 5.0 (5 mW · cm^{-2}), and the % initial fluorescence was measured at the desired time points.

2.9. Cytotoxicity of PBAE NPs Against HeLa Cells

To evaluate the cytotoxicity of the NPs, HeLa cells were seeded in a 96-well plate at a density of 1×10^4 cells per well and cultured at 37 °C in a humidified atmosphere with 5% CO_2. The cell medium was removed and 100 μL of fresh cell medium was added. NP dispersions (100 μL) were added to the medium in the wells resulting in the final concentrations of NPs of 10, 50, and 100 μg · mL^{-1}. The cells were incubated for 4 h with the NPs and then further incubated for 20 h after refreshment with cell medium. The samples were incubated for 4 h for the MTT assay and the absorbance was recorded at 562 nm.

3. Results and Discussion

3.1. Photo-Degradability of PBAE

A drug delivery platform, which utilizes external stimuli, is highly attractive as the stimuli can be applied with a high temporal and spatial precision in a noninvasive, on-demand fashion. However, payload release for many stimuli-responsive polymeric systems is not often instantaneous due to the incomplete scission of the polymers and insufficient sensitivity. In order to improve the responsivity of these systems, highly sensitive stimuli-responsive functional groups can be introduced in each repeating unit of PBAEs.[8] We envision that the NPs formed by these PBAEs would rapidly be disintegrated by external triggers and lead a burst release of the encapsulated compounds.

The photolabile property of PBAE was evaluated by GPC as a function of UV irradiation time. The degradation of the polymer was strongly dependent on the irradiation time and demonstrated high sensitivity. When the polymer was treated with 20 mW · cm^{-2} of UV light, a dramatic shift in the elution time was observed after 60 min (Figure 2a). This is mostly due to the decrease in MW of the polymers as a result of rapid cleavage of the polymer backbone, indicating that the polymer degradation was completed. The PBAE was degraded into oligomers or polymer fragments by the photocleavage reaction of nitrobenzyl groups in PBAE.[28] Upon UV irradiation, a clear DMF solution of PBAE turned yellow and then brown due to the nitrosobenzaldehyde production (Figure 1). In contrast, the molecular weight of PEG-PLA did not change by the 60 min UV treatment, demonstrating that the PEG-PLA was photostable under the same condition (Figure 2b).

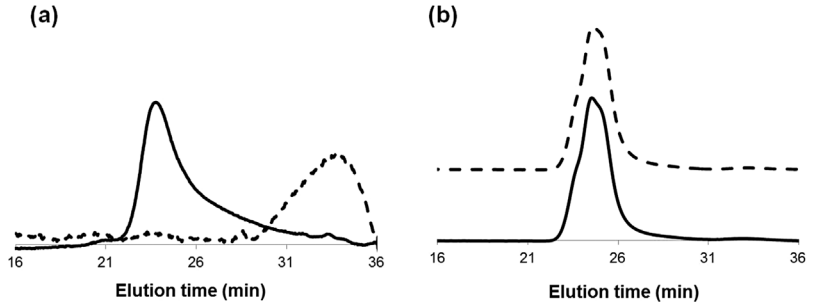

Figure 2. The GPC trace of the PBAE and PEG-PLA in DMF (10 mg · mL^{-1}) as a function of UV irradiation time (20 mW · cm^{-2}). The GPC curves for (a) PBAE before (solid) and after UV treatment for 60 min (dashed) are represented. (b) PEG-PLA before (solid) and after irradiation for 60 min (dashed) are also represented.

Table 1. Size and polydispersity index (PDI) of NPs depending on the composition.

Code	PBAE[a]:PEG-PLA[b]	Mean diameter [nm]	PDI
NP1	100:0	Precipitated	–
NP2	90:10	226.1	0.069
NP3	75:25	167.7	0.103
NP4	50:50	186.8	0.223
NP5	25:75	112.9	0.211
NP6	0:100	75.9	0.181

PDI, polydispersity index.
[a]PBAE MW = 12 000 g · mol^{-1}; [b]PEG-PLA MW = 6 300 g · mol^{-1}.

3.2. Formation of NPs

NPs were prepared by precipitating PBAE, PEG-PLA, or their mixture in DI water. NPs with a diameter from 76 to 226 nm were obtained with narrow size distributions (PDI < 0.25) (Table 1). NPs prepared by 100% of PBAE (NP1) immediately aggregated and precipitated. Similar result was observed for NPs made of 90% of PBAE and 10% of PEG-PLA (NP2), but the precipitation occurred after several hours (data not shown). PBAE has been demonstrated to cause particle fusion and aggregation even at room temperatures.[5,29] Generally, larger particles were formed as more PBAE was used (Table 1 and Figure S4). Stable NPs were obtained when more than 25% of PEG-PLA was used for the formulations (NP3-6), indicating that the presence of PEG stabilized the NPs and prevented particle aggregation. The PEG brush on the particle surface may also contribute to prolonged blood circulation times as previously reported.[30] NP3 was chosen for the SEM and DLS analyses due to their high PBAE content and stability among the NPs prepared in the study.

3.3. Size and Morphology of NPs Upon UV Irradiation

In order to demonstrate the UV-responsive properties of PBAE NPs, particle morphology and size distribution were monitored by SEM and DLS, respectively. In SEM images, it can be seen that spherical NP3s were formed by the nano-precipitation method (Figure 3a). After 30 min UV irradiation, NP3s were visibly swollen, showing initial disintegration (Figure 3b). The particles fully degraded and fused, losing their spherical morphology when irradiated for 2 h (Figure 3c) and eventually aggregated after 3 h (Figure 3d). Morphology of NP6s did not change by UV treatment (Figure 3e and f), meaning the administered UV irradiation time and strength had no effect on the degradation of control NPs (100% PEG-PLA). After UV treatment, a turbid dispersion of NP3 became a brownish clear (see Figure S5) as particle degradation followed photocleavage of the nitrobenzyl groups on the PBAE.

Consistent results were obtained by DLS, which correlated UV administration time with a widening size distribution of degrading NPs. After 3 h post-UV irradiation, the size and PDI of NP3s significantly increased and Kcps (light scattering intensity) decreased over time, demonstrating UV-triggered aggregation and precipitation (Figure 4). Although the average diameter of the NPs did not seem to change significantly over 2 h, a gradual increase in PDI is evident as the size distribution of NP3s became broader with UV irradiation (Figure 4 and S6). The

Figure 3. Scanning electron micrographs of NP3s (PBAE:PEG-PLA = 75:25) irradiated by UV (10 mW · cm^{-2}) for 0 h (a), 0.5 h (b), 2 h (c), and 3 h (d), and NP6s (100% PEG-PLA) before (e) and after irradiation for 3 h (f).

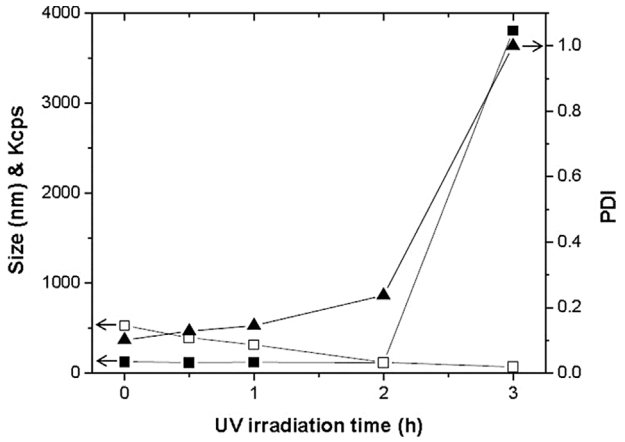

Figure 4. Size, kilo count per second (Kcps, light scattering intensity), and polydispersity index (PDI) of NP3s (PBAE:PEG-PLA = 75:25) as a function of irradiation time of UV (10 mW · cm^{-2}). Arrows indicate the corresponding *y*-axis to size (■), Kcps (▲), and PDI (□) data.

precipitation of the particle is responsible for the continuous reduction in Kcps.

3.4. Size and Zeta-Potential Measurements at Different pH

In order to assess the pH responsivity of the NPs, size, Kpcs, and PDI of NP3s were monitored while incubation at different pH. At pH 4.0, the size and PDI of the NPs dramatically increased just after 1 h, and the light scattering was recorded near zero, indicating that the NP3s instantly formed aggregates and precipitated (Figure 5). This is primarily due to the rapid protonation of PBAE at low pH and a following destabilization of PBAE in the particles. Particle aggregation was also fast at pH 5.0 (≤3 h), but slower than at pH 4.0. At pH 6.0 and 7.4, degradation was much slower, mostly due to the partial protonation of PBAE at near-neutral pH. As shown in Figure 6, zeta-potential of NP3s gradually increased up to 15 mV by acidification, indicating a pH-dependent protonation of the particles. At neutral pH, surface charge was slightly negative due to PEG but became positive below pH 6.5 since pKa of the PBAE is around pH 6.0 (Figure S2a). PBAE (1 mg · mL^{-1}) is clearly dissolved in water below pH 6.0 but precipitated above the pH (Figure S2b).

3.5. UV- and pH-Triggered Release

The fluorescence intensity of NR-loaded NPs (NR-NPs) was measured under UV treatment. As shown in Figure 7a, a rapid decrease in the fluorescence intensity was observed after exposure to UV for 3 h, indicating UV-mediated

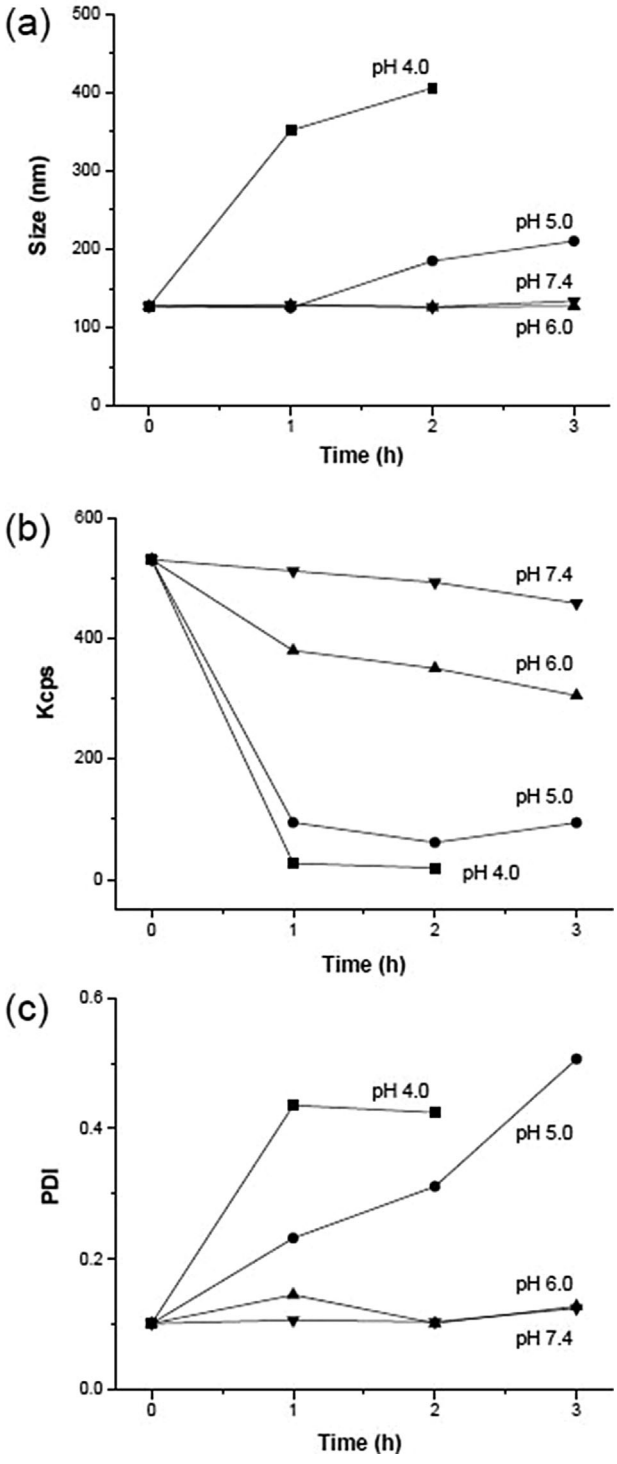

Figure 5. DLS results for NP3s (PBAE:PEG-PLA = 75:25) at pH 4.0 (■), 5.0 (●), 6.0 (▲), and 7.4 (▼) as a function of incubation time. Changes in size (a), kilo count per second (Kcps) (b), and polydispersity index (PDI) (c) for the NPs are represented.

photolysis of PBAE followed by a rapid escape of NR from NPs. NR is a hydrophobic dye whose fluorescence is strongly quenched by the exposure of polar environment when

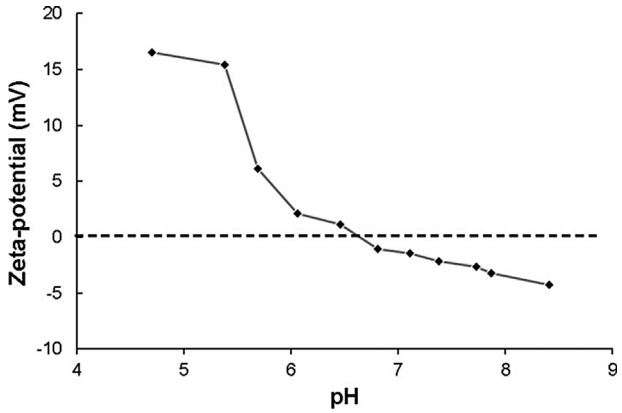

Figure 6. Zeta-potential of NP3s (PBAE:PEG-PLA = 75:25) while titrated the pH.

released.[31] The reduction in % fluorescence upon UV irradiation was shown to be higher when PBAE was a predominant polymer species in the particle composition, correlating the burst release kinetics of PBAE NPs to their photo-degradation properties. Contrastingly to PBAE NPs, PEG-PLA NPs demonstrated a constant fluorescence intensity with and without UV irradiation.

The PBAE NPs developed in this study showed higher UV susceptibility as compared to the previously reported systems due to the presence of multiple light-sensitive groups in the polymer chains. Katz et al. had prepared polymersomes using di-block copolymers with a photo-labile 2-nitrophenylalanine (2NPA) linker connecting two polymer blocks. Biocytin was encapsulated into the polymersomes and released over 6 h by much stronger UV irradiation ($\approx 55\ mW \cdot cm^{-2}$).[32] Polymeric micelles based on 100% of poly(2-nitrobenzyl methacrylate)-b-PEG showed only 20% decrease in the fluorescence intensity of the encapsulated NR by the UV irradiation at $50\ mW \cdot cm^{-2}$.[33]

The fluorescence intensities of NR-NP suspensions at pH 7.4 were compared with those at pH 5.0 as a function of time. Figure 7b shows that fluorescence intensity quickly decreased at pH 5.0 within an hour followed by slow rates of decrease for 2 h, whereas the intensity at pH 7.4 remained constant over the same time period. The rapid reduction in fluorescence intensity is driven by the protonation of a number of secondary amino groups in the polymer backbone. This may explain why the NR release induced by pH change is faster than that induced by UV light. The particles positively charged by the acidic pH may become more rapidly permeable to water than particles with shortened polymer chains by UV treatment.

Notably, the release of dye was not completed to 100% for any NPs in the study. This is probably due to the formation of separate domains by PBAE and PLA within the particles; it has been shown that blending of two polymers into one

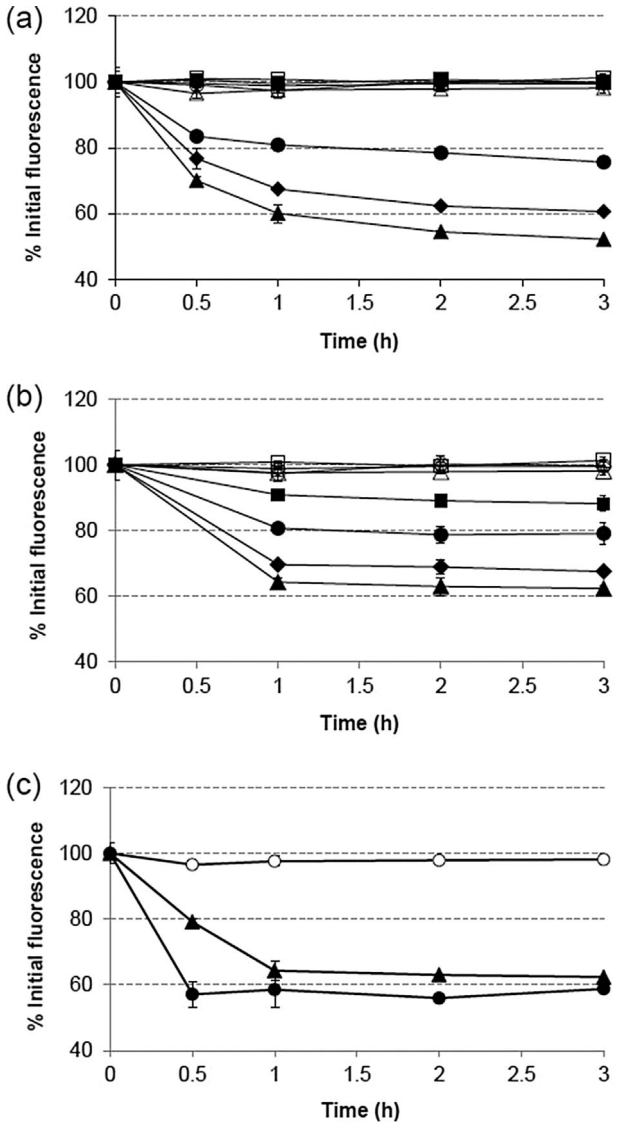

Figure 7. Normalized %fluorescence of Nile red (NR) in NP dispersions: (a) NP3 with (▲) or without UV (Δ), NP4 with (◆) or without UV (◇), NP5 with (●) or without UV (○), and NP6 with (■) or without UV (□). (b) NP3 at pH 5.0 (▲) or 7.4 (Δ), NP4 at pH 5.0 (◆) or 7.4 (◇), NP5 at pH 5.0 (●) or 7.4 (○), and NP6 at pH 5.0 (■) or 7.4 (□). (c) NP3 without triggers (○), NP3 at pH 5.0 (▲), and NP3 at pH 5.0 with UV (●). All experiments performed in triplicate.

matrix could induce a phase separation by the differences in the crystallinity of the polymers.[25] NR loaded in the PBAE domain was released by the stimuli, whereas the dye associated with the PLA domain may not be influenced. This can be corroborated by the result that more dye was released when more PBAE was incorporated for the NP formulations. The completion time for burst release of NR was similar between the NPs both in the UV- and pH-triggered release studies due to the high responsivity of

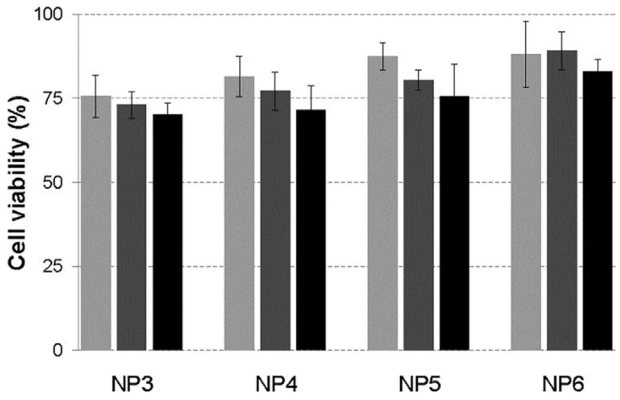

Figure 8. PBAE NP composition- and concentration-dependent HeLa cell viability after 24 h incubation. NPs with a concentration of 10 μg · mL^{-1} (light gray bars), 50 μg · mL^{-1} (dark gray bars), and 100 μg · mL^{-1} (black bars) were applied for the study. The experiment was carried out in triplicate.

PBAE in the particles. Dual "combinatory" trigger system was also synthesized and compared with a single trigger system in Figure 7c. The release kinetics was particularly accelerated by the incorporation of both UV- and pH-responsive groups in PBAE, even with irradiation strength as low as 5 mW · cm^{-2}. It is unambiguous that the high sensitivity of PBAE NPs would be very attractive especially in clinical applications, since penetration of UV light through the skin is less than few centimeters in depth.[34]

3.6. Cytotoxicity of PBAE NPs Against HeLa Cells

Toxicity of NPs based on PBAE and PEG-PLA was evaluated in HeLa cells using the MTT assay. More than 75% cell viability was observed at a NP concentration of 10 μg · mL^{-1} (Figure 8). Addition of PBAE to the particles slightly increased the cytotoxicity as previously reported.[5] A dose-dependent toxicity was also observed in which cell viability was lower as more NPs were applied. In general, PBAE NPs used for the study showed low cytotoxicity, with less than 30% even at NP concentration as high as 100 μg · mL^{-1}.

4. Conclusion

On-demand release of payload from NPs has been an interesting area of study in drug delivery. Here, we report the design of photo- and pH-dual responsive PBAEs and the subsequent studies of using such PBAE in formulating NPs that are capable of on-demand burst release of cargos in response to both UV and pH triggers. PBAEs, since their first design by Lynn, Anderson, and Langer, have been an extremely useful material in gene delivery. Numerous PBAE structures have been screened and key structures have been identified relevant to their efficiency in gene delivery. Based on the original design of PBAEs, we recently expanded the library of PBAEs, incorporated UV-responsive 2-nitrobenzene-1,3-dimethanol moiety to the backbone of PBAE, and used the resulting materials in the design of trigger-responsive, fast-degradable PBAE for gene delivery application. In this current study, we explored the potential of using this material to prepare photo- and pH-dual-responsive NPs. Excellent dual responsiveness was demonstrated, evidenced by the burst release of encapsulated small molecule cargos when treated with UV or by lowering the pH to change the protonated (charge) states of the PBAE backbone amine group. UV light was utilized for the proof-of-principle study, but PBAEs that are sensitive to near-infrared light (NIR) are currently under investigation because NIR responsibility is undoubtedly more attractive to clinical applications. We believe that this class of materials should not be limited to formulation of nanoparticulate drug delivery systems; dual responsive PBAE that can be mass produced inexpensively can potentially be used in many other areas such as design of responsive hydrogels and general controlled release applications in agroscience, cosmetic, and pharmaceutical industries.

Acknowledgements: This work is supported by the National Institute of Health (Director's New Innovator Award 1DP2OD007246-01).

Received: March 27, 2015; Revised: May 3, 2015; Published online: June 2, 2015; DOI: 10.1002/mabi.201500111

Keywords: nanomedicine; on-demand release; pH-sensitive; poly(β-amino ester); UV-sensitive

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