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Biotinylated Photocleavable Polyethylenimine: Capture and Triggered Release of Nucleic Acids from Solid Supports

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A *b*iotinylated *p*hoto*c*leavable *p*oly*e*thylen*i*mine (B-PC-PEI) was designed and synthesized for the capture and controlled release of nucleic acids from solid supports. B-PC-PEI was synthesized via a three-step reaction process and verified by ^{1}H NMR and mass spectrometry. In aqueous solution, the *o*-nitrobenzyl group within B-PC-PEI was efficiently cleaved by 5 min of 365 nm light exposure from a distance of 20 cm (9 mW/cm^2). When coupled to streptavidin-coated beads, the PEI domain of Cy5-labeled B-PC-PEI was released by 365 nm light exposure. In contrast, a Cy5-labeled biotinylated PEI (B-PEI) was used as a control and negligible fluorescence loss was observed. Cy5-labeled siRNA was electrostatically captured to streptavidin-coated beads preabsorbed with B-PC-PEI or B-PEI, and flow cytometry demonstrated significant loss of fluorescence from the bead surface after 5 min of light exposure only for B-PC-PEI, demonstrating controlled release of siRNA from the bead surface. Finally, the release of the Cy5-labeled siRNA into the supernatant was quantified. The release of Cy5-siRNA into the supernatant was significantly greater after 5 min of light exposure for B-PC-PEI/streptavidin beads compared to 0 min exposure and remained unchanged for B-PEI/streptavidin beads. B-PC-PEI facilitates capture and triggered release of surface-tethered nucleic acids with light exposure and is fully compatible with streptavidin-based applications.

INTRODUCTION

Photolabile protecting groups function by rendering a molecule of interest inert until photolysis and have been used extensively since their introduction in 1962 (*1*) for controlled release and kinetic studies (*2*–*13*). For biological systems, this approach allows control of concentration, location, and timing of the compound's release into a system. Furthermore, this technology, when applied to biomedical release applications, may mitigate problems such as off-target effects of a therapeutic agent. The potential to use photolytic release for gene delivery was first described by Monroe et al., who synthesized a 1-(4,5-dimethoxy-2-nitrophenyl)diazoethane caged plasmid DNA (pDNA) for light-activated gene delivery. However, the caging group limited transcription of pDNA because of direct modification of the pDNA backbone, and photolysis caused pDNA nicking. Also the caged pDNA had to be formulated with liposomes or precipitated onto gold beads for transfection (*8*).

Spatially controlled delivery of nucleic acids often involves substrate-mediated delivery or encapsulation within a polymeric scaffold. For substrate-mediated delivery of DNA, biotinylated polymers such as polyethylenimine (PEI)[1] and polylysine (PLL) have been attached to avidin-coated supports and provided for increased and positionally controlled transfection compared to polymer/DNA polyplexes absorbed nonspecifically onto the surface (*14*, *15*) or delivered via standard transfection methods (*14*). The increased function of surface-tethered polyplexes could be due to inhibition of aggregation and consequent intracellular sequestration (*15*) or to elevated local concentration of nucleic acids at the cell surface, which can increase transfection efficiency (*16*). Nucleic acids have also been immobilized in degradable scaffolds for localized delivery. There are many examples of both biological and synthetic scaffolds in the literature (as reviewed by Pannier and Shea (*17*)). The delivery of pDNA to cells from polymer scaffolds is controlled by the polymer degradation rate, a property that can be engineered (*18*).

2-Nitrobenzyl groups for photolability have been commonly used for biomedical applications. While the exact mechanisms and chemical intermediates leading to photolability are not fully resolved, light exposure does cause an intramolecular H-atom transfer which leads to an *aci*-nitro tautomer followed by nitroso group formation. This process is discussed in detail in McCray and Trentham (*7*), Pillai (*19*), Il'ichev et al. (*20*), and Schwörer and Wirz (*21*). In particular, α-substituted 2-nitrobenzyl esters exhibit highly efficient cleavage and are stable in biological systems (*4*, *6*, *22*, *23*) and under synthetic conditions for biomolecules such as peptide synthesis (*19*). Moreover, the near-UV light used to activate the photolysis is not harmful to many biomolecules and biological systems (*24*).

Photocleavable biotin molecules (B-PC) have been previously developed to combine both photolability and high affinity capture (*25*). The biotin–avidin association is essentially irreversible ($K_a = 10^{15}$ M^{-1}) and commonly used. Subsequent release of the biomolecule after isolation remains a challenge, as the association is relatively unaffected by pH, temperature, and 8 M guanidine-HCl at neutral pH. Typically, harsh conditions (e.g., detergents in combination with high temperature) succeed in dissociating the biotin–avidin interaction. B-PCs eliminate this shortcoming because exposure to light at specific wavelengths can cleave the biomolecule from the biotin. B-PCs have been used for several applications, including isolation and release of small peptides (*25*) and nucleotides for DNA extension products (*22*), and phosphorylation and purification of oligonucleotides (*26*).

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[1] Abbreviations: B, biotin; PC, photocleavable; PEI, polyethylenimine; PLL polylysine; siRNA, small interfering RNA.

In this report, we present a new approach that combines photolability and surface-tethering technology for the capture and controlled release of nucleic acids without covalent modification of the nucleic acids. PEI was covalently modified with a photolabile biotin group. This new compound was attached to the surface of streptavidin-coated beads and then mixed with siRNA to form tethered polyplexes. Exposure at 365 nm to the functionalized beads caused the release of PEI/siRNA complexes from the surface of the bead into the supernatant. Spatially and temporally controlled release of metered doses of nucleic acids from a solid surface can be potentially useful for biomedical applications, determination of rate-limiting factors for gene delivery, and for screening studies where temporally and spatially controlled release could offer insight into cellular processes.

EXPERIMENTAL PROCEDURES

Synthesis of Biotin–PC-PEI. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise specified. All experiments were performed under dimmed light conditions and with foil coverings.

Biotin–PC-Linker **3** was synthesized as previously described in Bai et al. (*22*). Briefly, 1-[5-(aminomethyl)-2-nitro]phenyle-thanol **1** (0.045 g, 0.23 mmol), a generous gift from Dr. Jingyue Ju of Columbia University, was dissolved in 1.5 mL of DMF. A volume of 0.325 mL of 1 M NaHCO$_3$ was added to the solution and stirred. To the mixture, biotin-NHS ester **2** (0.045 g, 0.10 mmol, Invitrogen, Carlsbad, CA) in 1.5 mL of DMF was slowly added while stirring. The reaction solution was stirred for 5 h (RT). The final product, **3** (0.048 g, 0.090 mmol, 90% yield), was purified using a silica gel column (80% CH$_2$Cl$_2$:20% MeOH (v:v)). ^{1}H NMR (300 MHz, MeOH-*d*) δ 7.89 (d, 1H), 7.80 (s, 1H), 7.34 (d, 1H), 5.35 (q, 1H), 4.52–4.48 (m, 3H), 4.33–4.29 (m, 1H), 3.23–3.18 (m, 3H), 2.95 (d, 1H), 2.70 (d, 1H), 2.30 (t, 2H), 2.20 (t, 2H), 1.78–1.47 (m, 16H).

Biotin–PC-NHS ester **4** was synthesized as previously described in Bai et al. (*22*). Briefly, **3** (0.011 g, 0.021 mmol) and *N,N′*-disuccinimidyl carbonate (0.0092 g, 0.036 mmol) were dissolved in 0.3 mL of anhydrous DMF. Triethylamine (0.008 mL, 0.057 mmol) was added, and the reaction mixture was stirred under Ar for 6 h (RT). The solvent was evaporated and the product dissolved in 1 mL of 1 M NaHCO$_3$. The solution was extracted 3× with EtOAc and concentrated in vacuo. The product mixture was dissolved in DMF and purified by preparative TLC (85% CH$_2$Cl$_2$:15% MeOH (v:v)). The product band was isolated from the TLC plate and soaked in DMF for 4.5 h (RT), after which it was centrifuged to separate the product from the silica. The solvent was removed in vacuo to yield **4** (0.0022 mg, 0.0032 mmol, 16% yield). ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 8.74 (s, 1H), 8.02 (d, 1H), 7.75 (t, 1H), 7.66 (s, 1H), 7,47 (s, 1H), 6.42 (s, 1H), 6.36 (s, 1H), 6.28 (q, 1H), 4.40 (d, 2H), 4.35–4.28 (m, 1H), 4.12 (m, 1H), 3.09–3.01 (m, 1H), 2.99 (s, 2H), 2.89–2.78 (m, 6H), 2.56 (s, 1H), 2.16 (t, 2H). 2.03 (t, 3H), 1.72 (d, 2H), 1.50–1.24 (m, 12H).

Biotin–PC-PEI (B-PC-PEI) **5**. Polyethylenimine (PEI, 800 Da, 0.0006 g, 0.00075 mmol) was dissolved in 0.3 mL of H$_2$O, to which 0.07 mL of 1 M NaHCO$_3$ was added dropwise. The reaction mixture was allowed to equilibrate and **4** (0.0011 g, 0.0016 mmol) in 0.3 mL of DMSO was slowly added. The reaction was stirred for 5 h and then concentrated in vacuo to yield **5**.

Synthesis of Biotin–PEI. PEI (800 Da, 0.0083 g, 0.010 mmol) was dissolved in 0.2 M NaHCO$_3$ (1 mL) and stirred to equilibrate. To the mixture was slowly added biotin-NHS ester **2** (0.010 g, 0.022 mmol) in anhydrous DMSO (1 mL). The reaction was stirred for 5.5 h and concentrated in vacuo to yield Biotin–PEI (B-PEI).
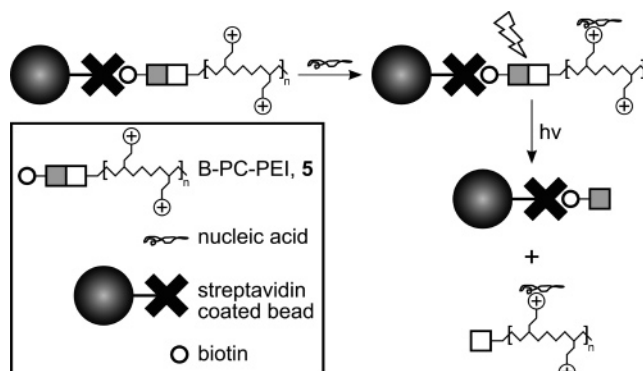

Figure 1. Schematic of B-PC-PEI bead formulation with nucleic acids and release with light exposure.

Testing for Photocleavability. UV irradiation was performed with a Model B-100A Hi Intensity UV Lamp (UVP Inc., Upland, CA) at a distance of 20 cm (emission peak at 365 nm, 9 mW/cm^2). B-PC-PEI, B-PEI, and PEI (0.01 mM) in H$_2$O receiving either 0, 5, or 10 min of 365 nm light exposure were analyzed by mass spectrometry on a 4700 Proteomics Analyzer with TOF/TOF optics (Applied Biosystems, Foster City, CA) at the University of Pennsylvania's Proteomics Core Facility.

Measuring Release of PEI from Streptavidin Microbeads. UV irradiation was performed, as described previously. For tracer studies, B-PC-PEI and B-PEI were covalently labeled with Cy5 NHS ester (Amersham Biosciences, Piscataway, NJ) according to the manufacturer's protocol using the ratio 1 mol Cy5 per 1 mol PEI primary amine. Streptavidin-coated magnetic beads (600 000 beads, Dynabeads M-280 streptavidin beads, Invitrogen) in 0.92 μL of phosphate-buffered saline (PBS, pH 7.4, Invitrogen) were added to 0.92 μL of 4.96 pmol/μL Cy5-labeled B-PEI or B-PC-PEI. A volume of 1.86 μL PBS was added, and the beads were allowed to incubate for 2 h (RT). The volume was brought to 300 μL, and 100 μL aliquots received either 0, 5, or 10 min of 365 nm light exposure. The samples were analyzed in a total volume of 400 μL of PBS on a BD FACSCalibur system (BD Biosciences, Franklin Lakes, NJ) at the University of Pennsylvania's Flow Cytometry and Cell Sorting Facility; 10 000 events were recorded per sample. The machine was calibrated to a negative control of unmodified streptavidin beads and a positive control of streptavidin beads directly labeled with Cy5 NHS ester. Flow cytometry data was analyzed with a custom Matlab (Mathworks Inc., Natick, MA) script designed to calculate the *x*-distance of the weighted maximum peak height for each sample. The fluorescence remaining on the bead after light exposure was calculated as a ratio comparing the *x*-distance of the maximum peak height at the specified time of 365 nm exposure to the *x*-distance of the maximum peak height for 0 min of 365 nm exposure.

Measuring Release of siRNA/PEI Polyplexes from Streptavidin Microbeads. Small interfering RNAs (siRNA) for lysosomal DNase II (siRNA ID = 121319, Ambion, Austin, TX) were labeled with Cy5 using a Label IT Nucleic Acid Labeling Kit (Mirus Bio Corporation, Madison, WI) according to the manufacturer's protocol and purified by ethanol precipitation. B-PC-PEI and B-PEI (unlabeled) were incubated with the streptavidin beads, as above, except for 1.5 h. The coated beads were diluted 10× with HEPES-buffered saline (HBS, 15 mM HEPES, 150 mM NaCl, pH 7.2) and added to the Cy5-labeled siRNA in HBS at varying N/P ratios. The polyplexes were allowed to form for 15 min (RT), and then the volume was adjusted to 300 μL with HBS. Aliquots (100 μL) were exposed to either 0, 5, or 10 min of 365 nm light (Figure 1). The samples

Scheme 1. Three-Step Synthesis of B-PC-PEI 5[a]
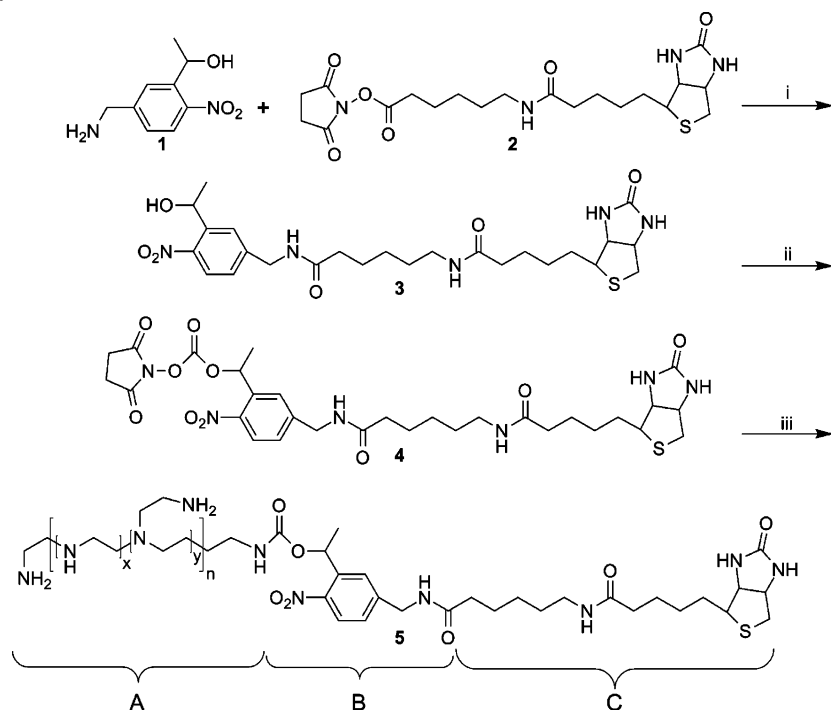
[a] Reagents and conditions: (i) NaHCO₃, DMF for 5 h; (ii) *N,N′*-disuccinimidyl carbonate, anhydrous DMF, Et₃N for 6 h; (iii) polyethylenimine, NaHCO₃, DMSO for 5 h.

were analyzed in a total volume of 400 μL of HBS on a BD FACSCalibur.

Quantifying the Amount of siRNA Released into the Supernatant. Streptavidin beads were prepared as above for siRNA/PEI polyplexes except after incubation with the Cy5-labeled siRNA the volume was brought to 100 μL with HBS. The samples were then placed on a Dynal MPC magnet (Invitrogen), and the supernatant was removed to remove any unbound siRNA/PEI polyplexes. The beads were then dissolved in 200 μL of HBS, and 100 μL aliquots received either 0, 5, or 10 min of 365 nm exposure. After exposure, the samples were placed on the magnet, the supernatant was separated from the beads, and the fluorescence of each was measured in 100 μL of HBS. The measurements were taken in a black 96-well plate (Nunc, Rochester, NY) on an EnVision Multilabel Plate Reader (Perkin-Elmer, Wellesley, MA). The fluorescence intensity (FI) data was used to calculate percent release [$100 \times FI_{supernatant}/(FI_{supernatant} + FI_{beads})$].

siRNA Stability. Negative control siRNA (0.4 μg, #4611G, Ambion) was tested alone, complexed with B-PC-PEI (N/P = 0.4), and complexed with B-PC-PEI attached to streptavidin beads (as described above). Each of these conditions received either 0 or 5 min of 365 nm light exposure, after which 2 μL of 6x loading dye (Promega, Madison, WI) was added. The samples (10 μL) were loaded into the wells of a 1% agarose gel in 1x TAE with SYBR Safe gel stain (Molecular Probes, Carlsbad, CA). The gel was run at 100 V for 30 min. Positive and negative controls were naked siRNA and B-PC-PEI, respectively.

RESULTS

Design, Synthesis, and Validation of Biotin−PC-PEI. Biotin−PC-PEI (B-PC-PEI) was designed to consist of three functional domains: (1) a cationic polymer domain (A in Scheme 1), in this case PEI, to electrostatically interact with and condense nucleic acids; (2) a photolabile domain (B in Scheme 1) to selectively release the polyplexes; (3) a biotin
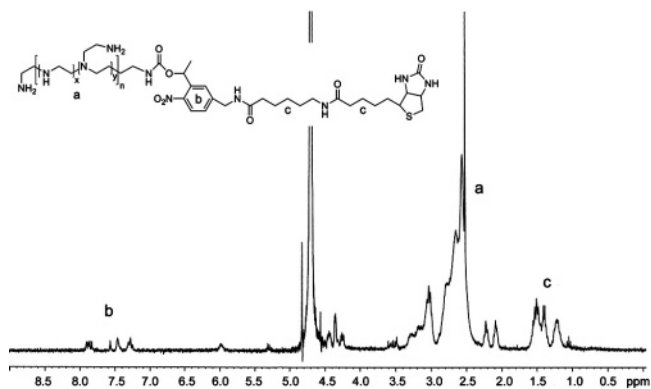

Figure 2. ¹H NMR of B-PC-PEI.

domain (C in Scheme 1) used to tether the compound to a streptavidin-coated surface or interact with free streptavidin. B-PC-PEI was synthesized via a three-step reaction process (Scheme 1) and was verified by ¹H NMR (Figure 2) and mass spectrometry (Figure 3A). By comparing mass spectrometry data from B-PC-PEI (Figure 3A) and unmodified PEI (not shown), it is estimated that the majority of the PEI received, on average, one photolabile-biotin arm (domains B and C, peaks centered around $m/z = 966.62$) and a smaller percentage received two photolabile-biotin arms (peaks centered around $m/z = 1527.86$).

After validation of its formation, B-PC-PEI was analyzed for the ability to breakdown after exposure to 365 nm light. B-PC-PEI was analyzed by mass spectrometry before and after 5 min of 365 nm light exposure (Figures 3A and 3B, respectively). After 5 min of light exposure, the peaks centered around $m/z = 1527.86$ completely disappeared and the peaks centered around $m/z = 966.62$ shifted to a lower m/z value, indicating that the photolabile biotin arms were cleaved from the PEI. Increasing the duration of light exposure to 10 min did not further increase the breakdown of B-PC-PEI (not shown). As a control, Biotin−PEI (B-PEI) was synthesized to have
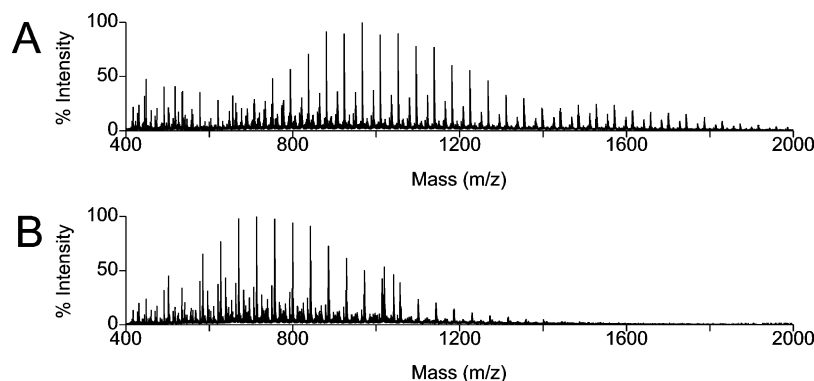
Figure 3. Mass spectrometry for B-PC-PEI without light exposure (A) and with 5 min of 365 nm light exposure (B).
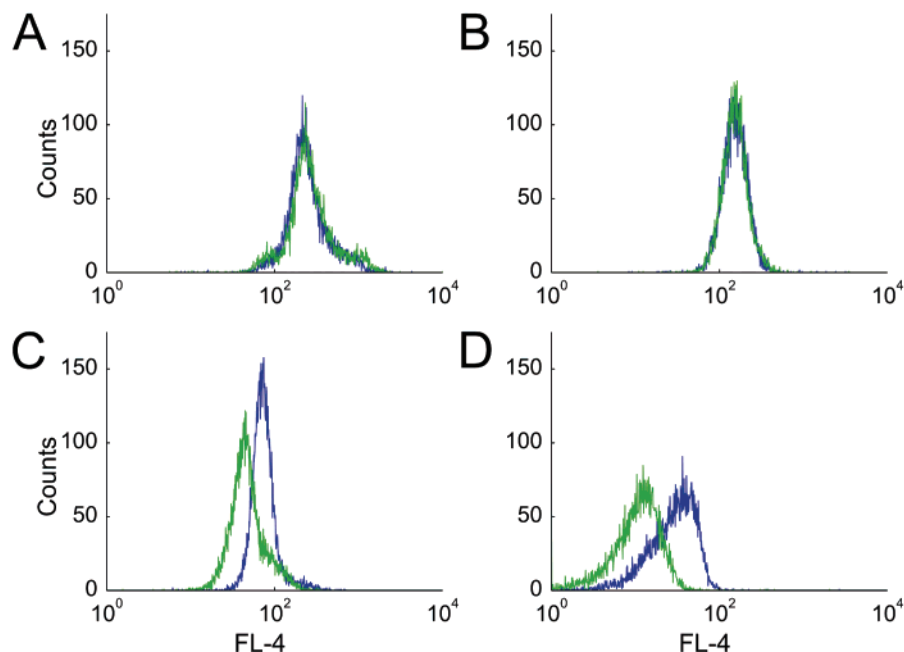

Figure 4. Flow cytometry data showing the fluorescence shifts of B-PEI/streptavidin beads without (A) or with (B) siRNA, and of B-PC-PEI/ streptavidin beads without (C) or with (D) siRNA due to light exposure (green line, 10 min (A and C) or 5 min (B and D) of 365 nm exposure; blue line, 0 min exposure).

domains A and C of B-PC-PEI (i.e., no photolabile domain B, see Scheme 1 for designations). Mass spectrometry validated the formation of this compound and showed no change in the spectra with exposure to 5 or 10 min of 365 nm light (not shown).

Release of PEI from Streptavidin Beads. B-PC-PEI was immobilized to the surface of streptavidin-coated beads as depicted in Figure 1. To determine release of the PEI domain from the streptavidin beads, the PEI primary amines of B-PC-PEI were labeled with Cy5 NHS ester prior to immobilization. The streptavidin beads bound with Cy5-labeled B-PC-PEI were then exposed to 0, 5, or 10 min of 365 nm light and analyzed by flow cytometry for fluorescence loss. As a control, B-PEI, labeled with Cy5, was subjected to the same procedure to differentiate between fluorescence loss due to photobleaching from the 365 nm light exposure and fluorescence loss due to actual release of the Cy5-labeled PEI domain from the surface of the bead.

As can be seen in Figure 4A and 4C, after 10 min of 365 nm exposure, there was a negligible loss of fluorescence due to photobleaching (Figure 4A, B-PEI) and a much larger shift (fluorescence loss) due to actual cleavage of the Cy5-labeled PEI domain from the surface of the bead (Figure 4C, B-PC-PEI). This data when looked at numerically as the fraction of remaining fluorescence associated with the bead (see

Experimental Procedures for details of the calculations) translates to values of 0.78 ± 0.15 and 0.90 ± 0.09 ($n = 6$, $p < 0.07$) for B-PC-PEI and B-PEI, respectively, after 5 min of light exposure, and 0.51 ± 0.12 and 0.97 ± 0.23 ($n = 6$, $p < 0.0008$) for B-PC-PEI and B-PEI, respectively, after 10 min of light exposure.

Release of siRNA from Streptavidin Beads. To quantify the release of nucleic acids from streptavidin beads, unlabeled B-PC-PEI was immobilized to streptavidin beads, as described above, and added to Cy5-labeled siRNA at an N/P ratio = 0.16 (Figure 1). The immobilized siRNA/B-PC-PEI polyplexes received either 0, 5, or 10 min of 365 light exposure and were analyzed by flow cytometry for fluorescence loss. As with the experiments measuring the loss of PEI from the surface of the streptavidin beads, B-PEI in place of B-PC-PEI was used as a control. As seen in Figure 4B and 4D, after 5 min of 365 nm light exposure, there was negligible fluorescence loss due to photobleaching (Figure 4B, B-PEI/siRNA), and significant fluorescence loss due to release of siRNA from the surface of the bead (Figure 4D, B-PC-PEI/siRNA). Quantitatively, the fraction of remaining fluorescence was 0.52 ± 0.14 and 0.94 ± 0.10 ($n = 3$, $p < 0.007$) for B-PC-PEI/siRNA and B-PEI/ siRNA polyplexes, respectively. Increasing the light exposure to 10 min further increased the release of the B-PC-PEI/siRNA polyplexes with the fraction remaining reduced to 0.27 ± 0.13
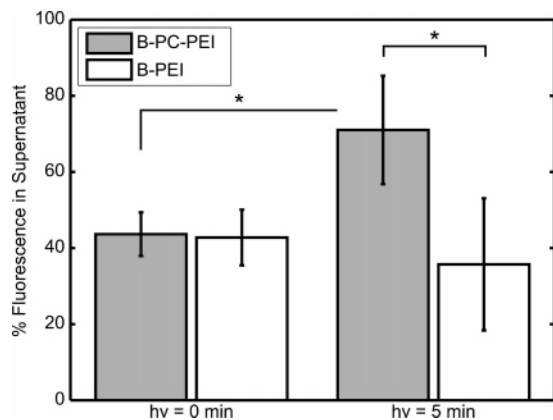
Figure 5. Release of surface bound siRNA/PEI complexes into the supernatant (* = $p < 0.03$).

($p < 0.05$ comparing B-PC-PEI/siRNA at 5 and 10 min light exposure). This data was consistent at N/P ratios of 1.6 and 4.8 for B-PC-PEI, but N/P = 0.16 showed optimal fluorescence readings for flow cytometry.

As an additional test, siRNA was immobilized to the streptavidin beads as above, but the fluorescence in both the supernatant and on the bead were measured. Fluorescence was measured as relative fluorescence units on a plate reader and converted into percent fluorescence in the supernatant (as described in the Experimental Procedures). After 0 min of 365 nm light exposure, both B-PC-PEI and B-PEI immobilized polyplexes (N/P = 0.4) released a similar amount of fluorescence in the supernatant (Figure 5). With 5 min of 365 nm light exposure, the amount of fluorescence in the supernatant was significantly higher for B-PC-PEI than for B-PEI immobilized polyplexes ($p < 0.03$, Figure 5). Increasing light exposure to 10 min did not enhance release of siRNA from B-PC-PEI polyplexes ($71.03 \pm 14.24\%$ and $78.34 \pm 7.59\%$ at 5 and 10 min of light exposure, respectively ($p > 0.05$)). Using these measurements, it is estimated that about 7 μg siRNA/mg bead is immobilized.

Finally, agarose gel electrophoresis was used to assess the integrity of the siRNA after exposure to the light-activated release conditions. The gels showed no difference in mobility between naked siRNA before or after light exposure, B-PC-PEI/siRNA polyplexes before or after light exposure, and B-PC-PEI/siRNA polyplexes released from streptavidin beads before or after light exposure (not shown).

DISCUSSION

Spatially and temporally controlled release of nucleic acids has many potential applications, including disease treatment and discovery of molecular processes involved in gene delivery. For nonviral gene therapy applications, spatial targeting has been attempted though inclusion of receptor targeting ligands to facilitate vector uptake into specific cells populations (27−30) and through the use of biotinylated cationic polymers for substrate-mediated delivery (14, 15).

We developed a novel compound, B-PC-PEI, that combines surface-mediated delivery with photolabile technology for capture and light-activated controlled release of nucleic acids from solid supports. B-PC-PEI consists of three functional domains designed to tether the compound to a surface, interact with nucleic acids, and release the nucleic acids from the surface through exposure to 365 nm light. This represents the first time this type of compound has been used for the controlled release of noncovalently modified nucleic acids from streptavidin-coated solid supports.

The use of biotinylated PEI for substrate-mediated delivery of pDNA can result in positive transfection (15). The retention of nucleic acids within a PEI polyplex depends on the molecular weight of the polymer used (31), the characteristics of the polymer (e.g., linear versus branched (32)), and the N/P ratio (33). Additionally, the extent of biotinylation will also affect the electrostatic interactions between PEI and nucleic acids because each biotin added should remove one free primary amine. The amount of release for a biotinylated PEI should be tunable by adjusting each of these properties, and therefore it should be easy to design the system to have negligible nucleic acid release from the polymer unless the whole polyplex is released from the substrate with light, as with B-PC-PEI. Further validating this point, the use of biotinylated PLL did not show enhanced transfection unless the majority of the polyplex was formed with a non-biotinylated PLL (15).

For B-PC-PEI, photolysis of the PEI domain from the remaining molecule occurred within 5 min when in free solution (Figure 3) and 10 min when attached to streptavidin beads (Figure 4C). It is possible that increased light exposure was necessary to release the Cy5-labeled PEI domain from the streptavidin beads because the beads or the Cy5 attenuated some of the light. Also, PEI has been thought to form nonspecific interactions with streptavidin (15) so it is possible that for this experiment, those forces needed to be overcome as well. Comparing the fluorescence loss from the Cy5-labeled B-PC-PEI (Figure 4C) and B-PEI (Figure 4A) beads upon light exposure shows clearly that the loss is due to actual cleavage of the PEI domain from the bead and not photobleaching.

In addition to the two causes of fluorescence loss described previously, for the siRNA there could also be loss due to release of weakly associated siRNA from the bead. Similar to the results with the Cy5-labeled biotin-compounds, there was negligible fluorescence loss due to photobleaching or diffusion after 5 min of 365 nm light exposure (B-PEI polyplexes, Figure 4B). For polyplexes made with B-PC-PEI there was a significant loss of bead-associated fluorescence after only 5 min of light exposure (Figure 4D). As with the PEI domain, increasing the duration of light exposure further increased polyplex loss; however, the majority of siRNA was released in the first 5 min. This differs from the PEI results possibly because PEI is less able to nonspecifically interact with the streptavidin when it is electrostatically condensing the siRNA.

The loss of siRNA fluorescence from the bead was further validated by a gain in fluorescence in the supernatant (Figure 5). At 0 min of 365 nm light exposure both B-PC-PEI and B-PEI polyplexes have a similar percent fluorescence in the supernatant (presumably due to diffusion), but by 5 min of light exposure the B-PC-PEI/siRNA polyplexes had released $71.03 \pm 14.24\%$ of the polyplexes into the supernatant while the B-PEI polyplexes remained unchanged from the 0 min time point. Additionally, increasing the duration of light to 10 min did not significantly enhance the release of the B-PC-PEI polyplexes. It is important to note that release of siRNA at 0 min for both B-PC-PEI and B-PEI polyplexes is most likely due to the low N/P ratio. Increasing the N/P ratio would cause greater retention of the siRNA within the polyplex; however, N/P = 0.4 gave optimal fluorescence readings. Additionally, since it is the PEI and not the siRNA that is directly coupled to the beads and the light-activated mechanism, any released siRNA should be released as polyplexes with the PEI.

Finally, the integrity of the siRNA was tested with agarose gel electrophoresis, and under all conditions tested, light exposure did not change the mobility or noticeably degrade the siRNA. This result is expected, as experiments conducted by Quick and Anseth showed negligible change in pDNA integrity after 365 nm light exposure (34). Additionally, Quick and

Anseth showed retained pDNA activity after light exposure at 365 nm (*34*).

While polymers have been previously engineered for the controlled release of nucleic acids (*35−39*), the release has largely relied on cellular process. In this report, we have demonstrated the synthesis and functionality of a compound, B-PC-PEI, that is able to selectively release nucleic acids from a solid support after light application and which has many potential applications ranging from biological studies to therapeutic applications.

ACKNOWLEDGMENT

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