ACS APPLIED MATERIALS & INTERFACES

Research Article

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Combining Fluorination and Bioreducibility for Improved siRNA Polyplex Delivery

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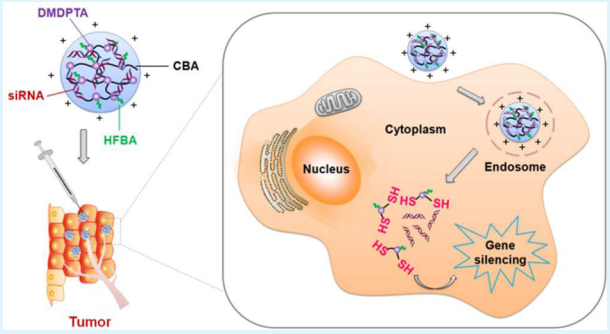
ABSTRACT: Polycations are promising vectors for the delivery of siRNA therapeutics but they often suffer from toxicity and low in vivo delivery efficacy. This study tests the hypothesis that combining fluorination and bioreducibility of polycations will overcome problems with both the toxicity and delivery efficacy. To test the hypothesis, we synthesized bioreducible (RHB) and nonreducible (NHB) poly(amido amine)s. The RHB were additionally fluorinated using reaction with heptafluorobutyric anhydride to obtain F-RHB. We found that both RHB and F-RHB showed significantly reduced cytotoxicity compared with NHB, which allowed their safe use in a wider range of doses than NHB. All three synthesized polycations formed polyplexes with siRNA. F-RHB achieved the best siRNA silencing efficacy in multiple cell lines in vitro, which was at least in part because of fluorination-induced enhancement of cellular uptake and improved endosomal escape. Lastly, F-RHB showed greatly improved Luc silencing efficacy in tumors in vivo when compared with polyplexes based on RHB, NHB, as well as control poly(ethylenimine) (PEI). This study suggests that combining fluorination with bioreducibility of polycations is a promising strategy to the design of siRNA delivery vectors with improved toxicity and in vivo activity profiles.



KEYWORDS: *fluorinated polymers, reducible polymers, polyplexes, siRNA delivery, cancer*

1. INTRODUCTION

Therapies based on nucleic acids, such as siRNA, are promising methods for treating many serious diseases, including cancer and various genetic disorders.[1,2] Success of nucleic acid therapies requires development of delivery systems that can facilitate transport across cell membranes and efficient transfection.[3] Polycations are promising delivery vectors for all the main types of nucleic acids, including siRNA.[4] Significant efforts have been devoted to the synthesis of safe and efficient polycations for nucleic acid delivery.[5]

Among the main challenges in the use of polycations is their toxicity. One way to overcome the toxicity of cationic polymers is the introduction of biodegradable bonds such as disulfides.[6] There is a difference in the redox potential between extracellular and intracellular environment.[7] Glutathione (GSH) is the most abundant intracellular reducing thiol, which is present in mM concentrations. The intracellular cleavage of disulfides in bioreducible polymers is mediated by exchange reactions with GSH.[8,9] In case of polyplexes, the disulfide reduction enhances intracellular disassembly and increases intracellular bioavailability, while decreasing cytotoxicity of the bioreducible polycations. The improved disassembly of bioreducible polyplexes typically enhances transfection efficacy.

Fluorination is a recently developed strategy for improving transfection efficacy of nucleic acid delivery vectors.[10−13] Fluorination imparts hydrophobic and lipophobic characteristics and results in a strong tendency toward phase separation in polar and nonpolar environments.[14,15] These unique features of the fluorous interactions promise to improve stability of polyplexes against both competing natural polyelectrolytes as well as lipids and lipoproteins in vivo.[16] Fluorination can also improve the ability of polycations to transport molecules across the cellular lipid bilayers, thereby facilitating their endosomal escape.[10,17] Previously, fluorinated liposomes showed improved transfection efficacy compared with nonfluorinated liposomes.[18] In recent studies, fluorination dramatically improved the transfection efficacy of PAMAM dendrimers to levels, which were superior to commercial lipid transfection reagents. The fluorinated dendrimers also exhibited excellent serum-resistance.[10] Because of the high affinity of the fluorous interactions, fluorinated compounds can assemble into nano- or micron-sized structures.[19] On the basis of this property, a class of fluorinated dendrimers were prepared, which could self-assemble into uniform nanoparticles combining the features of

Received: November 5, 2016
Accepted: January 19, 2017

DOI: 10.1021/acsami.6b14184
ACS Appl. Mater. Interfaces XXXX, XXX, XXX−XXX

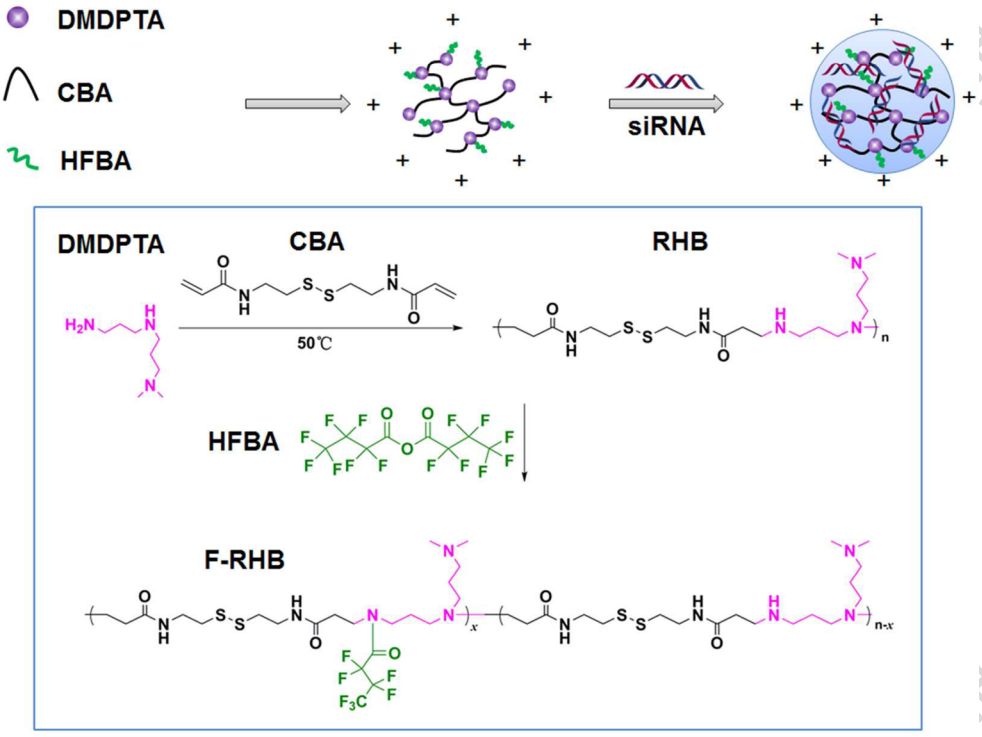


Figure 1. Polymer synthesis and formation of siRNA polyplexes. (DMDPTA, *N,N*-dimethyldipropylene-triamine. CBA, *N,N′*-Cystaminebis-(acrylamide). RHB, bioreducible hyperbranched poly(amido amine)s. HFBA, heptafluorobutyric anhydride).

lipid and polymeric nonviral vectors.[20] Fluorocarbon chains were also used to form the core of the micelles, and the polycations were concentrated at the micelle surface, which increased surface charge density and the binding affinity with DNA for improved transfection.[13]

In this study, we hypothesized that combining fluorination and bioreducibility features in a single polycation will improve siRNA delivery because of the ability to transport molecules across lipid bilayers and easy intracellular release of siRNA due to the bioreducible disulfides. To test the hypothesis, we synthesized nonreducible hyperbranched polycation (NHB), together with bioreducible (RHB) and fluorinated bioreducible (F-RHB) analogs. We evaluated the combined effect of fluorination and bioreducibility on cytotoxicity, cellular uptake, and trafficking, and siRNA transfection efficacy in vitro and in vivo.

2. MATERIALS AND METHODS

2.1. Materials. *N,N′*-Bis(acryloyl)cystamine (CBA), *N,N′*-hexamethylene-bis(acrylamide) (HMBA), *N,N*-dimethyldipropylene-triamine (DMDPTA), branched poly(ethylenimine) (PEI, 25 kDa) and heptafluorobutyric anhydride (HFBA) were from Sigma-Aldrich. Fluorescently labeled FAM-siRNA, negative control siRNA (siScr), siRNA targeting firefly luciferase (siLuc), siRNA targeting EGFP (siEGFP), and Cy3- and Cy5-labeled siScr were from GenePharma. LysoTracker Red, DAPI and Luciferase Assay kit were from the Beyotime Institute of Biotechnology. Trypsin, penicillin, streptomycin, RPMI-1640, and fetal bovine serum (FBS) were from Hyclone (Waltham, U.S.A.). Unless otherwise stated, all other reagents were from Nanjing Wanqing Chemical Glassware Instrument.

2.2. RHB and NHB Synthesis and Fluorination. RHB and NHB were synthesized and characterized as described in our previously published paper, using Michael-type polyaddition of DMDPTA and CBA or HMBA.[7] The molecular weight (from GPC) of NHB was 19.3 kDa and that of RHB was 15.8 kDa.

To fluorinate RHB, the polymer (0.100 g) solution in methanol was added to 0.120 g HFBA in methanol and triethylamine (0.030 g). The mixture was stirred at room temperature for 48 h and then extensively dialyzed against water and 10 mM HCl. The fluorinated polymer F-RHB was obtained by lyophilization. The fluorine content in F-RHB was determined by fluorine elemental analysis (Center of Modern Analysis, Nanjing University, China).[10,21,22]

2.3. Formulation of siRNA Polyplexes. siRNA solution (20 μg/mL in 10 mM HEPES, pH 7.4) was mixed with the polymers to achieve the desired polycation/siRNA (w/w) ratios. The mixture was vigorously vortexed for 30 s and left standing at room temperature for 30 min prior to use.

To evaluate polyplex formation, the polyplexes were run on a 1% (w/v) agarose gel containing JelRed at 90 V for 45 min and siRNA was visualized by UV illumination. Particle size and zeta potential of the siRNA polyplexes were determined by Zeta Plus (Brookhaven, U.S.A.). The polymers (0.5 mg/mL) were also titrated to assess buffering:the pH was adjusted to 12 with 1 M NaOH and the solution was titrated with 0.1 M HCl.[23,24]

2.4. Reduction-Triggered Release of siRNA from Polyplexes. Polyplexes were incubated at 37 °C for 2 h with 100 mM GSH or 200 mM NaCl analyzed on agarose gel as above.

2.5. Cytotoxicity. B16F10 cells were from Dr. Singh (University of Nebraska Medical Center). Mouse breast carcinoma 4T1 cells were from Dr. Miller (Wayne State University). CHO cells were from Miss Xu (Zhenjiang Hospital of Traditional Chinese and Western Medicine). Podocytes were from Dr. Gao (Jiangsu Province Hospital of TCM). The cytotoxicity of the polycations was measured by MTT assay. Briefly, the cells were incubated with increasing concentrations of the polymers and incubated for 24 h. Then, the MTT reagent was added and the cells incubated for additional 4 h before DMSO addition and absorbance measurement at 570 nm.

2.6. GSH-Induced Intracellular Disassembly of Polyplexes by FRET. The redox-trigged release of siRNA in the cells was studied using fluorescence resonance energy transfer (FRET). Cy3-siRNA and Cy5-siRNA were co-condensed within the polyplexes and used as a FRET pair.[25,26] The ratio of Cy3-siRNA and Cy5-siRNA was set to 1:1 and final siRNA concentration during incubation with the cells was

DOI: 10.1021/acsami.6b14184
ACS Appl. Mater. Interfaces XXXX, XXX, XXX−XXX

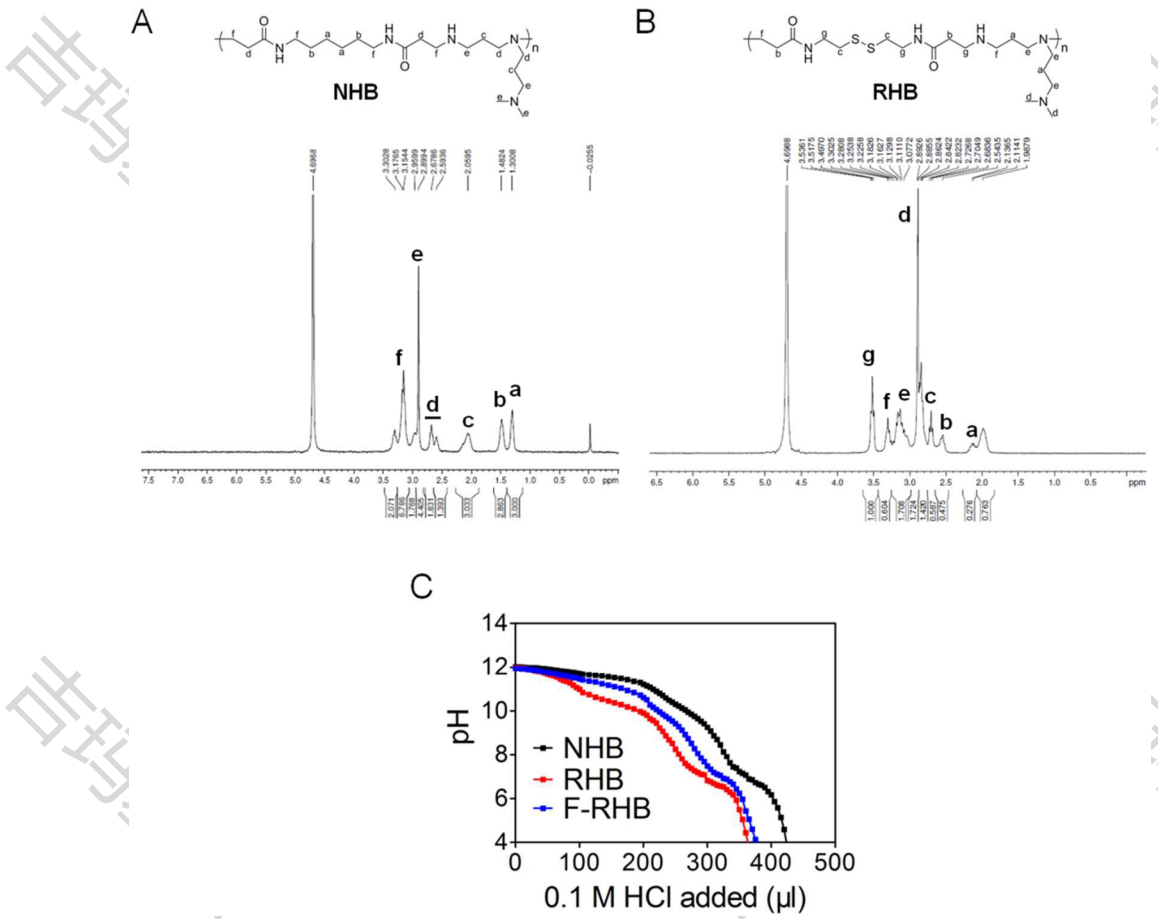


Figure 2. Physicochemical characterization of polymers. ^{1}H NMR spectra of NHB (A) and RHB (B). (C) Acid−base titration curves. Volume of HCl used to adjust the pH value of the polymer solutions from pH 12 to 4. (NHB, nonbioreducible hyperbranched poly(amido amine); RHB, bioreducible hyperbranched poly(amido amine)).

100 nM. *N*-ethylmaleimide (NEM) was used to deplete the intracellular GSH.[27] B16F10 were exposed to polyplexes for 6 h, either with or without 30 min pretreatment with 1 mM NEM, and the cells were imaged. The FRET of GSH-triggered disassembly of the polyplexes was determined using a Zeiss CLSM (Cy3 $\lambda_{exc/em}$ 550/570 nm, Cy5 $\lambda_{exc/em}$ 550/670 nm. The confocal microscope settings and parameters were the same in all groups.

2.7. Luciferase and EGFP siRNA Silencing in Vitro. Luciferase silencing in vitro was performed in B16F10 and 4T1 cells stably expressing luciferase (Luc). The cells (4×10^4) were seeded in 48-well plates and treated (4 h) with the polyplexes. Polyplexes were removed, and the cells were grown in the presence of 10% FBS for 24 h. Then, the Luc activity was measured using an assay kit as directed by the supplier. The relative light units (RLU) were measured and expressed per mg of cell protein determined by the bicinchoninic acid assay.

CHO-EGFP cells in black 96-well plates (1×10^4 cells per well) were treated with polyplexes (100 nM siRNA) in serum-free conditions for 4 h. Then, the cells were washed and cultured in medium with 10% FBS for 48 h. EGFP in the cells was observed by fluorescence microscopy.

2.8. Cellular Uptake and Intracellular Trafficking. To investigate the intracellular distribution, polyplexes were prepared with FAM-siRNA. B16F10 cells were incubated with the polyplexes (100 nM FAM-siRNA) in the absence of serum, nuclei stained with DAPI, and the intracellular distribution visualized by confocal microscopy with $\lambda_{exc/em}$ 346/460 nm for DAPI and $\lambda_{exc/em}$ 494/522 nm for FAM. The confocal microscope parameters were the same in all groups. To measure uptake, the cells were detached by trypsin, washed with PBS, and analyzed on BD FACSCalibur flow cytometer.

Endosomal escape of the polyplexes was measured after different incubation times in B16F10 cells stained with LysoTracker Red for 30 min. The cells were washed thrice with PBS and imaged by confocal microscopy.[28] The endosomal escape of polyplexes was visualized at $\lambda_{exc/em}$ 581/596 for LysoTracker Red and $\lambda_{exc/em}$ 494/522 nm for FAM. The confocal microscope parameters were the same in all groups.

2.9. siRNA Silencing in Vivo. C57BL/6 mice (6 weeks old, female) were used in compliance with the Institutional Animal Care and Use Committee of China Pharmaceutical University. One $\times 10^5$ B16F10 cells in PBS were subcutaneously injected. When the tumor size reached ~100 mm^3, the mice were injected (i.p.) with 200 μL D-luciferin (15 mg/mL) and imaged using Tanon 5200 Multi imaging system (Tanon Science & Technology Inc., Shanghai, China) to measure the tumor Luc bioluminescence on day 0. Imaging of the tumor-associated luciferase imaging was performed by Tanon 5200 Multi-imaging System with CCD camera (16 bit) at temperature −30 °C. The exposure time was set to 10 s for all live animal imagings. The parameters were the same in all groups and all time points. The mice were assigned to 7 groups (n = 3) and administered with 100 μL polyplexes (w/w 1 (PEI), 4 (NHB), or 8 (RHB and F-RHB)) by intratumoral injection using siRNA dose of 1.2 mg/kg. The injections were repeated on day 2 and bioluminescence of the tumors measured. Then, the mice were euthanized, and the tumors homogenized in a lysis buffer (5 mL of lysis buffer/g tumor). The lysate was centrifuged at 12 000g for 10 min and Luc expression measured as above.

2.10. Statistics. Statistical assessment was conducted by two-sided Student's t test for two groups and one-way analysis of variance for multiple groups ($P < 0.05$ was considered statistically significant).

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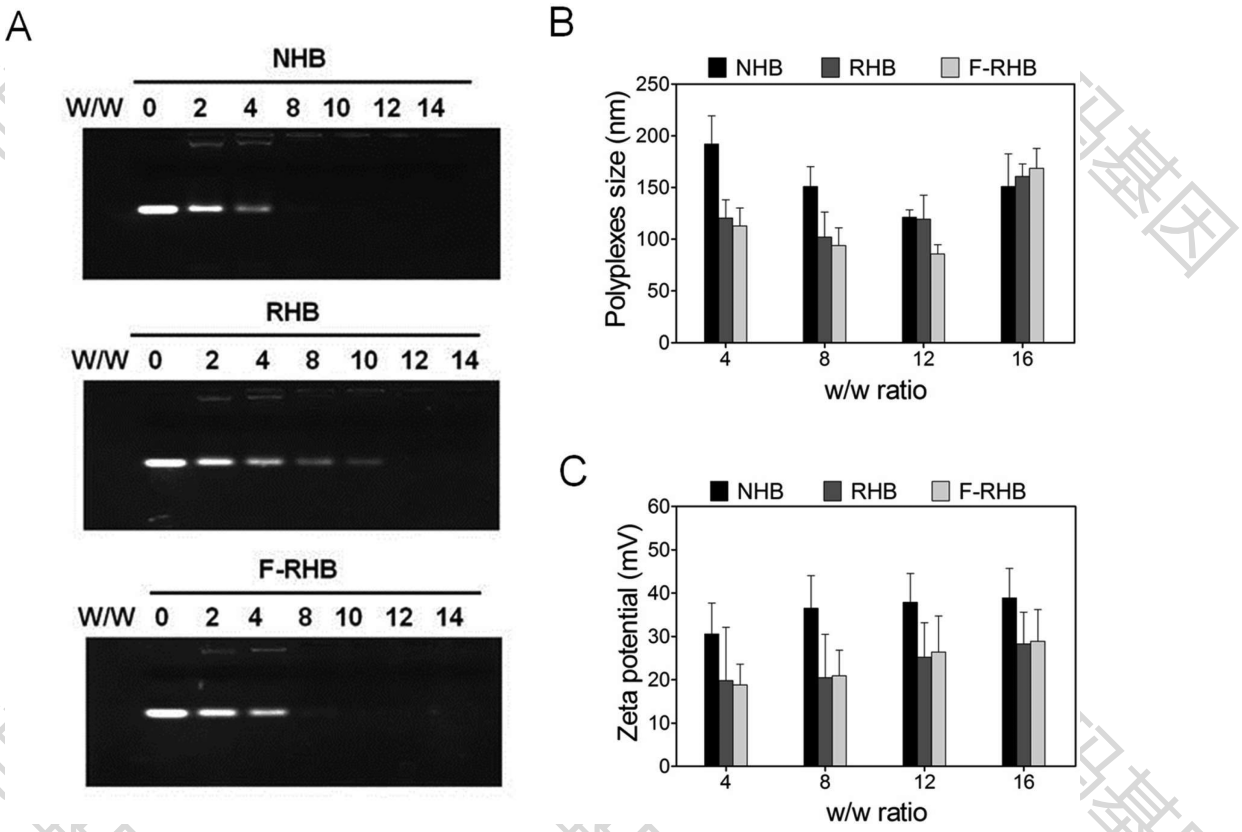

Figure 3. Polyplex formation and characterization. (A) siRNA complexation assessed by agarose gel electrophoresis of polyplexes prepared at different w/w ratios. Size (B) and ζ potential (C) of the polyplexes.

3. RESULTS AND DISCUSSION

3.1. Synthesis and Characterization of Polymers. We first prepared RHB and NHB by Michael-type polyaddition of triamine DMDPTA monomer with a suitable bis(acrylamide), CBA for RHB, and HMBA for NHB (Figure 1). We have selected the reaction conditions to prepare branched polymers. In this type of polyaddition, the polymer architecture (linear vs branched) can be easily controlled by the reaction stoichiometry and temperature.[29,30] Multiple previous studies documented the benefits of branched polycations for improved delivery of nucleic acids.[31] DMDPTA provides polymers with amines in the side chain to enhance charge density and improve binding with siRNA. The synthesized polymers with combination of three types of amines are known to have high transfection activity. Excess DMDPTA was added before the end of polymerization to react with unreacted acrylamides. The polymers were purified and converted to HCl salts by dialysis against HCl-acidified water. ^{1}H NMR spectrum confirmed the branched structure of the polymers (Figure 2A,B). The disappearance of the CH_2═CH— signal (5−6.5 ppm) confirmed complete consumption of the acrylamides. RHB and NHB were synthesized with similar molecular weight to allow for direct head-to-head comparison of their properties and siRNA delivery activity. To evaluate the combined effect of fluorination and bioreducibility on the siRNA delivery, fluoroalkyl chains were conjugated to RHB by reacting the available secondary amines with HFBA (Figure 1). The fluorine content in the synthesized F-RHB was measured by elemental analysis and found to be 19% (w/w). On the basis of the molecular weight of RHB and the fluorine content we calculated that about 50% of the secondary amines in RHB were modified with the fluoroalkyl chains.

The proton sponge hypothesis suggests that buffering by polycations is important for enhanced endosomal escape.[32] We analyzed buffering capacity to evaluate the effect of the combined fluorination and bioreducibility on the properties of RHB.[7] We measured buffering capacity by titration with HCl (Figure 2C). The results indicated that the buffering capability of NHB was slightly higher than that of RHB and F-RHB in the pH range of 5−7.4. The fluorination appears to slightly increase the buffering capacity. We believe this observation is the result of partial micellar assembly of F-RHB. The assembly is driven by the fluorous interactions and increases the local amine density in the assemblies, which decreases the apparent pK_a of the polymer.[13−19]

3.2. Formulation of siRNA Polyplexes. The effect of fluorination and bioreducibility on the ability of the polycations to form polyplexes was investigated by electrophoresis (Figure 3A). The w/w ratios of F-RHB/siRNA polyplexes in this study are shown as equivalent RHB/siRNA w/w ratios, excluding the fluorocarbon content. All three tested polycations could fully condense siRNA. NHB showed the best siRNA condensing ability, although the difference between the NHB, RHB, and the fluorinated analog was small.

Size and ζ potential of the polyplexes were measured by light scattering at w/w ratios 4 to 16 (Figure 3B,C). From the results, all the polyplexes had particle size under 200 nm and positive surface charge (ζ potential) ranging from 19 to 40 mV. The ζ potential increased with increasing w/w ratio due to excess polymer binding to the surface of the polyplexes.

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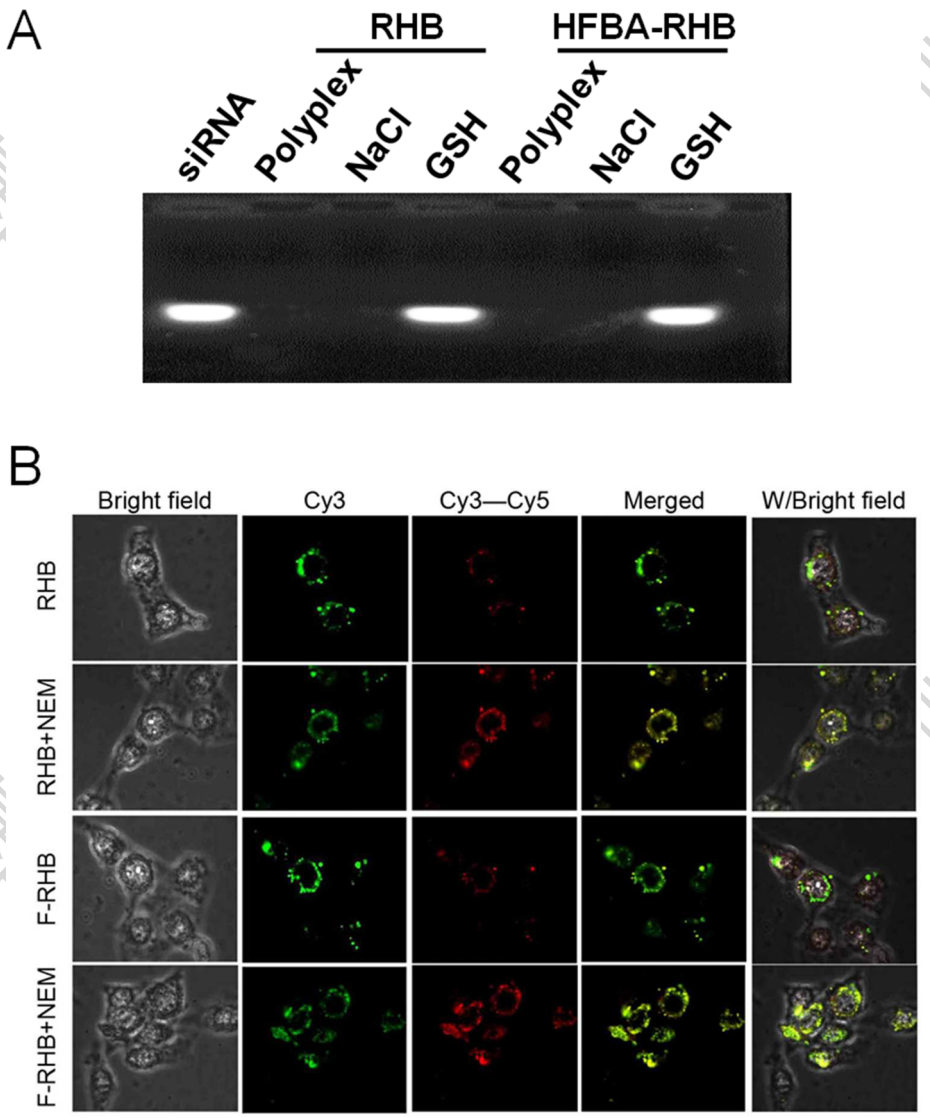

Figure 4. Reduction-triggered disassembly of polyplexes. (A) Reduction-triggered siRNA release from RHB and F-RHB polyplexes analyzed by agarose gel electrophoresis. (B) FRET analysis of GSH-triggered intracellular disassembly of polyplexes prepared from a mixture of Cy3-siRNA and Cy5-siRNA.

3.3. Redox-Triggered siRNA Release from Polyplexes. RHB and F-RHB contain disulfide bonds, which could be degraded in the cytoplasm and such degradation facilitates intracellular siRNA release from the polyplexes. As shown in Figure 4A, GSH treatment of the RHB/siRNA and F-RHB/siRNA polyplexes triggered siRNA release due to the disulfide breakage and degradation of the polymers. The results suggested that fluorination had no adverse effect on the accessibility and degradability of the disulfide bonds in RHB or within the formed polyplexes. No released siRNA from the RHB and F-RHB polyplexes was observed in the presence of up to 200 mM NaCl, indicating the polyplex stability under physiological salt conditions.[13]

We then used FRET to validate intracellular reduction-triggered disassembly of the RHB and F-RHB polyplexes. Polyplexes were prepared using a FRET pair based on Cy3- and Cy5-labeled siRNA co-condensed at equimolar ratios within polyplexes. FRET emission of the acceptor is active only in the presence of both fluorophores within the same polyplex. As shown in Figure 4B, after 6 h incubation with polyplexes, the strong green (Cy3) and weak red (Cy5) fluorescence were observed. This indicated that at least some of the polyplexes disassembled and the energy of the Cy3-siRNA donor could not be transferred to the Cy5-siRNA acceptor. In order to evaluate the importance of GSH on the observed disassembly, we have depleted intracellular thiols using NEM.[27] When the cells were pretreated with NEM, the Cy5-siRNA red fluorescence remained strong, suggesting inefficient polyplex disassembly in the cytoplasm of cells with depleted intracellular GSH. These findings were in agreement with the results in Figure 4A and validated that intracellular redox environment can effectively trigger the disassembly of the RHB and F-RHB polyplexes and the release of siRNA.

3.4. Cytotoxicity. Nonviral siRNA carriers with high transfection efficacy typically exhibit severe side effects, which is one of the main hurdles that prevents their clinical advancement.[33] As reported previously, bioreducible polycations show significantly decreased cytotoxicity in multiple cell lines.[9,34,35] Here, we evaluated if fluorination negatively affects the polycation cytotoxicity in a panel of cell lines, including

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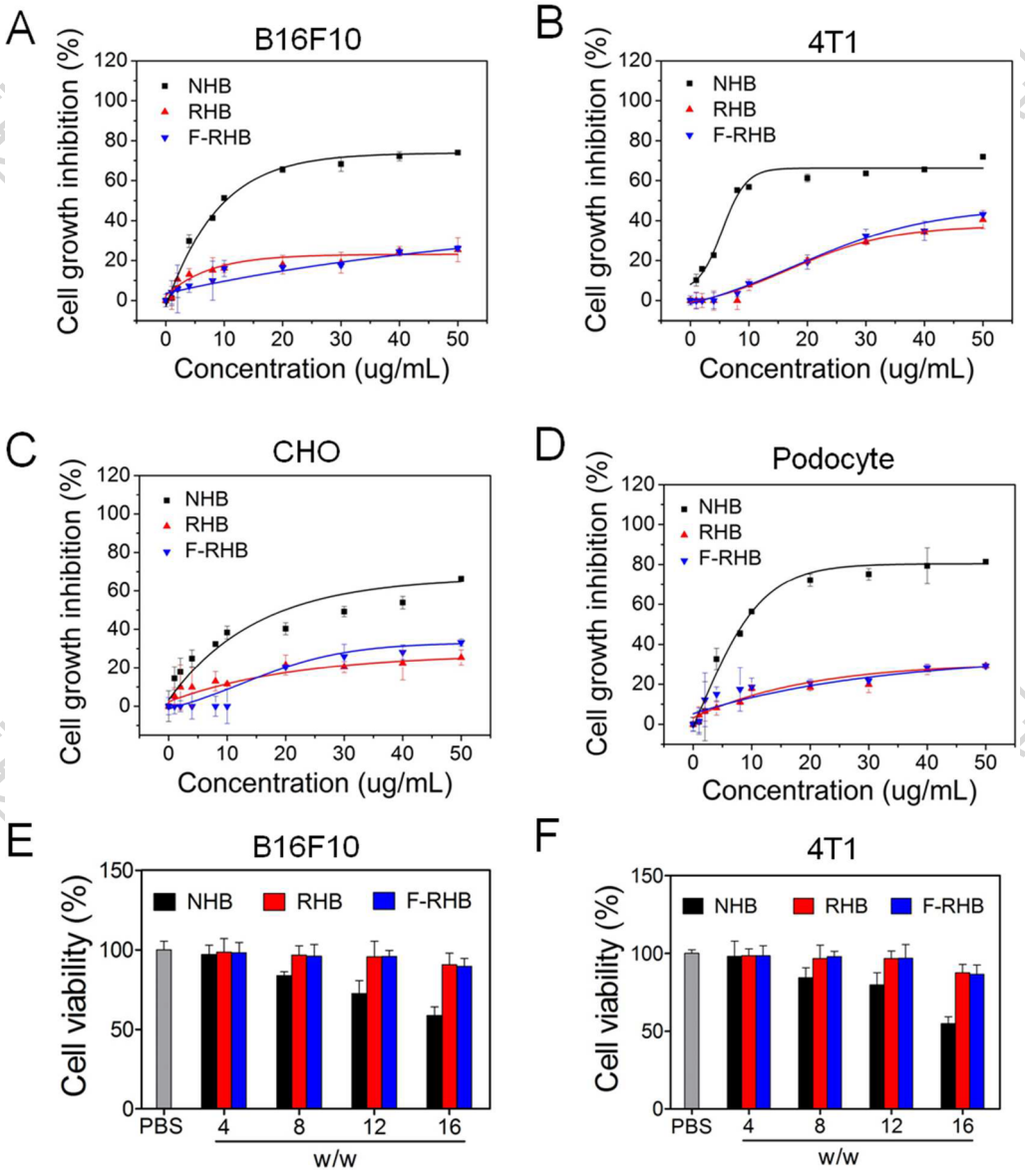

Figure 5. Cytotoxicity of polycations and their siRNA polyplexes. Cell growth inhibition (%) by the polycations in (A) B16F10, (B) 4T1, (C) CHO, and (D) podocyte cells. Cytotoxicity of polyplexes formed with siScr in B16F10 (E) and 4T1 (F) cells. Results shown as mean ± SD (n = 3).

B16F10, 4T1, CHO, and podocyte cells. The cell-growth inhibition curves of the polymers are shown in Figure 5A–D. No significant differences could be observed from the results with any of the four cell lines. As expected, the presence of disulfide bonds in RHB caused significant lowering of the cytotoxicity when compared with nondegradable NHB. This finding was consistent with previous reports which proposed that the decrease in cytotoxicity of reducible polycations is associated with intracellular GSH levels and rapid degradation of the reducible polycations.[9] Fluorination of RHB showed no adverse effect on the polycation toxicity as suggested by similar cytotoxic profiles of both RHB and F-RHB in all the used cell lines. We have also evaluated the cytotoxicity of the NHB/siScr, RHB/siScr, and F-RHB/siScr polyplexes in B16F10 and 4T1 cells at different w/w ratios (Figure 5E,F). The results confirmed that the polyplexes based on reducible polycations displayed considerably decreased cytotoxicity compared with nonreducible counterparts. These results could be used as guidance for further luciferase gene silencing.

3.5. siRNA Gene Silencing by Polyplexes. We next evaluated siRNA transfection activity of the polyplexes using safe w/w ratios determined from the above cytotoxicity study. The use of NHB/siRNA polyplexes was restricted to w/w 4 because of the NHB cytotoxicity, while RHB and F-RHB could be used safely up to w/w 12. The results showed that fluorination of RHB improved siLuc transfection efficacy in B16F10 cells at all studied w/w ratios (Figure 6A,B). NHB/siLuc was more effective than RHB and F-RHB polyplexes at its highest safe w/w ratio of 4 (60% Luc silencing). The improved safety permitted the use of RHB and F-RHB up to w/w 12, which then allowed us to achieve up to 74% and 83% Luc silencing, respectively. The Luc gene silencing activity of all studied formulations was lower in 4T1 cells and beneficial effect of fluorination was limited to F-RHB/siLuc prepared at w/w 12 only (75% silencing). These results demonstrated clear benefits of the safer RHB and F-RHB formulations compared with the NHB control. The best performing RHB and F-RHB

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ACS Appl. Mater. Interfaces XXXX, XXX, XXX−XXX

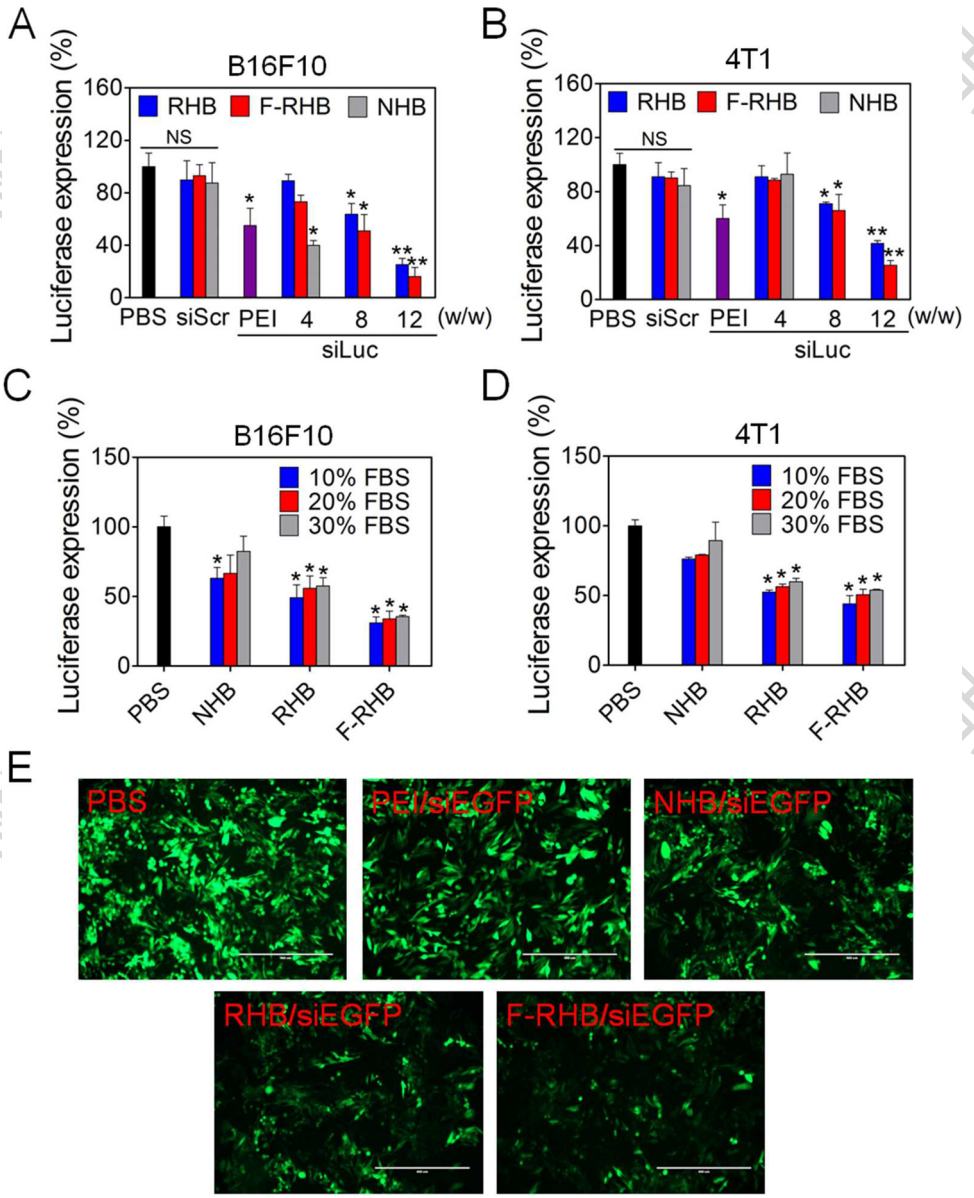

Figure 6. Transfection efficacy of F-RHB/siRNA polyplexes. Luc gene silencing F-RHB/siLuc polyplexes vs NHB/siLuc and RHB/siLuc in B16F10 cells (A) and 4T1 cells (B). Effect of serum (FBS) on transfection activity of the polyplexes in B16F10 (C) and 4T1 (D) cells. RHB/siLuc and F-RHB/siLuc were prepared at w/w 12. NHB/siLuc was prepared at w/w 4. (E) EGFP gene silencing by the polyplexes in CHO-EGFP cells by confocal microscopy. Scale bar = 400 μm. *$p < 0.05$, **$p < 0.01$, versus PBS group. NS, not significant versus PBS group.

polyplexes demonstrated also significantly higher transfection activity than control PEI.

One of the main expected benefits of fluorination was enhanced serum stability of the polyplexes due to the lipophobic nature of the fluorous interactions. To test the validity of the hypothesis, we evaluated the effect of serum on the siLuc transfection activity (Figure 6C,D). Both RHB and F-RHB polyplexes prepared at w/w 12 retained their improved silencing activity in the presence of serum when compared with the NHB polyplexes prepared at w/w 4. Notably higher levels of gene silencing were observed in both cell lines for the fluorinated polymer compared with the parent nonfluorinated polymer, even in the medium containing 30% FBS.

To validate the Luc silencing findings in another target gene and cell line, we have also tested silencing of EGFP in CHO cells (Figure 6E). Among all the groups, F-RHB polyplexes showed the highest EGFP silencing effect.

These results validated the benefits of fluorination on improving siRNA silencing efficacy and serum resistance of the F-RHB polyplexes. At the same time, due to the lowered cytotoxicity of bioreducible RHB and F-RHB, these polycations could be used safely at higher concentrations than the NHB.

3.6. Cellular Uptake and Intracellular Trafficking. For successful silencing, the polyplexes have to translocate across cell membranes and distribute into cytoplasm. We used polyplexes prepared with FAM-labeled siRNA to study the effect of fluorination on uptake and intracellular trafficking of the polyplexes in B16F10 cells. As above, we used safe polyplex formulations prepared at w/w 4 (NHB) and 12 (RHB and F-RHB). As shown in Figure 7A, F-RHB polyplexes exhibited increased cell uptake and more efficient cytoplasmic translocation than polyplexes based on RHB or NHB. F-RHB polyplexes also showed the highest efficacy of delivering siRNA into the B16F10 cells. These findings were confirmed by

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ACS Appl. Mater. Interfaces XXXX, XXX, XXX−XXX

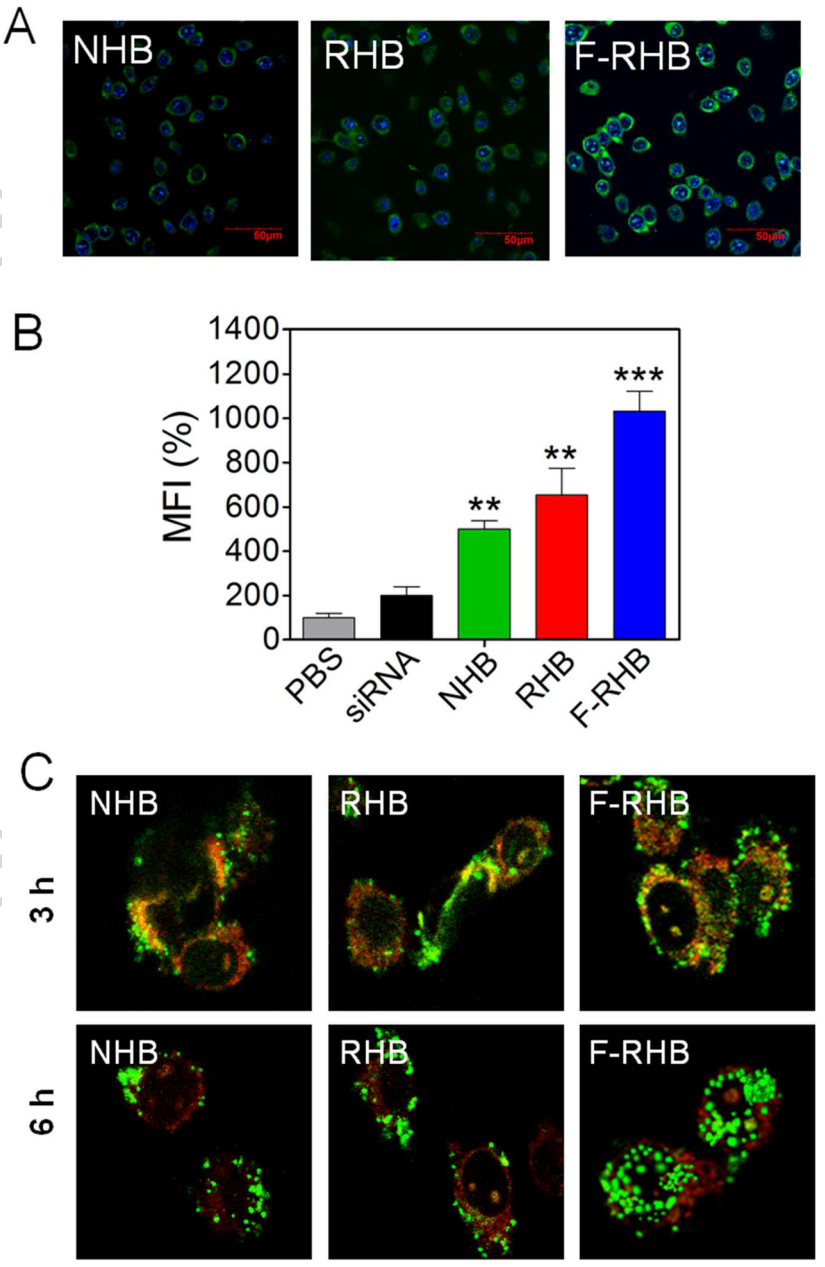

Figure 7. Intracellular trafficking and uptake of polyplexes. (A) Intracellular distribution of NHB/siRNA, RHB/siRNA, and F-RHB/siRNA polyplexes in B16F10 cells using FAM-siRNA (green). Cell nuclei stained with DAPI (blue). (B) Cell uptake determined by flow cytometry at 3 h postincubation with the polyplexes. (C) Endosomal escape of polyplexes in B16F10 cells 3 and 6 h after incubation. Lysosomes were stained with Lysotracker Red. $^{**}p < 0.01$, $^{***}p < 0.005$, versus PBS group.

analyzing the cell uptake using flow cytometry (Figure 7B). These results suggest that fluorination contributes to improved cell uptake and more favorable cytoplasmic distribution of the polyplexes, and these factors contributed to the overall enhanced gene silencing activity.

In addition to effective cell uptake, endosomal escape and transport into cytoplasm is another important step required for achieving high gene silencing efficiency of polyplexes. Therefore, we measured the endo/lysosomal escape of the polyplexes prepared with FAM-siRNA. Lysosomes were stained with LysoTracker at 3 and 6 h. As shown in Figure 7C, most of the endocytosed FAM-siRNA was located in lysosomes after 3 h, as indicated by the yellow fluorescence indicative of the signal overlay between the FAM-siRNA and LysoTracker. At 6 h, most of the FAM-siRNA fluorescence could be seen separated from the LysoTracker signal, suggesting efficient endo/lysosomal release. Taken together, F-RHB polyplexes were able to enter B16F10 cells and facilitate efficient endosomal escape.

3.7. In Vivo Luc Silencing. To examine the Luc silencing effect of the F-RHB polyplexes in vivo, Luc-expressing B16F10 tumor model was used. The tumor bioluminescence was analyzed before and after treatment with the polyplexes. Control animals were also injected with PBS, free siLuc, and PEI/siLuc. As shown in Figure 8, F-RHB/siLuc polyplexes showed excellent silencing effect 2 days after injection (88% Luc silencing). In contrast, RHB/siLuc and NHB/siLuc polyplexes only showed limited silencing activity (20−30%).

DOI: 10.1021/acsami.6b14184
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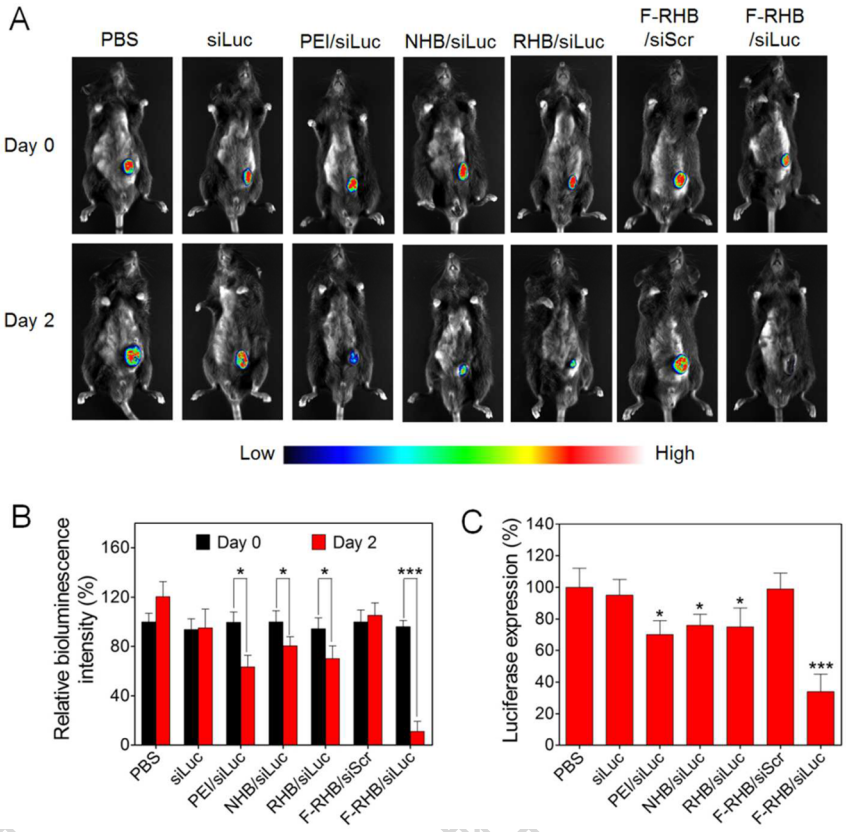

Figure 8. Luc gene silencing in vivo. (A) Bioluminescence images of mice with B16F10-Luc tumors before and after gene silencing. (B) Quantification of Luc expression from the whole-body images. (C) Ex vivo analysis of the Luc activity in the isolated tumor tissues. Data are shown as mean ± SD (n = 3). *p < 0.05, ***p < 0.005, versus PBS group in Figure C.

No silencing effect was observed in the PBS and free siLuc groups. To confirm the Luc silencing, the tumors were excised, homogenized and Luc activity was measured. The performance of F-RHB was confirmed by the observed 66% silencing (Figure 8C). Taken together, these results suggest fluorinated and bioreducible polymers had promising potential as nonviral vectors in vivo.

4. CONCLUSIONS

We have synthesized fluorinated bioreducible polycations. The polymers exhibited good siRNA binding ability. Due to the lower cytotoxicity, F-RHB could be used safely at higher concentrations and doses than control NHB and achieve significantly improved both in vitro and in vivo siRNA silencing. Thus, combining fluorination and bioreducibility can effectively improve the ability of polycations to deliver siRNA. The reported polymers have the potential for further development as siRNA delivery systems. Future development of these polymers will focus on studies of potential systemic toxicity, immunogenicity, and genotoxicity as well as improvement of gene silencing activity following intravenous injection.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This study was supported by the China National Science Foundation (No. 81373983, 81573377) and by the Changjiang Scholar program.

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DOI: 10.1021/acsami.6b14184
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