In this work, we used the advantage of the versatile function of cationic poly(p-phenylene vinylene) (PPV) derivative to develop a white light-enhanced siRNA delivery in a remote control manner. Conjugated polyelectrolyte PPV, which possesses cationic and amphipathic features, can effectively self-assemble with siRNA and deliver siRNA into living cells. More importantly, PPV can sensitize surrounding oxygen into reactive oxygen species (ROS) upon exposure to white light and disrupt the endosomal membrane, and the resulting enhanced endosomal escape increases the gene silencing ability of siRNA. Besides, due to high fluorescent emission, PPV can also be used to image the siRNA delivery and intracellular location. Therefore, by taking full advantage of PPV, the strategy provides a new enhanced siRNA delivery in a non-invasive and spatiotemporal photochemical manner.

Gene therapy has attracted much attention not only in fundamental biology but also in clinical treatment in recent decades.\textsuperscript{[1–3]} Compared with viral vectors with an inherent risk for clinical use, non-viral vectors have been widely developed owing to the advantages of biosafety, simplicity, and controllability.\textsuperscript{[4–6]} However, to allow therapies to be successfully applied, the genes should be effectively delivered. It is well known that the endosome is the main physiological barrier to obtain efficient transfection activity. Several strategies were proposed to overcome this obstacle, such as modulators of endosomal rupture (chloroquine),\textsuperscript{[7]} cationic nanoparticles,\textsuperscript{[8, 9]} and so on.\textsuperscript{[10, 11]} However, the inherent cytotoxicity and complexity of these strategies severely hinder their clinical application as gene vectors.\textsuperscript{[12–14]} Hence, new gene vectors or strategies for enhanced gene delivery are still required.

Photochemistry has been widely considered as a light platform in biological applications owing to its non-invasive and highly spatiotemporal-resolved character.\textsuperscript{[15–19]} With remote control capability and biosafety, photochemical pattern is also promising to be applied in photoresponsive drug release as well as photodynamic and photothermal therapy.\textsuperscript{[20–22]} More recently, using light activity, a strategy termed photochemical internalization (PCI) has been successfully developed.\textsuperscript{[23–26]} PCI could enhance the cytoplasmic delivery of a small molecule and macromolecular drug through endosomal disruption by light-active reactive oxygen species (ROS). Although PCI has been developed to work for gene transfection, the conventional PCI should integrate an additional photosensitizer (PS) into the complex of vector and gene.\textsuperscript{[27–29]} Thus, the development of novel multifunctional and simple gene vectors with incorporated light-active ROS ability becomes an imminent mission.

Recently, conjugated polyelectrolytes with excellent properties including high fluorescence brightness, good photostability, low cytotoxicity and generation of ROS have been developed as useful therapy platforms for biological applications.\textsuperscript{[30–33]} Owing to their charged side chains, conjugated polyelectrolytes can self-assemble with siRNA by electrostatic interactions. The unique properties endow conjugated polyelectrolytes as novel materials to serve as multifunctional gene vectors.\textsuperscript{[34, 35]} In this work, we used the advantage of the versatile function of cationic conjugated polyelectrolytes and developed a multifunctional platform for white light-enhanced siRNA delivery though remote control manner.

The mechanism of white light-enhanced endosomal escape strategy for high siRNA delivery is schematically shown in Scheme 1. Cationic PPV, which possesses cationic and amphipathic features, is selected as the multifunctional platform as it not only can effectively self-assemble with siRNA and deliver siRNA into cells but also sensitize surrounding oxygen into ROS when exposed to white light to photochemically disrupt the endosomal membrane. Endosomal disruption enhances the endosomal escape of siRNA, which helps siRNA to avoid enzymatic degradation and enhances the influx of siRNA into the cytoplasm for RNA interference.

The siRNA loading capability of PPV was firstly studied. Gel retardation assay was performed to explore the siRNA loading and compressibility of PPV. As shown in Figure 1a, siRNA could not run out from gel wells after binding with PPV at a low N/P...
ratio even to 2. Meanwhile, PPV can self-assemble with siRNA owing to its amphipathic characteristic and positively charged side chains. The size of PPV/siRNA polyplexes at an N/P ratio of 10 is 59.2 ± 6.7 nm in diameter, while that of PPV was 197.2 ± 10.5 nm (Figure 1b and Figures S1–S2). The size decrease between PPV and PPV/siRNA polyplexes further confirms that PPV can effectively assemble into compact nanoparticles after assembly with siRNA. Furthermore, the positively charged surface of PPV/siRNA polyplexes at various N/P ratios makes sure that PPV/siRNA polyplexes could easily bind to the negatively charged cell membrane and is suitable for endocytosis by cells (Figure 1c). These results demonstrate that PPV could load siRNA effectively and maintain a positively charged surface for high cellular uptake. Then we investigated whether PPV has the ability to sensitize oxygen into ROS upon exposure to white light. The ROS probe 2,7-dichloro fluorescein diacetate (DCFH-DA) was chosen to probe ROS production. The irradiation of DCFH-DA was carried out in the presence or absence of PPV (1 μm) under white light (400–800 nm), and the fluorescent intensity of DCF (an activated product of DCFH-DA) at 525 nm was monitored. The PPV group showed a markedly fluorescence intensity compared to the control group (Figure 1d). Moreover, the ROS production in living cells sensitized by PPV was confirmed (Figure S3). These results indicate that PPV has a favorable ROS-sensitizing capability even in the intracellular surroundings. To determine whether PPV could photochemically disrupt the endosomal membrane and enhance the endosomal escape in living cells upon white light irradiation, HeLa cells were incubated with PPV for 1 h and irradiated with or without white light for another 1 h. After labeling with Lysotracker, the intracellular location of PPV was assessed by confocal laser scanning microscopy (CLSM). As shown in Figure 1e and Figure S4, after white light irradiation, the endosome is effectively disrupted and PPV fully escaped from the endosome, while PPV without white light irradiation is nearly co-localized with the endosome. Moreover, the co-localization ratio of PPV with late endosome/lysosome is significantly reduced from 0.89 to 0.16 at 4 h after irradiation (Figure 1f). Thus, this PCI-triggered endosomal disruption of PPV may result in the enhanced endosomal escape. We next examined the biosafety of this strategy by MTT assay. As shown in Figure 1g, the cell viability is barely influenced at various concentrations of PPV (0–20 μM) under white light irradiation at a fluence rate of 3 mW cm⁻². Furthermore, PPV also shows a favorable biocompatibility compared with the classic gene vector PEI25kD (Figure S5).

To further explore the ROS-enhanced endosomal escape of PPV/siRNA polyplexes, we next investigated the intracellular distribution of PPV/siRNA polyplexes by CLSM. As depicted in Figure 2a, the PPV/siRNA polyplexes-treated HeLa cells show an apparent fluorescence emitted from Cy5-labeled siRNA and PPV, and both the fluorescence of siRNA and PPV mainly overlap with Lysotracker, which is indicative of the co-localization effect of PPV/siRNA polyplexes with the endosome. After light irradiation, the majority of siRNA molecules distribute in the cytoplasm of light-treated cells, indicating that siRNA in PPV/siRNA polyplexes shows an evident endosomal escape effect.
after white light irradiation. More interestingly, PPV, which emitted high fluorescence, can work as fluorescent probe for the visualization of siRNA delivery. Furthermore, single-cell imaging was used to observe the precise intracellular distribution of PPV/siRNA polyplexes. As shown in Figure 2b and Figure S6, the siRNA had completely escaped from the endosome when the cells were incubated for another 20 h after light irradiation. These results confirm that PPV can not only effectively deliver siRNA into the intracellular endosome but also assist siRNA to escape from the endosome through ROS sensitized by PPV disrupting the endosomal membrane. Moreover, the transfection efficiency of PPV/siRNA polyplexes was further quantified by flow cytometry using Cy5-labeled siRNA. PPV/siRNA polyplexes (93.7 ± 5%) show a higher transfection efficiency than PEI25kD (28.8 ± 13%) and lipofectamine 2000 (77.0 ± 9%) (Figure 2c). Next, the gene silencing ability of PPV/siRNA polyplexes upon light irradiation was evaluated by luciferase assay with HeLa-Luc cells. Compared with siRNA only and PEI25kD, a higher silencing efficiency of PPV/siRNA polyplexes was obtained, indicating that PPV is an excellent siRNA vector for gene silencing (Figure 2d), while PPV itself does not interfere with the luciferase activity (Figure S7). Furthermore, the silencing efficiency of PPV/siRNA polyplexes with and without light irradiation was also investigated. The results showed that gene silencing is evidently higher with light irradiation at the same conditions, which is presumably due to the enhanced endosomal escape of siRNA loaded by PPV, demonstrating the advantage of photochemistry based on ROS-sensitized conjugated polymer PPV.

In conclusion, we developed a novel multifunctional platform based on a conjugated polyelectrolyte for enhanced siRNA delivery by light-enhanced endosomal escape. In this design, the conjugated polyelectrolyte PPV, which possesses cationic and amphipathic features, can effectively self-assemble with siRNA and deliver siRNA into the target cells. Furthermore, upon light irradiation, PPV is able to light-sensitize surrounding oxygen into ROS which can disturb the endosomal membrane, and thus an enhanced endosomal escape is obtained to increase the gene silencing ability of siRNA. Meanwhile, PPV is highly fluorescent, thus allowing the imaging of siRNA delivery and intracellular location through confocal microscopy. This strategy takes full advantage of PPV, providing a new enhanced siRNA delivery in a non-invasive and spatiotemporal manner.

**Experimental Section**

**Preparation and characterization of PPV/siRNA polyplexes**

To determine the siRNA loading capacity of PPV, siRNA (0.2 µg) and PPV at various N/P ratios were incubated for 30 min at room temperature to produce PPV/siRNA polyplexes. All samples were
tested by 0.8% agarose gel electrophoresis and stained by using
Greeniner. Gel images were used to quantify the appropriate
proportion of conjugated polyelectrolyte and siRNA using the gel
imaging system GELDOCR XR-1 (Bio-Rad, USA). The sizes and zeta
potential of PPV/siRNA polyplexes at various N/P ratios were mea-
 sured using a Zetasizer Nano ZS 90 system (Malvern, UK) at room
temperature. Solutions of complexes containing 1 μg of siRNA
were prepared and diluted with 1 mL of ultrapure water before
characterization.

In vitro gene silencing
To assess the gene silencing efficacy of PPV/siRNA polyplexes,
anti-Luc siRNA was used to target firefly luciferase. HeLa-Luc cells
were plated in 24-well plates with 5 × 10^4 cells/well and incubated
overnight. Lipofectamine 2000 and branched PEI were used as con-
trols. The transfection assay was performed as described previously
with three wells per sample. After transfection for 24 h, the cells
were washed twice with PBS. Then the cells were lysed using 200 μL of
1× reporter lysis buffer (Promega, Madison, USA) followed by three
freeze-thaw cycles to ensure complete lysis. The cell lysate was centrifuged for 2 min at 12000 rpm at 4°C. Then the supernatant was collected for luminescence measurement. The relative light units (RLUs) were measured with a fluorometer (Synergy HT, BioTek, USA). The total protein was measured by using a BCA protein assay kit (Tiangen Biotech, Beijing, China) according to the manufacturer’s protocol.

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