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Shell-detachable nanoparticles based on a light-responsive amphiphile for enhanced siRNA delivery†

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Novel light-responsive nanoparticles based on an amphiphile with a single photolabile linker between its hydrophilic head and hydrophobic tail was developed for small interfering RNA (siRNA) delivery. Upon UV exposure, cleavage of the linkage resulted in rapid shell detachment of the nanoparticles, which facilitated siRNA release and enhanced gene silencing efficiency.

Small interfering RNA (siRNA) is one of the most powerful technologies for sequence-specific suppression of genes, and is considered highly promising to treat a variety of human disease.[1] However, its application is markedly hampered by inherent drawbacks, including susceptibility to degradation by serum nucleases and poor cellular uptake.[2] Successful applications of siRNA-based therapy rely on the development of safe and efficient delivery vectors. Up to now, various promising delivery systems, including cationic polymers,[3] lipid-based vectors,[4] and inorganic nanoparticles[5] have been developed for siRNA delivery.

One great challenge for successful gene silencing is to address an inherent dilemma in designing ideal vectors; that is, the vectors should on the one hand form a tight and stable complex with siRNA during circulation, and on the other hand they should be able to facilitate siRNA release in the cytoplasm.[6] To address the former aspect, hydrophobic modification of cationic vectors has been considered a useful solution. Several studies have confirmed that hydrophobic modification of water soluble polycations significantly improves the binding ability, stability and gene silencing efficacy of siRNAs as compared to the parent vectors.[3,7] Furthermore, the incorporation of hydrophobic segments in polycations could produce siRNA complexes with enhanced stability, improved blood circulation and biodistribution for *in vivo* studies.[8–10] To solve the latter aspect of intracellular siRNA release, versatile smart vectors with stimuli-triggered sensitivities have been developed. For example, various pH-, light- or redox-responsive delivery vectors have been constructed and demonstrated to be capable of enhancing gene silencing efficiency by facilitating siRNA release in the cytoplasm.[11–16]

Despite these impressive achievements, either hydrophobization or stimuli-triggered siRNA release only meets one aspect of the design dilemma. Studies that simultaneously solved the two aspects are rarely reported. The design of siRNA delivery systems can still be advanced and optimized. In the present study, we propose a light-responsive amphiphile as a proof-of-concept model in an attempt to address such a design dilemma. Light-responsive linkage was used because it can be remotely applied with high spatial and temporal precision and has been widely used as an external stimulus to control cargo release from delivery systems.[17–19] The amphiphile in this study has a long hydrophobic tail and a cationic head with a single photolabile 2-nitrobenzyl (NB) ester bond as a bridge. In aqueous solution, the amphiphile self-assembles into UV-sensitive nanoparticles (USNPs) with a hydrophobic core and a cationic shell. The hydrophobic core maintains the stability of the nanoparticles, while the cationic shell provides complex sites for siRNA condensation. When exposed to UV light irradiation, cleavage of the NB bond causes detachment of the hydrophilic shell and subsequently facilitates siRNA release from the complex to eventually enhance gene silencing efficiency Scheme 1.

The cationic amphiphile **1** was synthesized through a multiple reaction procedure, as illustrated in the ESI (Scheme S1†). Its chemical structure was verified by ^{1}H NMR and ESI-MS data (Fig. S1†). USNPs of amphiphile **1** were prepared by a dialysis method. As shown in Fig. 1a, the transmission electron microscopy (TEM) image indicates that USNPs were well-dispersed with an average diameter approximately 230 nm, which is in good agreement with the dynamic light scattering (DLS) results shown in Fig. 1b.

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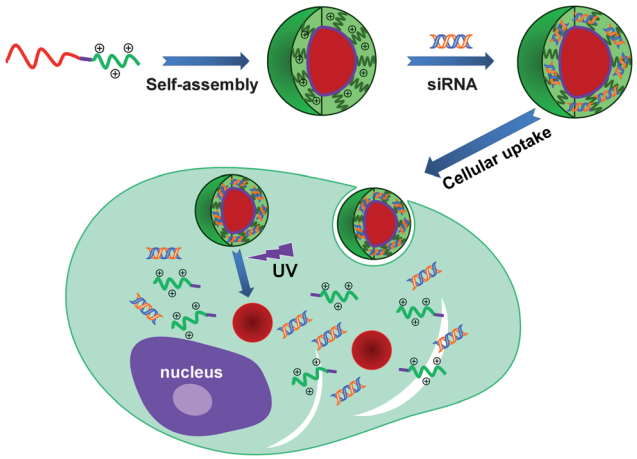

Scheme 1 Illustration of the preparation of UV-sensitive nanoparticles/siRNA complexes (USNP/siRNA). The complexes can be taken up by cancer cells and effectively release siRNA by the trigger of UV light.

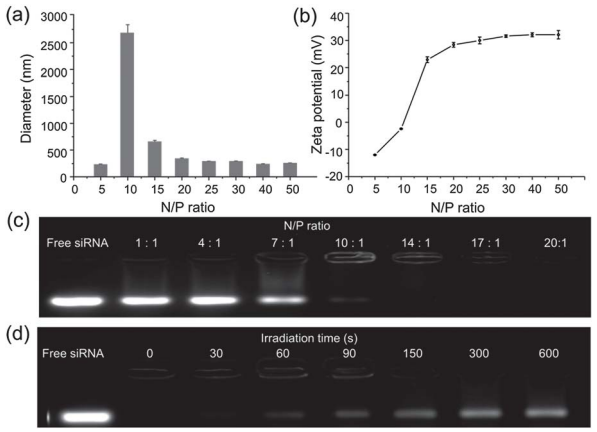

Fig. 2 (a) Size and (b) zeta potential measurements of USNP/siRNA complexes at different N/P ratios. (c) Agarose gel analysis of siRNA migration of the USNP/siRNA complexes at N/P ratios varying from 1/1 to 20/1. (d) Effect of UV irradiation time on siRNA migration from the USNP/siRNA complexes at N/P = 25.

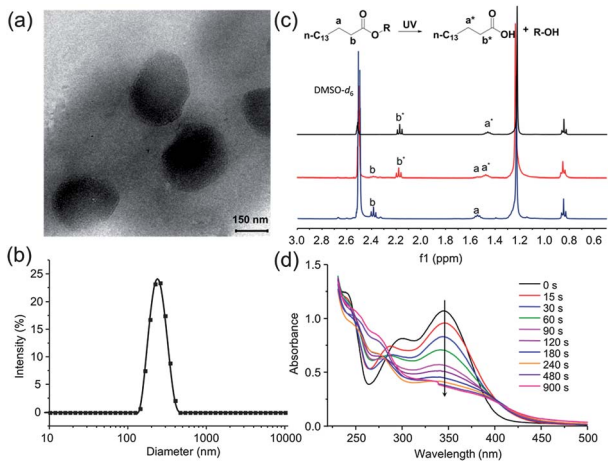

Fig. 1 Size measurement of USNPs by (a) TEM and (b) DLS. (c) Partial ^{1}H NMR spectra of amphiphile **1** before UV irradiation (bottom), after UV irradiation for 300 s (middle) and pure palmitic acid (top). (d) UV-Vis spectra of USNPs after exposure to UV light at times varying from 0 to 900 s. The USNPs solution was subjected to UV irradiation at the intensity of 10 mW cm^{-2} (λ_{max} = 365 nm).

The UV-light triggered degradation of USNPs was studied by ^{1}H NMR and UV-Vis absorption methods. Upon UV irradiation, the NB ester bond was cleaved, which causes characteristic changes in both the ^{1}H NMR spectrum and UV-Vis absorption curve. As shown in Fig. 1c, after UV irradiation for 300 s, the chemical shifts of peak *a* and *b* shifted from 1.55 and 2.38 ppm to 1.50 (*a**) and 2.18 ppm (*b**), respectively. By comparing the ratio of peak *b* and *b** of the middle ^{1}H NMR spectrum, the degradation efficiency was calculated to be 72% after 300 s of UV irradiation. Meanwhile, the UV-Vis absorption spectra also showed a rapid decrease at 345 nm along with a concomitant increase at 265 nm upon UV irradiation (Fig. 1d). This spectral change was attributed to cleavage of the linker and the formation of nitrosobenzaldehydes.[20] By plotting the absorbance at 345 nm against irradiation time, one can see that the absorbance decreased rapidly in the initial 200 s and flattened gradually after 300 s (Fig. S2†), demonstrating the rapid degradation of the NB ester bond upon UV irradiation.

Due to the presence of amino groups on the surface of USNPs, the zeta potential of the nanoparticles was highly positive (52.2 ± 0.5 mV). As siRNA is negatively charged, the USNPs were expected to bind siRNA through electrostatic interactions. After mixing USNPs with siRNA at various nitrogen/phosphate (N/P) ratios, the size and zeta potential of the complexes were determined. As shown in Fig. 2a and b, with an increase in the N/P ratio, the size of the complexes changed dramatically, transforming from 234.3 nm at N/P = 5 to 2687.2 nm at N/P = 10, then decreased and remained less than 300 nm when the N/P ratio was greater than 25. Meanwhile, the zeta potential of the complexes increased consistently with an increase in the N/P ratio. It should be noted that the maximum particle size at N/P = 10 is caused by neutral surface charge induced particle aggregation.[21] In accordance with these results, the gel retardation assay in Fig. 2c also shows that the migration of siRNA could be completely halted by the USNPs when the N/P ratio was greater than 10, demonstrating successful siRNA binding by USNPs. In comparison, the pure water soluble cationic molecule diethylenetriamine hydrochloride was also used; this molecule has an identical structure to the cationic head of amphiphile **1** but could not bind siRNA, even at an N/P ratio up to 50 (Fig. S3†). This result implies that the formation of nanoparticles by introducing a hydrophobic tail indeed improves siRNA binding capacity.

siRNA release from the complex is a crucial step for effective gene silencing. One key design of our USNPs was the UV-induced degradation of the carrier to facilitate siRNA release. To test this, USNP/siRNA complexes at an N/P ratio of 25 were subjected to UV irradiation for a predetermined period of time and analyzed by agarose gel electrophoresis. As shown in Fig. 2d, with an increase in irradiation time, more free siRNAs

were observed to migrate from the loading position, indicating that UV-induced nanoparticle degradation facilitated siRNA release.

Next, cellular uptake and intracellular siRNA release of the USNP/siRNA complexes were studied by flow cytometry (FACS) and confocal laser scanning microscopy (CLSM) in MDA-MB-231 cells. As depicted in Fig. 3a, compared to free FAM-labeled siRNA (FAM-siRNA), USNP/FAM-siRNA complexes showed much stronger fluorescent signals, whether the incubation time was 3 or 5 h, demonstrating that USNPs were effective in delivering siRNA into cancer cells. CLSM images also confirmed this result. As shown in Fig. 3b, with free siRNA, no detectable FAM-siRNA fluorescence could be seen in the cells. In contrast, clear green fluorescence could be observed when cells were treated with USNP/FAM-siRNA complexes. Furthermore, by careful observation, it was found that the green fluorescence of the complexes without UV irradiation was mainly in an aggregated form, whereas that upon UV exposure was much more dispersed throughout the entire cytoplasm and even in the nuclei. These results confirm our initial expectation that USNPs could promote intracellular siRNA release in response to UV light irradiation.

Based on these observations, we further studied whether the enhanced cellular uptake and intracellular siRNA release would result in enhanced *in vitro* gene silencing. The siRNA sequence targeting the luciferase gene (si*Luc*) was chosen to form a complex with USNPs, and the silencing efficiency was tested in stable luciferase-expressing MDA-MB-231 cells. As shown in Fig. 3c, compared with the PBS control, neither free si*Luc* nor the UNSP complex with negative control siRNA (USNP/si*N.C.*) generated a gene silencing effect. Furthermore, the gene silencing efficiencies did not change considerably, even after UV irradiation for 300 s, implying that UV irradiation did not induce cell cytotoxicity under these experimental conditions. In contrast, the USNP/si*Luc* complexes showed better performance after UV irradiation. Without UV irradiation, USNP/si*Luc* only exhibited a slight gene silencing effect (~15%). However, after UV irradiation for 300 s, the gene silencing effect was significantly enhanced up to 50%, which could be attributed to the enhanced intracellular siRNA release induced by UV-triggered nanoparticle degradation. Additionally, an MTT assay revealed that the USNPs did not induce obvious cell cytotoxicity at the test concentrations (Fig. S4†).

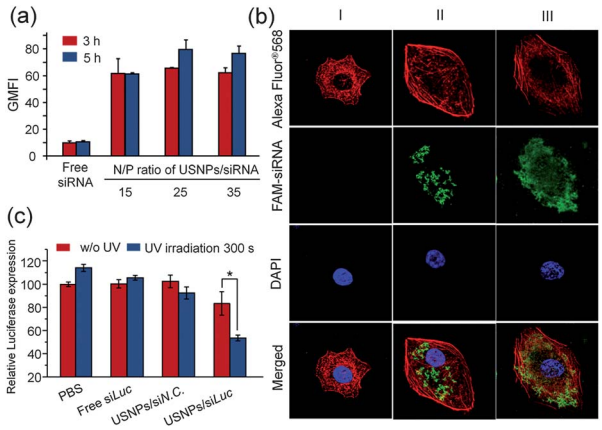

Fig. 3 (a) Cellular uptake of USNP/FAM-siRNA complexes measured by FACS after 3 or 5 h of incubation with MDA-MB-231 cells. (b) CLSM images of the intracellular localization of USNP/FAM-siRNA complexes in MDA-MB-231 cells. Red: F-actin stained by Alexa 568-phalloidin; green: FAM-siRNA; blue: cell nuclei counterstained with 4′,6-diamidino-2-phenylindole (DAPI). I: Free FAM-siRNA, II: USNP/FAM-siRNA complexes without UV irradiation, III: USNP/FAM-siRNA complexes with UV irradiation for 300 s. (c) Relative luciferase expression with various si*Luc* formulations with or without UV irradiation. The study was conducted in a stable luciferase-expressing cell line (MDA-MB-231) with the concentration of si*Luc* at 200 nM ($n = 3$, $^*p < 0.05$). The p values were calculated according to Student's t-test. The cells were first transfected for 3 h. After exposure to UV irradiation for 0 or 300 s, the cells were further incubated for 1 h before CLSM observation and 48 h before luciferase analysis.

In summary, we have successfully developed a novel light-responsive shell-detachable nanoparticle based on an amphiphile with a photolabile linker for enhanced siRNA delivery. The formation of nanoparticles enhanced siRNA binding when compared to the water-soluble cationic counterpart at identical N/P ratios. More importantly, when exposed to UV light, cleavage of the linker caused rapid shell detachment of the nanoparticle and accordingly facilitated intracellular siRNA release and enhanced gene silencing efficiency. Our design provides a new strategy to manipulate delivery systems for advanced performance. Just recently, near-infrared (NIR) light with deeper tissue penetration and lower toxicity has attracted more and more attentions in biomedical applications. By combining UV-sensitive leaving groups with upconversion nanoparticles, a couple of materials have been reported to be capable of responding to NIR light to promote cargo release.[22] Based on these progresses, our newly designed siRNA vector can extend to NIR responsiveness and further expands its applications in the future.

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