A Yolk–Shell Nanoplatform for Gene-Silencing-Enhanced Photolytic Ablation of Cancer

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Noninvasive near-infrared (NIR) light responsive therapy is a promising cancer treatment modality; however, some inherent drawbacks of conventional phototherapy heavily restrict its application in clinic. Rather than producing heat or reactive oxygen species in conventional NIR treatment, here a multifunctional yolk–shell nanoplatform is proposed that is able to generate microbubbles to destruct cancer cells upon NIR laser irradiation. Besides, the therapeutic effect is highly improved through the coalition of small interfering RNA (siRNA), which is codelivered by the nanoplatform. In vitro experiments demonstrate that siRNA significantly inhibits expression of protective proteins and reduces the tolerance of cancer cells to bubble-induced environmental damage. In this way, higher cytotoxicity is achieved by utilizing the yolk–shell nanoparticles than treated with the same nanoparticles missing siRNA under NIR laser irradiation. After surface modification with polyethylene glycol and transferrin, the yolk–shell nanoparticles can target tumors selectively, as demonstrated from the photoacoustic and ultrasonic imaging in vivo. The yolk–shell nanoplatform shows outstanding tumor regression with minimal side effects under NIR laser irradiation. Therefore, the multifunctional nanoparticles that combining bubble-induced mechanical effect with RNA interference are expected to be an effective NIR light responsive oncotherapy.

1. Introduction

Noninvasive physically stimuli-responsive therapeutic modalities have become the new trend of cancer treatment over the past decades. Usually by utilizing nanoscale agents that are responsive to external physical stimuli including ultrasound, light, magnetic field, and radiofrequency, etc., these therapies can selectively kill the cancer cells while leave the normal cells and tissues undamaged. Among the various stimulus, near infrared (NIR) light shows superior tissue penetration ability and accurate spatiotemporal control, which have attracted extensive attention from many research groups. The representative NIR laser responsive treatment modalities mainly convert optical energy into heat or reactive oxygen species by photothermal agents or photosensitizers to destruct cancer cells. However, some drawbacks such as the hyperthermia to normal tissues induced by high power density laser and the low therapeutic effects caused by instability of most photosensitizers largely restrict the development of these two modalities.

Recently, an interesting strategy of optical droplet vaporization has been proposed to construct NIR light responsive cancer treatment platform. Instead of producing heat or reactive oxygen, plenty of gas microbubbles are generated under laser irradiation in this system. The rapidly expanding gas generation and violent collapse process have been demonstrated to induce mechanical destruction to cancer cells and form enhanced ultrasound imaging, showing excellent diagnostic capability and cancer treatment effect. Nevertheless, the short circulation lifetime and poor vascular permeability of the microbubbles seriously affect the real treatment results. More importantly, some cancer cells could escape death under the external stress through secreting protective proteins such as heat shock proteins, that greatly increase the odds of cancer recurrence. Therefore, it remains urgent to explore novel therapeutic platforms to tackle these issues.

RNA interference is a highly regulated, enzyme-mediated process that plays an important role in silencing gene expression. The predominant strategy is to use small interference RNA (siRNA), a double-strand RNA with 21–23 nucleotides in length, to degrade complementary messenger RNAs and inhibit protein translation concomitantly. Hence, harnessing application of synthetic siRNA to knock down the expression of tumor-associated proteins has emerged as an attractive supplementary modality for cancer treatment. During the past decade, siRNA therapy has already been proved to effectively enhance the conventional oncotherapy efficacy such as chemotherapy and radiotherapy, as well as some newly developing
cancer treatment modalities including phototherapy and immunotherapy. Although plenty of siRNA therapeutic platforms have been developed and some of these have demonstrated tremendous potential in clinical application, inefficient systemic delivery of siRNA is a terrific obstacle to overcome.

In this paper, we report the preparation of a yolk–shell structure photothermal carrier (PTC) to codeliver energy and siRNA for gene silencing-enhanced photolytic ablation of cancer. The composite PTC nanoparticles consist of polypyrrole (PPy) as the core, mesoporous silica (mSiO2) as the shell, and a middle hollow space. Here, the PPy core acts as photothermal agent, which could effectively absorb NIR light and transform it into ample heat with high photothermal efficiency. The mesoporous silica shell and hollow space are utilized to load perfluorohexane (PFH), which is a low boiling point compound with high biocompatibility, and also shows great potential in enhanced ultrasonic imaging. The nanoparticles are further loaded with heat shock protein 70 (HSP70) siRNA by electrostatic attraction. HSP70 is a protective protein that could be upregulated by toxic chemicals, heavy metals, and especially heat stress, while HSP70 siRNA is able to suppress the synthesis of HSP70 and the protein level. After modifying the particle surface with polyethylene glycol (PEG) chains and transferrin (Tf) molecules, the PTC can selectively target to cancer cells. As a result, HSP70 siRNAs are effectively delivered into cancer cells to silence HSP70 gene expression. Upon NIR laser irradiation, the generated local heat can gasify PFH to form plenty of microbubbles to kill cancer cells by mechanical effect. Meanwhile, the delivered siRNA can induce low protective protein level, which further enhances the photolytic therapy effect. Additionally, owing to the unique properties of PPy and PFH, the platform possesses ultrasound (US) and photoacoustic (PA) imaging abilities. Compared with core–shell, metal, and semiconducting nanoparticles, this yolk–shell nanoplatorm realize the simultaneously delivery of PFH and siRNA. The therapeutic effects and poisonousness of the multifunctional nanoplatorm have been evaluated on a small-animal model. The encouraging results suggest immense potential of this novel yolk–shell nanoplatorm in oncotherapy.

2. Results and Discussion

2.1. Fabrication and Characterization of PTC-siRNA/PFH

The strategy used to fabricate the yolk–shell nanoplatorm (PTC-siRNA/PFH) is illustrated in Figure 1a. First, PPy nanoparticles are constructed through the emulsion polymerization method and characterized by transmission electron microscopy (TEM). As depicted in Figure 1b(i), the PPy nanoparticles show uniform size of about 80 nm in diameter. Then according to our previous work, poly(tert-butyl acrylate) (PTBA) layer is coated on the surface of PPy core, acting as a sacrificial template, to achieve the hollow middle layer in the yolk–shell structure. The average PTBA layer thickness is 25 ± 5 nm from the TEM image (Figure 1b(ii)). Next, by a sol–gel nanocoating process (tetrathiooxysilane (TEOS) as the siliceous source, cetyltrimethylammonium bromide (CTAB) as the porogen to produce mesopores in the shell), a mesoporous outer shell is fabricated on the surface of PPy@PTBA and then –NH2 groups are conjugated with to obtain a positively charged surface. Subsequently, PTBA middle layer and CTAB are removed simultaneously in a mixture solution of ethanol and NH4NO3 to obtain the yolk–shell structure PPy@mSiO2. The diameter and shape of PPy@mSiO2 are uniform and mSiO2 layer thickness is 30 ± 5 nm (Figure 1b(iii),(iv)). The mesoporous structure of PPy@mSiO2 is determined from the N2 sorption isotherms (Figure S1, Supporting Information), revealing a typical type IV curve with a surface area of 632 m² g⁻¹ and an average pore diameter of 2.7 nm. In the process of preparation of PPy@mSiO2, the size of PPy nanoparticles can be adjusted by changing the molecular weight of polyvinylpyrrolidone (Table S1, Supporting Information). As a result, the size of PPy@mSiO2 decreased with the decreasing size of PPy core (Figure S2, Supporting Information). Due to the size of nanoparticles playing an important role in vivo cancer treatment, we choose the smallest particles for subsequent study.

Insufficient enrichment of nanoparticles at tumor region will greatly decrease the therapeutic effects of nanogents. Hence, the PEG chains and Tf molecules are conjugated on the surface of PPy@mSiO2 following procedures we previously published, to endow the particles with long term circulation time and specific cancer targeting ability. Hereafter, the PEG- and Tf-functionalized PPy@mSiO2 (PPy@mSiO2-PEG/Tf) is denoted as PTC. The amount of PEG and Tf conjugated in PTC is also measured through the thermogravimetric analysis (TGA) experiments (Figure S3, Supporting Information), in which PTC shows an additional weight loss of 6.57 wt% when compared with the PPy@mSiO2 particles. HSP70 siRNA is loaded on the PTC through electrostatic attraction and PFH is loaded into the mesoporous shell and the middle hollow space under rapid ultrasonic condition. The loading content of siRNA is measured with fluorescence intensity and calculated to be 50 × 10⁻⁹ mol (particle concentration 100 µg mL⁻¹) and PFH loading amount is estimated to be 1.0 µL mg⁻¹ particles. The zeta potential of the nanoparticles changes from the positively charged to negatively charged after absorbing HSP70 siRNA (Figure S4, Supporting Information), indicating the absorption of negatively charged RNA on particle surface. Variation of the particle size and polydispersity after each step of process is also monitored by dynamic light scattering (DLS, Table S2, Supporting Information). The PTC-siRNA/PFH shows a hydrodynamic diameter of 237 nm with narrow distribution and also exhibits high colloidal stability in the physiological aqueous condition including serum free media and serum-containing media as demonstrated from the DLS results (Table S3, Supporting Information).

2.2. Photothermal Performance of PTC-siRNA/PFH

The excellent photothermal effect of PPy-related materials have been demonstrated in a great deal of research. Owing to the PPy core, the PTC-siRNA/PFH is expected to absorb NIR light and then convert it into heat to gasify PFH. To establish suitable operation conditions for cancer treatment, photothermal performance of the PTC-siRNA/PFH is first investigated. The UV–vis-NIR spectra could reflect the ability of light absorption of materials. As shown in Figure 1c, the phosphate buffer saline (PBS) dispersion of PTC-siRNA/PFH exhibits obvious absorption
in the same wavelength range (400–1000 nm), although the signal density is less than that obtained from the PPy suspension. The less absorption density is likely caused by the relatively low proportion of PPy in the composite PTC materials when compared with the pure PPy nanoparticles of the same mass concentration. To evaluate the heat generation ability of the PTC nanoplatform, different samples (at an equivalent concentration of 100 µg mL⁻¹) are exposed to the 808 nm NIR laser with a
power density of 1 W cm\(^{-2}\) for 500 s. As shown in Figure 1d, the PTC-siRNA/PFH exhibits very close photothermal performance to that of the PTC sample, suggesting that the loading of siRNA and PFH in the particles has little influence on the photothermal performance of the materials. According to formula described by Roper et al.,\(^{[26]}\) the photothermal conversion efficiency (\(\eta\)) of PTC-siRNA/PFH is calculated to be 37.4%.

Besides, this sample shows a final temperature of \(\approx 43^\circ C\), which could greatly decrease the thermal damage to normal cells and tissues surrounding the tumor. However, the temperature around the nanoparticles is relatively high that can be sufficient to kill cancer cells through gasifying PFH to form microbubbles.\(^{[27]}\)

### 2.3. Microbubble Generation and siRNA Delivery

The PFH can be readily converted into gas through phase-shift process since its boiling point is just about 56 °C.\(^{[16,28]}\) In order to confirm the generation of PFH microbubbles upon exposure to laser irradiation, the PTC-siRNA/PFH suspension (100 µg mL\(^{-1}\)) is prepared and treated with 808 nm laser (1 W cm\(^{-2}\), 5 min). The confocal laser scanning microscope (CLSM) images of the sample in bright field are captured. As shown in Figure 2a, it is obvious to see plenty of microbubbles occurred in the field, suggesting the PFH could be effectively vaporized and released from PTC-siRNA/PFH under laser irradiation.

![Figure 2. a) Bright field CLSM images of PTC-siRNA/PFH before and after NIR laser irradiation (808 nm, 1 W cm\(^{-2}\), 5 min). Scale bars indicate 100 µm. b) CLSM images to visualize the gene delivery effect (red color from the Cy3 dye-labeled HSP70 siRNA). Scale bars indicate 200 µm. c) Western blot for the detection of HSP70 expression.](image)

After the PTC-siRNA/PFH entering into cancer cells, the HSP70 siRNA are supposed to specific target HSP70 mRNA to inhibit the production of HSP70.\(^{[29]}\) To verify the siRNA delivery effect of PTC-siRNA/PFH in vitro, human adenocarcinomic alveolar basal epithelial cell line (A549 cells) is used as a model. To intuitive observe the amount of siRNA within the cells, Cy3 (a high-quantum-yield NIR dye) labeled HSP70 siRNA is adsorbed on the surface of nanoparticles. Meanwhile, a commercial transfection reagent Lipo2000 mixed with the siRNA (Lipo2000-siRNA) is utilized as control group. A549 cells are cultured with PTC-siRNA/PFH or Lipo2000-siRNA for 6h and then subjected to CLSM. The CLSM images are depicted in Figure 2b, in which the red fluorescence raised from Cy3 represents the Cy3-labeled HSP70 siRNA. Compared with the Lipo2000-siRNA group, almost the same fluorescence intensity is observed in the PTC-siRNA/PFH group, indicating this yolk–shell nanoparticle possess excellent siRNA delivery ability. Flow cytometry results (Figure S5, Supporting Information) indicate that the accumulation of nanoparticles in A549 cells are far above that in 293T cells (normal cells), indicating the Tf-functionalized PTC-siRNA/PFH could specifically target cancer cells.

To evaluate the efficiency of the PTC-siRNA/PFH as HSP70 inhibitors, the western blot assay is carried out to measure the expression of HSP70. A549 cells are incubated with PTC, PTC-siRNA, PTC-PFH, and PTC-siRNA/PFH nanoparticles respectively, then exposed to 808 NIR laser irradiation. The
western blot results show that the groups treated with HSP70 siRNA-loaded PTC have much lower HSP70 levels than other groups (Figure 2c), which almost drop by about two-thirds (Figure S6, Supporting Information). These results suggest that the HSP70 is significantly down-regulated, thereby favoring the gene-silencing efficiency achieved by the siRNA-loaded PTC.

2.4. In Vitro Cytotoxicity

Inspired by its exceptional photothermal-induced microbubbles generation properties and efficient siRNA delivery ability, the therapeutic efficacy of PTC-siRNA/PFH in vitro to A549 cells and MCF-7 cells (human breast cancer cell line) is evaluated. The cytotoxicity of the PTC-siRNA/PFH was investigated by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. No significant toxicity of the nanocarriers was observed both in cancer cells and normal cells after 48 h incubation without laser irradiation (Figure S7, Supporting Information), indicating gratifying biocompatibility of the yolk–shell platform. However, cytotoxicity of the particles could be very different in presence of the NIR laser illumination, since ample heat will be generated in this condition and the raised temperature can trigger the PFH gasification and HSP70 siRNA release. To analyze the impact of every individual component on cell cytotoxicity, A549 cells and MCF-7 cells are cultured with different samples including PTC, PTC-siRNA, PTC-PFH, and PTC-siRNA/PFH, respectively and then illuminated by 808 nm laser. As shown in Figure 3a,b, PTC alone has little influence on the cell viability, suggesting the heat

Figure 3. A comparison of the growth inhibition of a) A549 cells and b) MCF-7 cells treated with PTC, PTC-siRNA, PTC-PFH, and PTC-siRNA/PFH with NIR laser irradiation (808 nm, 1 W cm⁻²) for 5 min. c) CLSM images of calcein-AM (green, live cells) and PI (red, dead cells) costained A549 cells or MCF-7 cells after different treatment. Scale bars indicate 1 mm.
generated by PPy core is not enough to efficiently kill cancer cells. Whereas, the PTC-siRNA group and PTC-PFH group decrease the cell viability to 46.1% and 35.9% for A549 cells and to 30.9% and 38.7% for MCF-7 cells, respectively, when the nanoparticles have a concentration of 100 µg mL⁻¹. These results indicate that the HSP70 siRNA could greatly reduce the tolerance of cancer cells to environmental damage, and the photothermal-induced microbubbles are able to kill cancer cells, but the only bubbles or siRNA is not good enough to thoroughly destruct tumor cells. Impressively, the PTC-siRNA/PFH group dramatically decreases the cell viability to 13% for A549 cells and 10.8% for MCF-7 cells under the same experiment conditions, suggesting the combination of siRNA and PFH can achieve excellent therapeutic effect in oncotherapy.

To further confirm the anticancer efficacy, the above treated A549 cells and MCF-7 cells are costained with calcein acetoxy-methyl ester (calcein-AM, a dye to stain live cells) and propidium (PI, a dye to stain dead cells). As depicted in Figure 3c, the green color in CLSM images represents live cells, while the red color represents dead cells. It clearly shows that both types of cancer cells treated with the PTC-siRNA/PFH are hardly alive, which is in consistent with the MTT data. The superior performance of the PTC-siRNA/PFH in killing cancer cells could be attributed to the combination of the HSP70 siRNA induced lower endurance of cancer cells and the PFH induced violent photothermal-based mechanical effect, suggesting great potential of using the PTC-siRNA/PFH in cancer treatment.

2.5. Photocoustic/Ultrasonic Imaging

The enrichment of nanoparticles at tumor site has essential roles in the therapeutic efficacy so that using imaging technique to aid in determining the right treatment time is important.⁴⁰ Due to the PPy core and loading of PFH, the PTC-siRNA/PFH platform is expected to possess both PA imaging and contrast-enhanced US imaging ability. PA imaging is a promising technique, which could provide preferable spatial resolution compared with traditional optical imaging modalities, while US imaging has been widely used in clinic with relatively low cost.⁴¹ To verify whether the mesoporous silica shell has influences on PA imaging, PA signals of PTC-siRNA/PFH are first detected in vitro. The results (Figure S8, Supporting Information) show a linearity enhancement of PA signal with

![Graph showing relative photocoustic signals over time](image)

Figure 4. a) PA (left) and US (right) images of tumor region before and after intravenous injection of 100 µL of PTC-siRNA/PFH nanoparticles in A549 tumor-bearing mice. b) Plot of average relative PA signals at tumor site versus time. The average PA signal at 0 h is set as 1. c) Plot of average US signals at tumor site (pointed out with yellow circles) versus time.
increasing particle concentration, suggesting the PTC-siRNA/PFH has reliable PA property.

With the help of the two imaging techniques, the real-time accumulation of nanoparticles in tumor region is recorded by using A549 tumor-bearing BALB/c nude mouse as animal model. The PTC-siRNA/PFH nanoparticles are administered to mice by intravenous injection at a dosage of 2 mg mL\(^{-1}\) (100 µL), and a multimode PA/US small animal imaging system is then used to capture PA/US images at various time intervals before and after (1, 2, 4, 6, 8, 12, and 24 h) the injection. As depicted in Figure 4a, gradually increased signals are observed within 6 h postinjection at tumor site from both the PA images and the US images. The signals are maintained at a high level between 6 and 8 h postinjection, indicating maximum nanoparticles enrichment in tumor region in this time window. Quantitative analysis of the PA and US signals is performed by calculating the average PA and US intensity of region of interest (yellow circles in Figure 4a). As shown in Figure 4b,c, the relative PA signals and US signals have quite similar variation trends, both of which exhibit the highest value at 6 h and still maintain a relatively high level even at 24 h postinjection. Furthermore, the in vivo biodistribution of nanoparticles (Figure S9, Supporting Information) is investigated by collecting the major organs and tumors for ICP-AES according to the Fe content in PPy cores, and accumulation of PTC-siRNA/PFH in tumors are calculated as 13.5 ± 2.2% ID/g at 6 h postinjection. These results demonstrate that with the assistance of Tf molecules and PEG chains, PTC-siRNA/PFH nanoparticles could effectively accumulate in targeted tumor tissue.

2.6. In Vivo Photolytic Therapy

Encouraged by the above results and analysis, then oncotherapy experiments in vivo are conducted. The A549-tumor bearing mice are randomly divided into five groups (n = 5): PBS group (control), PTC group, PTC-siRNA group, PTC-PFH group, and PTC-siRNA/PFH group. Suspension of different nanoparticles at a particle concentration of 2 mg mL\(^{-1}\) is injected into mice via tail vein at a dosage of 100 µL. Based on the PA/US imaging results, PTC-siRNA/PFH would have the maximum enrichment at tumor site around 6 h postinjection, thereafter all the groups of mice are exposed to 808 nm laser irradiation for 5 min at a laser power density of 1 W cm\(^{-2}\) at 6 h postinjection. During the illumination, the real-time temperature of tumor region is recorded by using an infrared thermal camera. As shown in Figure 5, the temperature of tumor tissue in PTC group or PTC-siRNA/PFH group shows no significant difference, suggesting that the loading of siRNA and PFH in the nanocarrier has little impact on the photothermal effect.

Figure 5. Thermal images of PBS (left panel), PTC (middle panel), and PTC-siRNA/PFH (right panel) injected, A549 tumor-bearing mice after exposing to laser irradiation (808 nm, 1 W cm\(^{-2}\)) for different time (0–5 min).
in vivo. Besides, the measured highest temperature in tumor region of the PTC-siRNA/PFH-injected group is around 44 °C, which is not likely to kill cancer cells by hyperthermia but will greatly reduce the side effects to normal tissues. The time-dependent temperature profile in the tumor site is also recorded (Figure S10, Supporting Information). These results indicate that the PTC-siRNA/PFH nanoplatform could effectively convert light energy into heat at a laser power density of 1 W cm\(^{-2}\) in vivo.

In order to evaluate the therapeutic effect of the yolk–shell nanoparticles, the tumor growth rate is determined by measuring tumor sizes every other day. As depicted in Figure 6a, compared to control group, the tumors still grow rapidly in the PTC group, which means that the heat itself only is not enough to eliminate cancer cells. However, in the PTC-siRNA group and the PTC-PFH group, tumor growth is obviously inhibited, indicating that the HSP70 siRNA could effectively weaken the endurance of cancer cells to heat and the violent PFH gasification process greatly enhance the lethality of the PTC nanoparticles. Most impressively, the tumors are completely eliminated when the mice are treated with PTC-siRNA/PFH. Meanwhile, no apparent weight loss can be seen in all the groups of mice (Figure 6b), suggesting the yolk–shell platform is reasonably safe. Besides, the tumor section of each group has been collected for image analysis (Figure S11, Supporting Information), further indicating the significant damage caused to tumor tissue in PTC-siRNA/PFH group. At the 20th day post-treatment, all mice are sacrificed due to the extensive tumor burden in the control group, and the tumors are removed and weighed. As shown in Figure 6c,d, the results are found to agree fairly well with the tumor growth curves. To further evaluate the safety of such nanoparticles, the major viscera including heart, liver, spleen, lung, and kidneys are also excised and stained by hematoxylin and eosin (H&E). The result from Figure 6e shows, compared to the control group, there is no obvious inflammatory lesion and organ damage in all other groups, indicating relatively low systemic toxicity of the PTC materials. All these data demonstrate the feasibility of using the PTC-siRNA/PFH nanoplatform for PA/US imaging-guided gene and photolytic therapy in vivo.

3. Conclusion

In summary, we have developed the yolk–shell nanoplatform that can not only efficiently deliver siRNA and PFH into cells but also convert light energy into heat to generate microbubbles for gene silence and photolytic cancer treatment. In vitro experiments show that the PTC-siRNA/PFH could effectively facilitate cellular uptake, down regulate HSP70 expression, gasify PFH liquid, and inhibit cancer cell proliferation. In vivo experiments demonstrate that the PTC-siRNA/PFH exhibit excellent PA/US dual-model imaging ability and could eliminate tumors totally with minimal side effects under NIR laser irradiation. We envisage that the multifunctional yolk–shell nanoplatform that is able to combine gene silencing with photothermal induced mechanical effect may provide new opportunities in the field of imaging-guided oncotherapy.

4. Experimental Section

Fabrication of Yolk–Shell PTC: First, PPy nanoparticles (NPs) were synthesized following method reported previously.[19,26] Briefly, 0.40 g polypyrrolidone \(M_n (58, 360, \text{ or } 1300)\) and 1.081 g FeCl\(_3\)·6H\(_2\)O were dissolved in 40 mL of deionized water and stirred for 30 min. Then 121 µL of pyrrole was added into the reaction solution and followed by stirring for 10 h to obtain the PPy NPs. The obtained particles were washed with tetrahydrofuran to remove the pyrrole monomer and other byproducts. The prepared PPy NPs (20 mg) were redispersed in 80 mL of deionized water with CTAB (0.2 g) and stirred for 30 min under the atmosphere of nitrogen at 70 °C. Then 43.3 mg of potassium persulfate and 2.0 mL of tert-butyl acrylate were added in the mixture. The reaction was carried out for 6 h and PPy@PTBA was obtained. Afterward, dispersing PPy@PTBA (20.0 mg) and CTAB (0.3 g) into a mixture of deionized water (60 mL) and ethanol (20 mL) with quickly stirring for 30 min at 40 °C. Later aqueous ammonia solution (25 wt%, 0.85 mL) and TEOS (0.15 mL) were added into the suspension to fabricate a mesoporous silica shell on the nanoparticles. After 24 h of reaction, PPy@PTBA@mSiO\(_2\) with CTAB-filled in the mesopores was prepared and then surface of the nanoparticle was modified with –NH\(_2\) groups through further reacting with 3-aminopropyltriethoxysilane (100 µL) in the ethanol dispersion (50 mL) for 6 h at 80 °C. The PPy@mSiO\(_2\)-NH\(_2\) was obtained after removal of the PTBA shell and CTAB through dispersing the particles in the ethanol solution of NH\(_4\)NO\(_3\) (10 mg mL\(^{-1}\)) at 80 °C for 6 h. Finally, the PTC is obtained after conjugating the PEG chains and Tf molecules to the PPy@mSiO\(_2\)-NH\(_2\) surface through the procedure reported previously.[18]

Synthesis of PTC-siRNA/PFH: The HSP70-siRNA with the sequence 5’-UCACUGCAAGAGCUUCUAACTC-3’ was synthesized by RebioBio Co. Ltd. To load siRNA onto the PTC, the positively charged PPy@mSiO\(_2\)-NH\(_2\) (10.0 mg) were mixed with siRNA (5.0 mmol) solution in diethyl pyrocarbonate (DEPC) water (10 mL). After 1 h of incubation, free siRNA was removed by centrifugation and then PEG (\(M_w = 2000\)) and Tf (purchased from Sigma-Aldrich) were conjugated onto surface of the siRNA-loaded PPy@mSiO\(_2\)-NH\(_2\) particles following the former procedures. The PTC-siRNA was washed with DEPC water and dried under vacuum. The loading content of siRNA was determined by using the fluorescence of Cy3-labeled siRNA. Then PFH (10.0 µL) was added into vial containing PTC-siRNA (10.0 mg) and the tube mouth was sealed tightly. After treating with ultrasonic condition (50 Hz, 50 W, 5 min) in ice bath, PBS solution (2.0 mL) was added into the mixture to redisperse the nanoparticles. The obtained PTC-siRNA/PFH suspension was sealed tightly and stored at 4 °C before use.

Particle Characterization: UV–vis-NIR absorption spectra of the nanoparticles were measured by a Perkin-Elmer Lambda 750 spectrophotometer. The size distribution and zeta potential of the particles were evaluated using a Zetasizer Nano-ZS (Malvern). The Tecnai G2 TEM (FEI, USA) was used to observe the morphology of the particles. The TGA profiles of samples were recorded by Pyris 1 (Perkin-Elmer) with a heating rate of 5 °C min\(^{-1}\) from 100 to 800 °C under air atmosphere. Nitrogen adsorption–desorption isotherms of nanoparticles were measured by a Micrometitics Tristar 3000 system at 77 K and the surface area was calculated using the Brunauer–Emmett–Teller method.

Photothermal Performance Assessment: To evaluate the photothermal effects of the samples, 100 µL of the nanoparticle suspensions (particle concentration 100 µg mL\(^{-1}\)) was placed in a well of a 96-well cell culture plate and irradiated using an 808 nm continuous-wave NIR laser for 500 s with a power density of 1 W cm\(^{-2}\) (spot size: 5 mm × 8 mm). The temperature was monitored in real time by a thermocouple probe at 20 s interval with an accuracy of 0.1 °C.

Release in Aqueous Medium: The suspension of PFH-siRNA/PFH with a particle concentration of 100 µg mL\(^{-1}\) was applied in confocal dishes. Then the dish was subjected to CLSM and images were captured under bright-field before and after laser irradiation.

Cell Culture: A549 cells and MCF-7 cells were provided by American type culture collection, and cultured in high-glucose Dulbecco modified eagle’s medium containing 10% (v/v) fetal calf serum, streptomycin...
Figure 6. a) Tumor growth curves and b) weight change curves of A549 tumor-bearing mice after various treatments. c) Photograph and d) weights of tumors after excision from each group. e) H&E stained images of major organs collected from mice (40×). *, p < 0.01; ***, p < 0.001.
(100 mg mL\(^{-1}\)) and penicillin (100 U mL\(^{-1}\)) at 37 °C in a humidified incubation chamber containing 5% CO\(_2\).

**Evaluation of siRNA Delivery Effect:** A549 cells were seeded in confocal dishes at a suitable density and cultured for 12 h. Afterward, PTC-siRNA/PFH (100 µg mL\(^{-1}\)) and Lipo2000-siRNA (siRNA concentration: 50 × 10\(^{-9}\) µ) were added into the wells, respectively, and coincubated for 6 h. Then, the cells were washed with serum-free medium several times and subjected to the CLSM (C2\(^{+}\), Nikon) experiment. All the CLSM images were captured in the same conditions (excitation at 488 nm and emission at 515 nm).

**Evaluation of HSP70 Expression:** To confirm the different levels of expression of HSP70 in A549 cells, western blot assay was used to determine the HSP70 expression. A549 cells were incubated in 12-well plates and coincubated with PTC-siRNA/PFH (100 µg mL\(^{-1}\)) for 6 h. Then, the cells were irradiated for 5 min and further cultured for 4 h. Afterward, the cells were lysed and collected after centrifugation. The total protein was determined using the bicinecinonic acid protein assay reagent, and the proteins of each sample were resolved via sodium dodecyl sulfate polyacrylamide gel electrophoresis and then transferred into a polyvinylidene fluoride membrane. The membranes were incubated with 5% nonfat milk in tris-buffered saline containing 0.1% TBST (tris-buffered saline with Tween) for 1 h and cultured with relevant primary antibodies (HSP70 and β-actin, 1:10,000 dilutions) at room temperature overnight. After being washed three times with TBST, the blots were incubated with secondary antibody for 30 min and stained using an electrochemiluminescence detection kit. The signals were photographed using a chemiluminescent imaging system.

**MTT Assay:** A549 cells and MCF-7 cells were seeded in 96-well plates at a density of 5 × 10\(^4\) viable cells per well and incubated for 24 h to allow cell attachment. Then, different amounts of nanoparticles were coincubated with the cells for 6 h, and the wells were subjected to 808 nm laser irradiation at 1 W cm\(^{-2}\) output power density for 5 min. After culturing for another 24 h, the medium was aspirated, and PBS containing 1 mg mL\(^{-1}\) MTT reagent was added into the wells. After further 4 h, dimethyl sulfoxide was added to dissolve the purple formazan crystals that were produced by the live cells. Subsequently, the 96-well plates were investigated using a multiwell scanning spectrophotometer, and relative cell viability was measured by comparing the absorbance differences at 570 nm between the control and experimental wells.

**Live/Dead Cell Viability Assay:** A549 cells and MCF-7 cells were plated with a proper density and exposed to 100 µg mL\(^{-1}\) PTC-siRNA/PFH for 6 h. After laser irradiation at 808 nm (5 min, 1 W cm\(^{-2}\)), the cells were stained by calcein-AM and PI. Green fluorescence and red fluorescence indicated live cells and dead cells, respectively.

**Animal Model:** BALB/c female nude mice (four to six weeks old) were purchased from Shanghai BK Laboratory Animal Co., Ltd., China. All animal experiments were conducted in accordance with the “Guide for the Care and Use of Laboratory Animals” of the Institute of Laboratory Animal Resources and approved by the ethics committee of Fudan University. Around 2 × 10\(^4\) cells of A549 mixed with 200 µL PBS were subcutaneously implanted in the right or left flank of each nude mouse.

**In Vivo Photoacoustic and Ultrasonic Imaging:** 100 µL of PTC or PTC-siRNA/PFH suspension (particle concentration, 2 mg mL\(^{-1}\)) was injected into the caudal vein when the tumor volume of the mice reached 100 mm\(^3\). Then, at specified time intervals, the mice were injected with a multimode photoacoustic/ultrasonic animal imaging system (Vevo LAZR, Fuji Film Visual Sonics Inc) to record PA and US images.

**In Vivo Biodistribution Studies:** 100 µL of PBS or PTC-siRNA/ PFH suspension (particle concentration, 2 mg mL\(^{-1}\)) was injected into A549 tumor bearing mice via tail vein. At different time points (6 and 24 h), mice were sacrificed and major organs and tumor tissues were collected after manually ground. Then 1 mL acid mixture (\(\text{HNO}_3\) and \(\text{HCl}\) at 1:4) was added into each sample to dissolve Fe content. After keeping at room temperature for 24 h, the samples were heated to 60 °C and kept for another 12 h. Then these solutions were carefully filtered through a 0.22 µm membrane and measured by using ICP-AES. The PBS group was utilized to subtract the baseline Fe\(^{3+}\) levels in different organs.

**In Vivo Photolytic Therapy:** When the tumors had grown to 100 mm\(^3\), the nude mice were randomly separated into five groups (n = 5 per group), and each was intravenously administered one of the following experimental treatments: PBS solution (control), PTC dispersion, PTC-siRNA dispersion, PTC-PFH dispersion, and PTC-siRNA/PFH dispersion. The dose of nanoparticles that was injected in each treatment modality was 100 µL of suspension (2 mg mL\(^{-1}\)). After 6 h, the tumors of the mice were exposed to the 808 nm NIR laser (1 W cm\(^{-2}\), 5 min), and the temperature of each tumor region was recorded using an infrared camera thermographic system (Infratec, VarioCAM hr research, Germany). Subsequently, the tumor volumes and body weights were measured using a digital Vernier caliper and a scale, respectively, every other day. The volume was calculated as \(V = d^2 \times D / 2\) (d: shortest diameter of the tumor; D: longest diameter of the tumor). On the 20th day, the tumors and major organs (heart, liver, spleen, lung, and kidney) of the mice were dissected and all the mice were euthanized. All tumors were weighted and parts of the organs were fixed in 4% formaldehyde solution for tissue slices.

**Statistical Analysis:** All data in this paper are expressed as mean ± SD. Unpaired student’s t-test was used for comparison between two testing groups and a probability (\(P\)) less than 0.05 was considered statistical significance.

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Keywords**

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