Graphene Oxide-Cyclic R10 Peptide Nuclear Translocation Nanoplatforms for the Surmounting of Multiple-Drug Resistance

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Multidrug resistance resulting from a variety of defensive pathways in cancer has become a global concern with a considerable impact on the mortality associated with the failure of traditional chemotherapy. Therefore, further research and new therapies are required to overcome this challenge. In this work, a cyclic R10 peptide (cR10) is conjugated to polyglycerol-covered nanographene oxide to engineer a nanoplatform for the surmounting of multidrug resistance. The nuclear translocation of the nanoplatform, facilitated by cR10 peptide, and subsequently, a laser-triggered release of the loaded doxorubicin result in efficient anticancer activity confirmed by both in vitro and in vivo experiments. The synthesized nanoplatform with a combination of different features, including active nucleus-targeting, high-loading capacity, controlled release of cargo, and photothermal property, provides a new strategy for circumventing multidrug resistant cancers.

1. Introduction

Cancer-related death, which is one of the most terrible killers around the world, will rise to around 150 million by 2020. The development of safer and more effective tumor treatment tactics is urgent but still formidable. Graphene, as the most widely used 2D nanomaterials, has shown extraordinary performance in the antitumor nanomedicine exploitation, which is attributed to its stability, photothermal property, high loading capacity, fast cellular uptake, and biodegradability. Although many graphene-based antitumor systems have been constructed and much progress has been achieved in this field, the situation becomes more complicated and challenging when it meets the multidrug resistance (MDR). The emergence of MDR is largely owing to the overexpression of ATP-binding cassette, in particular, P-glycoprotein (P-gP), on the plasma membrane. These proteins reduce the internalization of therapeutic agents into the cells and even mediate the efflux of these agents from the cells. In recent years, a variety of functional nanomaterials and smart nanodevices were developed to incapacitate P-gP and to deliver anticancer drugs across the plasma membrane. Despite the efficient cellular uptake and improved drug concentration inside the cytoplasm of cancer cells, the “efflux pump” still frustrates the chemotherapy. This shortage is more serious when it comes to therapeutic agents with specific targeting organelles, such as doxorubicin (DOX), whose anticancer mechanism is to intercalate with DNA in the nucleus. Therefore, efficient tumor therapy could hardly be achieved if MDR is not efficiently suppressed and therapeutic agents are not localized into the target organelles.

Recently, nucleus-targeting strategies were proposed for the chemotherapy of MDR tumors by DOX. By this strategy, DOX...
could be delivered into nucleus and the “efflux pump” on the plasma membrane could be hopefully avoided. Arginine-rich cell-penetrating peptides (CPPs), particularly the TAT peptide, which originates from the HIV-1 virus, are shown to be efficient vectors to translocate nanoparticles including mesoporous silica nanoparticles,[21,22] upconversion nanoparticles,[23] copper sulfide nanoparticles,[24] and polymer nanoparticles[25,26] into the cell nucleus. Cyclization of CPPs has been demonstrated as a new strategy to improve their ability as transmembrane carriers and nucleus targeting agents.[27–31] For example, cyclic TAT (cTAT) has been proven to show higher ability to facilitate the cellular translocation of proteins than linear TAT at 4 °C.[30] The internalized cTAT-decorated protein is distributed both in the cytoplasm and in the nucleolus, a nuclear compartment.[30] Moreover, cyclic R10 peptide (cR10) even has displayed higher transduction location efficacy than cTAT.[32] Accordingly, cR10 peptide is supposed to be an effective molecule for the nuclear targeting and translocation of nanocarriers into cells.[32] Furthermore, the size of nanoparticles restricts their ability to enter the cellular nucleus.[33,34] In general, nanoparticles bigger than 40 nm can scarcely cross the nuclear membrane.[20,33,34] Graphite could be exfoliated to small sheets, 15–30 nm, via intensified oxidation processes.[35,36] The obtained nanographene oxide (nGO) contains a large number of epoxy and hydroxyl functional groups on the basal plane and carboxyl on its edges.[36] Moreover, hyperbranched polyglycerol (hPG) with great potential in a wide range of biomedical applications, including drug delivery, tissue engineering, and pathogen interactions,[37,38] has been used to improve the biological safety and performances of nanomaterials, such as graphene.[39–41]

In this work, we have synthesized polyglycerol-functionalized nGO derivatives with the ability of the intra-nucleus delivery and release of antitumor therapeutic agents and MDR suppression (Figure 1). nGO with a 15–30 nm average size was produced and functionalized by hPG and conjugation of peptides (Figure 2B,C). The small average size (20 nm) is a significant factor that can affect the cellular uptake and nucleus localization of GOPR.

The composition of nGO, GOP, and GOPR was investigated by X-ray photoelectron spectroscopy (XPS), UV-vis absorption, and thermogravimetric analysis (TGA). In Figure 2D, the C1s component peak at 284.6 eV is assigned to sp² hybridized carbon atoms.

Figure 1. Suppression of MDR by polyglycerol-covered nGO sheets. After efficient uptake by the tumor cells, nanosheets localize inside the nucleus by cR10 peptide. Consequently, an intra-nucleus release of DOX triggered by NIR irradiation resulted in efficient chemophototherapy against MDR tumors.
Scheme 1. Synthesis routes for the production of nGO, GOP, and GOPR. Detailed information for step i, ii, iii, and iv are as follow: i) dispersed in mixture (v/v = 3:1) of H₂SO₄ (98%) and HNO₃ (65%), 120 °C, 2 d; ii) with hPG(NH₂)₁₀%, dispersed in MES solution including EDC.HCl, room temperature (RT), overnight; iii) with BCN, dispersed in dimethylformamide (DMF) including triethylamine (TEA), RT, overnight; iv) with cR₁₀ peptide, dispersed in PBS (7.4), RT, overnight.

Figure 2. The TEM images and size profiles of A) nGO, B) GOP, and C) GOPR nanomaterials. The scale bars correspond to 200 nm. The highly resolved C1s XPS spectrum of D) nGO, E) GOP, and F) GOPR nanomaterials.
carbon bonds of the graphene platform. Component peaks at 286.2 and 288.1 eV were attributed to C−O and C=O bonds created after oxidation of graphite. This result confirmed the disruption of the plane and edges of graphene and formation of a large number of carbonyl, hydroxyl, carboxyl, and epoxy functional groups upon oxidation (Figure 2D).Cls XPS spectra of GOP and GOPR showed successful stepwise conjugation of hPG and cR10 peptide to the nGO. (Figure 2E,F, Table S1, Supporting Information). The appearance of C−N bond related component peaks in the survey spectra of GOP and GOPR were attributed to the amino groups, amide groups, and guanidine groups in hPG(NH2)10% and cR10. In order to confirm the reaction between the thiol group of cR10 and GOPBCN, a control reaction was performed. Accordingly, cR10 without thiol functional group was incubated with GOPBCN and the product of the reaction was characterized by XPS. Significant changes in the XPS spectrum of the product were not found, which indicated that peptide was not conjugated to the graphene sheets (Table S2, Figures S1 and S2, Supporting Information). This control reaction proved the critical role of thiol group for the conjugation of cR10 to GOPBCN.

The composition of the synthesized nanomaterials, as well as their peptide contents, were further investigated by UV−vis experiments. They showed a characteristic absorption peak at 225 nm, which was used to determine the concentration of nGO in the aqueous solutions (Figure S3A, Supporting Information). According to these UV−vis experiments, the nGO and hPG contents of GOP were around 46% and 54%, respectively. After conjugation of cR10 peptide to GOP, the nGO decreased to around 43.8%, indicating around 4.8% peptide content for GOPR (Figure S3B, Supporting Information). Data related to the composition of nanomaterials can be found in Supporting Information, page 9.

2.2. Cellular Uptake and Intracellular Distribution

The characterized materials were used for the cell experiments, including cytotoxicity and intra-nucleus localization. According to Cell Counting Kit-8 (CCK-8) assays, the toxicity of GOP and GOPR against multidrug-resistant HeLa (HeLa-R) cells was negligible up to 300 µg mL−1 (Figure S5, Supporting Information). The low toxicity of these nanomaterials holds promise for biomedical applications. Therefore, we labeled the nanomaterials by fluorescein isothiocyanate (FITC) (Figure S6, Supporting Information) and investigated their uptake by HeLa-R cells using confocal laser scanning microscopy (CLSM) (Figure 3A,B) and fluorescence-activated cell sorting (FACS) (Figure 3C). GOP showed very low cellular uptake after 6 h incubation due to the hPG coverage. However, GOPR was efficiently taken up by HeLa-R cells, indicating a significant role for cR10 in the cellular internalization of functionalized nanosheets (Figure 3B). The quantitative data obtained by FACS showed an increase in the
uptake efficiency ten times after conjugation of cR10 peptide to nanosheets (Figure 3C).

It has been shown that conjugation with cyclic cell-penetrating peptides (cCPPs) like cR0 or cTAT enhanced the transduction of protein-cargo in living cells.[30,32] Additionally, non-cleavable cCPP conjugates have shown the ability to accumulate in the nucleoli within the nucleus.[32] Along these lines, we proposed that the conjugation of cR10 peptide to nanomaterials is effective for the nuclear localization of their cargos. Interestingly, the intranuclear translocation of GOPR was very efficient and cR10 was able to facilitate the localization of nanosheets inside the nucleus (Figure 3B). Statistical analysis was applied to calculate the percentage of the cells with nucleus-translocated graphene sheets (Figure 3D). While localization of GOP into the nucleus of cells after 6 h incubation was negligible, GOPR was effectively transferred into the nucleus and around 80% of cells affected by the nucleus-targeting peptide.

### 2.3. Preparation of DOX-Loaded Nanosheets and Their Release Profiles

The action site of DOX is inside the nucleus, where it inhibits the macromolecular biosynthesis of DNA and causes apoptosis of carcinoma cells.[18,19] Therefore, translocation of this therapeutic agent inside the nucleus, after cellular internalization, is a crucial step. However, many nanocarriers discharge DOX molecules in the cytoplasm, which causes failure of chemotherapy against MDR cells.[14,15] The DOX signal in GOPD-treated cells, before and after laser irradiation, was assigned to the disrupted graphene basal plan and limited sites for the efficient hydrophobic and π-π interactions with DOX molecules.[46] However, the loading capacity was still higher than most of the polymer-based nanocarriers.[45]

The photothermal effect is an unique characteristic for the graphene-based nanomaterials,[8,46] which is also beneficial for the controlled release of their cargo.[47,48] GOP and GOPR solutions at different concentrations were irradiated with the near-infrared (NIR) laser and the temperature variations were recorded (Figure S7, Supporting Information). After 10 min irradiation, the temperature of the aqueous solutions of GOP and GOPR (1 mg mL−1) increased to 50 °C, which was enough for triggering the release of their cargo as well as an efficient photothermal therapy.[8,15] The ability of laser irradiation to accelerate the release of DOX from GOPD and GOPRD was investigated at pH 5.6 and 7.4 (Figure 4). As expected, the release rate increased upon NIR laser irradiation, which was ascribed to the increased temperature of the nanosheets.[15] However, the effect of pH on the release rate of DOX from graphene sheets was not significant. This is an advantage for this system, because it results in a minimum leaking of drugs in the cytoplasm, optimum nucleus localization, and laser-triggered release of DOX. These spatiotemporally controlled release properties are very helpful for precise tumor therapy and to mitigate the unwanted side effects.[49,50]

### 2.4. DOX Cellular Uptake and Antitumor Effect

GOPD and GOPRD were incubated with HeLa-R cells for 12 h to investigate the intracellular DOX release and distribution. The cells were irradiated with NIR laser (808 nm, 0.5 W cm−2) for 10 min after 6 h incubation and non-irradiated cells were used as control. For the DOX.HCl-incubated HeLa-R cells, a significant difference between the irradiated and non-irradiated cells was not observed (Figure 5A). In both cases, the amount of DOX inside the cells and its therapeutic effect against cancer cells was low. The half maximal inhibitory concentration (IC50) value for DOX.HCl against HeLa-R cells with and without laser irradiation was 18.6 and 20.9 µg mL−1, respectively (Figure 5C,D). The resistance of HeLa-R cells against DOX is due to many factors, especially overexpressed P-gP on the plasma membrane as efflux pumps.[14,15]

The DOX signal in GOPD-treated cells, before and after laser irradiation, was similar to free DOX (Figure 5A). The reason for weak DOX signal is either low uptake of this drug or the lack of targeting ligand in GOPD and localization in the cytoplasm, where most of the released DOX molecules were “pumped” into the external cellular environment. However, the IC50 value for the cells incubated with GOPD...
The enhanced therapeutic effect was attributed to the photothermal effect of nGO sheets and promoted release of therapeutic agents. Laser irradiation of cells treated with GOPRD resulted in a high DOX signal in nucleus, indicating the efficient nucleus translocation by cR10 peptide (Figure 5A). Before irradiation, the signal of DOX was weak, because its fluorescence was partially quenched by nGO sheets (Figure S9, Supporting Information). This result confirmed that DOX molecules were majorly associated with nGO, and they were not considerably released during their journey inside the cytoplasm. The low IC50 of GOPRD without laser irradiation (9.4 µg mL\(^{-1}\)) is another proof for the successful targeting of this carrier and associating cargo with the carrier until nucleus internalization (Figure 5C). The IC50 of GOPRD plummeted to 1.1 µg mL\(^{-1}\) when NIR laser was employed. The high DOX content of the GOPRD-treated cells (Figure 5B) and high therapeutic effect of this carrier (Figure 5D) proved the incapacity of MDR cells to discharge released DOX molecules into the external cellular environment after nuclear localization. From the in vitro studies, it can be concluded that the cR10-functionalized graphene sheets were able to efficiently circumvent the MDR, due to the combination of a series of unique physicochemical and biological properties.

### 2.5. Biodistribution and Photothermal Effect

Although promising therapeutic effects were obtained in vitro, they cannot afford an efficient nGO-based nucleus-targeting and anticancer ability in vivo. However, the hPG coverage of the sheets decreases their unspecific interactions with the biosystems and effectively relieves the possible injuries,\(^{[33]}\) which was reported for the bare graphene-treated animals.\(^{[51]}\) Nanocarriers were labeled with 1,1′-bis(4-sulfobutyl) indodicarbocyanine-5-carboxylic acid (IDCC, a derivative dye of Cy5), and their biodistribution was studied. The labeled carriers were injected into the HeLa-R tumor-bearing nude mice. Mice were sacrificed after 24 h, and their tumors and main organs were isolated. The fluorescence intensities were measured quantitatively (Figure 6B and Table S3, Supporting Information). The fluorescence signal in tumors was stronger than other organs, such as liver and kidney, confirming an effective tumor localization of carriers. Interestingly, the accumulation of GOPR in tumors and organs was higher than GOP, which may have been attributed to the higher cellular internalization efficacy of this system.

In addition to triggering the release of the cargo, laser can induce a photothermal effect of nGO sheets, which improved the anticancer efficacy of these systems synergically.\(^{[8]}\) In order to investigate the photothermal effect of carriers, saline, DOX. HCl, GOPD, and GOPRD were intravenously administrated into nude mice. After 24 h, the NIR laser (808 nm, 0.5 W cm\(^{-2}\)) irradiation was applied in the tumor sites, and tumor local temperature was monitored with a thermal camera. While the tumor temperature of the saline- and DOX.HCl-treated mice increased from 30 to 37 °C, it reached 44.9 and 48.6 °C for GOPD- and GOPRD-treated mice, respectively (Figure 6A). This temperature range is reported to show an effective photothermal effect.\(^{[52]}\)
2.6. In Vivo Anticancer Effect

The tumor size (Figure 6C) and body weight (Figure S12, Supporting Information) were recorded in the following 16 days after treatment. The tumors of saline- and DOX.HCl-treated mice grew very fast, and DOX.HCl alone only exhibited limited tumor suppression. In the case of GOPD-treated mice, the antitumor efficacy slightly improved but still was far away from satisfaction, even with irradiation. This was due to the low uptake efficacy and intra-cytoplasm release of drug, as well as its moderate photothermal effect. However, GOPRD showed much higher antitumor efficacy even without laser irradiation, and the tumor size was only 32.4% compared with the DOX.HCl-treated mice. This could be attributed to the efficient cR10 targeting and intra-nucleus release of the loaded DOX molecules. The most exciting therapeutic results came from the combination of GOPRD and laser irradiation at the tumor sites. The tumors kept shrinking during the whole treatment (the tumor size was only 10.1% compared with the DOX.HCl-treated mice after the therapy), which indicated the rational strategy to conquer MDR tumors.

The adverse side effects, including hair loss, bone marrow suppression, inflammation of the mouth, and other things, are always the concern of DOX when it is applied for clinical patients. Body weight as the most representative indicator, was recorded to analyze the side effects of GOPRD (Figure S12, Supporting Information). It could be seen that the weight loss for the DOX.HCl-injected mice was quite apparent, particularly in the initial treatment stage. In addition, obvious diseased regions could be found in the heart histological staining of DOX.HCl-treated mice, indicating its serious cardiotoxicity (Figure S13, Supporting Information). These side effects could...
rarely be observed in the GOPD- and GOPRD-treated mice, confirming the low side effects of these nanocarriers.

In order to investigate the antitumor therapeutic mechanism of these nucleus-targeting nanosheets, histological staining analyses were applied to the isolated tumors, including hematoxylin and eosin (H&E), Ki67, and TUNEL. Compact tumor tissue was found for saline- and DOX.HCl-treated mice, while a remarkable reduction of tumor cells and more vacanies were observed after GOPD administration (Figure S13, Supporting Information). Furthermore, less Ki67-positive cells (proliferative cells) and more TUNEL-positive cells (apoptotic cells) could be found in the GOPRD-treated tumors (Figure 6D). On contrary, more proliferative tumor cells and less apoptotic tumor cells were observed in the case of saline- and DOX.HCl-treated mice (Figure 6D). These results confirmed the successful antitumor therapy by nucleus-targeting graphene-based nanoplatorms.

3. Conclusion

We demonstrated that the MDR in cancer could be successfully bypassed both in vitro and in vivo by nucleus targeting polyglycerol-covered graphene nanosheets. The effectivity of these systems to incapacitate MDR was due to the successful combination of different aspects, including high ability of cR0 for nucleus targeting, strong association of DOX with nGO sheets, and a laser-triggered release of DOX and photothermal effect. GOPRD with and without laser irradiation showed the best and second best results, pointing out the critical role of cR0 to circumvent the MDR effect. An abrupt increase in the signal of nucleus-localized DOX and synergic photothermal and chemotheraphy upon laser irradiation was a solid proof for the association of cargo and carriers even after nuclear internalization. This work introduced a newly developed, cyclic peptide-directed nucleus-translocation nanomedicine and opens a new gate to conquer MDR tumors in the future.

4. Experimental Section

Preparation of DOX-Loaded GOP and GOPR: Hydrophobic DOX was prepared based on the reported protocol. GOP (20 mg) was dispersed in PBS (7.4, 20 mL), and then DOX (10 mg) dissolved in DMSO (1 mL), was added to this dispersion dropwise. The mixture was stirred overnight in PBS (7.4, 20 mL), and then DOX (10 mg) dissolved in DMSO (1 mL), was added to the HeLa-R cell with a series of concentrations (from 1 to 500 µg mL^-1), respectively. The culture medium solutions were removed after 24 h incubation, and the wells were rinsed with PBS. Afterward, medium-containing CCK-8 (100 µL, 10%) was added to each well. After a 2 h incubation, the absorbance of each well at 450 nm was measured. Cells without any treatments were considered as a negative control, and the cytotoxicity of DOX.HCl (2 and 50 µg mL^-1) was measured in the same way as the positive controls. Each test was triplicated.

The antitumor tests for GOPD and GOPRD were performed by almost the same procedure. However, the concentration of DOX was changed from 0.01 to 100 µg mL^-1. The tests were replicated as 2 groups, and NIR laser irradiation (808 nm, 0.5 W cm^-2) was employed to one group for 10 min after 6 h incubation.

Confocal Laser Scanning Microscopy: HeLa-R cells were seeded in 8-well plates with the density of 3 x 10^4 cells per well and incubated for another 24 h before CLSM experiments. Celluar uptake of GOP and GOPR: GOP and GOPR were dispersed in DMEM (20 µg mL^-1) and incubated with the cells at 37 °C for 1, 3, and 6 h, respectively. Subsequently, the wells were rinsed with PBS, and then the nucleus of HeLa-R cells was stained with Hoechst for 30 min. Afterward, cells were observed with CLSM (Leica TCS SP8). Hoechst was excited at 350 nm with the emission at 460 nm, and FITC was excited at 488 nm with the emission at 520 nm. Intracellular DOX release: DOX.HCl, GOPD, and GOPRD were dispersed in DMEM and then added to the wells. The concentration of DOX for all experiments was set at 5 µg mL^-1. Each experiment was replicated as two groups, and the cell culture medium was refreshed after 6 h incubation. Subsequently, one group was treated with NIR laser irradiation (808 nm, 0.5 W cm^-2) for 10 min, while the other group was not treated and used as control. Afterward, the cells were incubated with the fresh medium for additional 6 h. Finally, cells were rinsed with PBS and the nuclei were stained with Hoechst before evaluation by CLSM. Hoechst was excited at 350 nm with the emission at 460 nm, and DOX was excited at 488 nm with the emission at 550 nm.

Flow Cytometer: HeLa-R cells were seeded in 24-well plates (5 x 10^4 cells per well), and they were incubated for 24 h before experiments. Cellular uptake of GOP and GOPR: GOP and GOPR were dispersed in DMEM (20 µg mL^-1) and then added to the cells. The concentration of DOX for all experiments was set at 5 µg mL^-1. Each experiment was replicated as two groups, and the cell culture medium was refreshed after 6 h incubation. Subsequently, one group was treated with NIR laser irradiation (808 nm, 0.5 W cm^-2) for 6 h before rinsing with PBS. Then the cells were treated with trypsin/EDTA for 4 min and centrifuged at 1000 rpm, and the supernatant was discarded. Cells were re-suspended in PBS, and fluorescence was measured (excitation: 488 nm; emission: 520 nm) using a BD FACS caliber flow cytometer. Intracellular DOX release: DOX.HCl, GOPD, and GOPRD were dispersed in DMEM and...
were intravenously injected with saline, DOX.HCl, GOP D, and GOPR D balance.

Female BALB/c nude mice (4 weeks old) were purchased from Model Animal Research Center of Nanjing University (Nanjing, China), and all animal experiments were conducted in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (China). $1 \times 10^5$ HeLa-R cells were subcutaneously inoculated into the right flank of one nude mouse to construct the tumor-bearing mice. Tumor size was measured (excitation: 488 nm; emission: 550 nm) using a BD FACS flow cytometer.

**Tumor Xenograft Models on Nude Mice:** Female BALB/c nude mice (2 weeks old) were purchased from Model Animal Research Center of Nanjing University (Nanjing, China), and all animal experiments were conducted in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (China). $1 \times 10^5$ HeLa-R cells were subcutaneously inoculated into the right flank of one nude mouse to construct the tumor-bearing mice. Tumor size was measured by electronic caliper, and the tumor volume ($V$) was calculated as $V = a^2 \times b/2$ (a and b represent the width and length of the tumor, respectively). And the body weight of nude mice was recorded by an electronic balance.

**In Vivo Photothermal Studies:** HeLa-R tumor-bearing nude mice were intravenously injected with saline, DOX.HCl, GOPD, and GOPRD (5 mg kg$^{-1}$). After 24 h, the tumors were irradiated by NIR laser (808 nm, 0.5 W cm$^{-2}$) for 10 min, and the thermal photos were recorded by an IR thermal imaging camera (FLIR, E40) during the irradiation every 2 min.

**In Vivo Biodistribution Studies:** For the quantitative drug distribution experiments, HeLa-R tumor-bearing nude mice were intravenously administrated with GOPD and GOPRD (5 mg kg$^{-1}$). After 24 h, the mice were sacrificed, and the organs were extracted and homogenized in 1 mL 1% Triton X-100. Extracting solution (HCl-IPA, 5 mL) was added to tissue lysates and the samples were kept at 4°C overnight. After that, the mixture was centrifuged at 11000 rpm for 15 min. The concentration of DOX was determined by the fluorescence measurement of the supernatant.

**In Vivo Antitumor Efficacy:** When the tumor volume reached about 100 mm$^3$, the nude mice were randomly divided into 6 groups with 6 mice in each group. Then the mice were intravenously administrated with saline (group 1), DOX.HCl (group 2), GOPD (group 3), GOPR (group 4), GOPD (group 5), and GOPRD (group 6). The ratio of DOX amount and body weight was 5 mg kg$^{-1}$. After 24 h, the tumors in groups 1, 2, 4, 5, and 6 were irradiated with NIR laser (808 nm, 0.5 W cm$^{-2}$) for 10 min. The tumor size and body weight were recorded every 2 days during the treatment. The mice were sacrificed after 16 days, and then the tumors and major organs were isolated and stained by hematoxylin & eosin (H&E). In addition, Ki67 and TUNEL immunostaining were also applied for all of the tumors.

**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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