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An Efficient Photo-Chemo Combination Therapeutic Platform Based on Targeted Reduction-Responsive Self-Crosslinked Polymer Nanocapsules

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Phototherapies, including photothermal therapy (PTT) and photodynamic therapy (PDT), have attracted enormous attention in cancer treatment.\textsuperscript{1-5} Notably, since single chemotherapy cannot achieve satisfactory treatment effects due to the inefficient tumor killing activity, the combination of phototherapy and chemotherapy was considered a most promising strategy to enhance the effectiveness of cancer therapies.\textsuperscript{6-10} In addition to directly killing cancer cells by hyperthermia and reactive oxygen species (ROS), phototherapy can also promote therapeutic molecule internalization into cells by the "photochemical internalization" (PCI) effect, resulting in reduced side effects and enhanced therapeutic effects.\textsuperscript{11-14} To date, although various delivery approaches have been established for delivering photo-chemo combination reagents, however the potential applications of those delivery systems are hampered by the poor structure stability, and lack of stimuli-responsive ability.

Covalently bonded polymer nanocarriers named as nanogels or nanocapules hold great prospects for targeted drug delivery because they can stably transport therapeutic agents to the disease location with minimal premature leakage, and controllably activate them by both endogenous and exogenous stimuli-factors, such as acidity, redox substances, enzymes, and light, to achieve an optimal efficacy.\textsuperscript{15-20} Owing to the heterogeneous redox potential gradient in malignant tumor tissues, such as glutathione (GSH) in the cytoplasm, polymer nanocarriers containing disulfide bond have been extensively explored for fabricating reduction-responsive drug delivery systems.\textsuperscript{21-25} In particular, bonding reaction of disulfide bond between thiols has mild and friendly reaction conditions, which is an easy-to-accomplishing process.

Over the past decade, poly(ethylene glycol) (PEG) have been widely utilized for fabricating drug nanocarriers due to its outstanding aqueous solubility, non-toxicity, non-immunogenicity, and electric neutrality, which significantly improved drug stability, blood circulation times, as well as tumor targeting ability.\textsuperscript{26-31} As compared to traditional liner PEG, branched multi-arm PEG has higher density of polymer chains that could achieve better protection for the loaded drugs in the treatment.\textsuperscript{32} Otherwise, multi-arm PEG provides more sites of end group, which can be useful for modification of different functional units for constructing high-performance drug nanocarriers.\textsuperscript{33-35}

Herein, we designed a four-arm poly(ethylene glycol)-poly(propylene sulfide)-cyclc(Arg-Gly-Asp-D-Phe-Lys) (PEG-PPS-cRGD) amphiphilic branched copolymer with end-capping
A photo-chemo combination therapy platform is introduced by incorporating both doxorubicin (DOX) and Indocyanine green (ICG) into a single PEG-PPS-cRGD nanocapsule for targeted treatment of squamous cell carcinoma. As shown in Figure 1, first, DOX and ICG were encapsulated into PEG-PPS-cRGD by a single-step solvent-antisolvent method to form DOX and ICG-coloaded DINCs. I, self-crosslinked DINCs were prepared via a solvent-antisolvent process under initiation of TEA; II, DINCs target cancer cells via the αvβ3-integrin receptor; III, DINCs internalize into cells by the receptor-mediated endocytosis pathway and PCI effect initiated under short-term light irradiation; IV, DINCs achieve PTT, PDT and release of DOX cargo under long-term light irradiation; V, DINCs can also significantly release DOX cargo by the high-level of GSH in cytoplasm; VI, released DOX enters the nucleus and plays a chemotherapy role by intercalation in DNA.

The generation of singlet oxygen ($^{1}\text{O}_2$) was measured via the method reported by Wöhrl et al. Briefly, 1 mL of PBS buffer, free ICG solution and DINCS solution (ICG concentration of 14.4 μg/mL) in a tube were irradiated by a 785 nm continuous-wave NIR laser (Beijing NBeT Group Corp, China) with an output power of 2.0 W cm$^{-2}$. The temperature was also recorded using a digital thermometer every 30 s during the irradiation period.

Detection of Single Oxygen Detection ($^{1}\text{O}_2$) of DINCs

The four-arm PEG-PPS-cRGD copolymer was synthesized via three steps according to our previous work. Furthermore, 4 mg of ICG, 2 mg of DOX-HCl and 4.5 μL of triethylamine (TEA) were dissolved in 0.4 mL of DMSO and mixed for 15 min with stirring. Additionally, 4 mL of PEG-PPS-cRGD aqueous solution (0.5 wt.%) was slowly added to the ICG and DOX solution. After addition of PEG-PPS-cRGD, the reaction solution was stirred at 45 °C for another 2 h. Finally, the products were dialyzed against ultrapure water through a dialysis bag (molecular cut-off: 3500 Da) to remove unloaded DOX, ICG and DMSO. The target product was then obtained by lyophilizing the solution at -55 °C for 72 h.

The typical structure of the nanocapsules was investigated by transmission electron microscopy (TEM, Hitachi HT7700, Japan). The size distributions of the nanocapsules were determined using dynamic light scattering (DLS, Malvern Nano ZS, UK), reported as the mean size of 3 mL. After vortexing for 10 s, the mixture solutions were monitored temperature changes under laser irradiation. PBS buffer, free ICG solution and DINCS solution (ICG concentration of 14.4 μg/mL) in a tube were irradiated by a 785 nm continuous-wave NIR laser (Beijing NBeT Group Corp, China) with an output power of 2.0 W cm$^{-2}$. The temperature was also recorded using a digital thermometer every 30 s during the irradiation period.

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The typical structure of the nanocapsules was investigated by transmission electron microscopy (TEM, Hitachi HT7700, Japan). The size distributions of the nanocapsules were determined using dynamic light scattering (DLS, Malvern Nano ZS, UK), reported as the Z-average (intensity-based mean particle diameter). The DOX and ICG loading capacity (LC) and entrapment efficiency (EE) of the DINCs were measured using a UV-visible spectrophotometer (Varian Cary 50, USA) with an absorption wavelength at 488 nm and 633 nm for DOX and ICG, respectively, and calculated according to the following formula:

$$\text{LC (wt%) = (weight of loaded drug / weight of initially added drug)} \times 100\%.$$ $$\text{EE (wt%) = (weight of loaded drug / weight of initially added drug)} \times 100\%.$$
generation of \( \text{O}_2 \) accompanying the absorbance decrease in DPBF was detected at 410 nm.

**In Vitro DOX Release from DINCs**

Two milligrams of DINCs were dispersed in 2 mL of PBS buffer and transferred into a dialysis bag (molecular cut-off: 3500 Da). The dialysis bag was then placed in 20 mL of PBS buffer containing 10 mM of GSH. For laser irradiation of the samples, the dialysis bag was placed in 20 mL of PBS buffer or 20 mL of PBS buffer containing 10 mM of GSH, and the samples were then irradiated with a NIR laser (785 nm, 2.0 W) for 5 min at the initial time. Subsequently, the samples were incubated at 37 °C for 24 h, and the cumulative release rate of DOX from the DINCs was calculated by determining the UV absorption of DOX at 488 nm.

**Cell Culture and Tumor Model**

The human squamous cell carcinoma cell line (SCC-15 cells) was cultured in DMEM (high glucose) with 10% fetal bovine serum (FBS) at 37 °C with 5% CO\(_2\). Female BALB/c nude mice were obtained from Beijing Vital River Laboratories. SCC-15 tumors were created by a subcutaneous injection of 2 \times 10^6 SCC-15 cells into each mouse. All the animal studies were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Beijing Vital River Laboratory Animal Technology Co., Ltd. and approved by the Institutional Animal Care and Use Committee of Beijing Vital River Laboratory Animal Technology Co., Ltd.

**In Vitro Cellular Uptake of DINCs**

SCC-15 cells (8×10^3 cells per well) were seeded into 8-well chambered cover glasses (Lab-Tek, Nunc, USA) in 200 µL of complete DMEM and cultured overnight. The cells were treated with the designated formulations (final DOX concentration of 3.6 µg/mL and ICG concentration of 14.4 µg/mL) with or without short-term laser irradiation (785 nm, 2.0 W cm\(^{-2}\), 1 min) and further incubated for another 4 h. Then, the cells were washed 3 times with PBS and fixed with 4% paraformaldehyde (100 µL) for 20 min. The cells were then washed with PBS and stained with DAPI (5 µg/mL, 100 µL) for 15 min in the dark. Finally, the cells were washed at least 3 times and observed by confocal laser scanning microscopy (CLSM, Leica TCS SP8, Germany) (DAPI, 360/460 nm; DOX, 488/515 nm; ICG, 633/780 nm).

**Detection of Intracellular ROS Generation of DINCs**

SCC-15 cells (8×10^3 cells per well) were seeded into 8-well chambered cover glasses in 200 µL of complete DMEM and cultured overnight. The cells were then treated with various designated formulations (final DOX concentration of 3.6 µg/mL and ICG concentration of 14.4 µg/mL) with or without laser irradiation (785 nm, 2.0 W cm\(^{-2}\), 1 min) and incubated for another 4 h. Next, the cells were harvested, detected, and quantified using a flow cytometer (FCM, Beckman Coulter MoFloXDP, USA).
Subsequently, the cells were washed, trypsinized, resuspended in PBS, stained using the Annexin V-FITC/PI apoptosis detection kit, and analyzed using FCM.

**Animal model**

All of the animal experiments were studied in accordance with the Guidelines for Care and Use of Laboratory Animals of Beijing Vital River Laboratory Animal Technology Co., Ltd. and approved by the Institutional Animal Care and Use Committee of Beijing Vital River Laboratory Animal Technology Co., Ltd.

**In Vivo Imaging and Biodistribution Analysis of DINCs**

The tumor-bearing nude mice were randomly divided into 2 groups (three per group). Next, 200 μL of free ICG and DINCs (containing 1 mg/mL ICG) was injected via the tail vein, respectively. Images and FL semiquantitative analysis of ICG in vivo were performed at 1, 3, 6, 12, 24, 36 and 48 h after injection using an IVIS Imaging Spectrum System (PerkinElmer, USA) at certain parameters (λex = 745 nm, λem = 820 nm, binning = 1, exposure time = 0.1 s). The mice were then sacrificed, and organs including heart, liver, spleen, lung, kidneys, brain and tumor were collected for FL imaging. After FL imaging, the tumors were isolated and immediately frozen at -80 °C. Further, frozen sections of tumor were prepared for fluorescence imaging and fluorescence intensity analysis.

**In Vivo Photothermal Evaluation of DINCs**

The tumor-bearing nude mice were randomly divided into 2 groups (three per group): the free ICG group and the DINC group. Two hundred microliters of free ICG and DINCs (containing 1 mg/mL ICG) was injected via the tail vein, respectively. At 0, 24, and 48 h post-administration, the tumor site was irradiated with a laser (785 nm, 2.0 W cm⁻²) for 5 min. Then, an infrared thermal imager was utilized to photograph and record the temperature change of the mouse body. Two-dimensional (2D) infrared thermographic images were processed with SmartView 3.14 software (Fluke, USA).

**In Vivo Antitumor Efficacy and Biosafety Evaluation of DINCs**

The tumor-bearing nude mice were randomly divided into 5 groups (three per group) to evaluate the antitumor efficacy. Two hundred microliters of PBS, free DOX (containing 0.25 mg/mL DOX) and DINCs (containing 0.25 mg/mL DOX and 1 mg/mL ICG) was injected via the tail vein 4 times (on days 1, 8, 15 and 22). For the laser treatment groups, the tumor site was irradiated with a laser (785 nm, 2 W cm⁻²) for 5 min on days 2, 9, 16 and 23. The tumor volumes and body weight changes of the mice were monitored.
were recorded, and the inhibition rate of the tumor (IRT) was calculated by the following formula:

\[
IRT(\%) = \left(1 - \frac{D}{S}\right) \times 100\%
\]

where D is the tumor volume of treatment group and S is the tumor volume of saline group.

After 28 days, the mice were sacrificed, and their main organs (heart, liver, spleen, lung, and kidney) were excised, sectioned, and stained with hematoxylin and eosin (H&E). Each section was imaged using a digital microscope to detect histopathological changes. Furthermore, the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) apoptosis assay kit was used to stain tumor slices for the evaluation of cell apoptosis by CLSM.

**Statistical Analysis**

Data are expressed as the mean ± standard deviation (SD). The two-tailed Student’s tests or one-way ANOVA was applied for statistical evaluations.

**Results and discussion**

**Preparation and Characterization of DINC**

DOX and ICG-coloaded PEG-PPS-cRGD nanocapsules (DINC) cross-linked by disulfide bond were synthesized using a self-crosslinked approach of disulfide exchange reaction, which is an easy-to-accomplishing and particularly meaningful strategy because it can completely avoid using multiple chemicals such as monomers, crosslinkers and initiators in traditional synthesis of polymer nanogels/nanocapsules that may significantly improve tedious purification process and serious toxicity of the products.

Owing to branched structure and higher PEG densities, four-arm PEG-PPS-cRGD nanocapsules exhibited outstanding properties for holding the loaded drugs, and drug loading capacity of 4.8% and 15.9% and drug encapsulation efficiency of 49% and 90% were detected for DOX and ICG in DINC, respectively. The DINC exhibited an obvious core-shell structure with a dark drug core coated with a gray polymer layer, suggesting that DOX and ICG were successfully encapsulated inside the polymer nanocapsules (Figure 2a). The TEM image also revealed that the particle size of the DINC was in the range from 100-200 nm. Consistent with the TEM result, an average diameter of approximately 152.6 nm was tested by DLS (Figure 2b), which confirmed that the DINC possessed a suitable size for passive tumor targeting through the enhanced permeability and retention effect (EPR). The zeta potential of DINC was -2.10 mV, indicating that this DINC has slight negative surface potential which would be perfect for prolonging its blood circulation time during the treatment. Furthermore, to assess aqueous phase dispersibility and stability, DINC were dispersed in PBS buffer for long-term treatment. An average particle size of 153.4 nm was observed for DINC incubated in PBS for 1 week (Figure S1), proving that PEG-PPS-cRGD nanocapsules can carry both DOX and ICG agents with excellent stability in the aqueous phase, benefiting their application by vein injection.

To evaluate the photothermal effect of DINC, we used a digital thermometer and infrared thermal imager to monitor temperature changes under NIR laser irradiation (785 nm, 2.0 W cm\(^{-2}\)). As shown in Figure 2c, the temperature of free ICG and DINC rapidly increased to 48.2 and 62.0 °C during the first 5 min of irradiation and retained an equilibrium for the remaining time, while the temperature of PBS only increased to 33.9 °C. Furthermore, the highest temperatures of 33.9, 50, and 63.4 °C were observed for PBS, free ICG and DINC using an infrared thermal imager after 5 min of irradiation (Figure 2d). Taken together, the DINC exhibited a higher temperature increase than free ICG under laser irradiation, suggesting that the encapsulation of ICG in polymer nanocapsules enhanced its photothermal conversion efficiency due to a more condensed concentration and slower heat dissipation process, which would benefit PTT and DOX release of DINC.

The ROS generation ability of DINC was investigated by measuring the change in concentration of \(^1\)O\(_2\) under NIR laser irradiation. DPBF, which could react with \(^1\)O\(_2\) to induce a reduced absorbance at 410 nm, was used to evaluate the
amount of \( \text{O}_2 \) using a UV-visible spectrophotometer.\(^{38}\) As shown in Figure 2e, the decrease in DPBF absorbance was negligible in the PBS group. However, the absorbance of DPBF in the DINCs group sharply dropped to 34.3% and 27.5% of its original value with short-term (1 min) and long-term (5 min) laser irradiation, respectively, demonstrating that DINCs could respond to laser irradiation to generate \( \text{O}_2 \), which can be used not only for PDT but also for promoting DINC internalization into cells by the PCI effect.

To assess the DOX release profiles of DINCs, various triggers including GSH and NIR laser were adopted, and the cumulative release rate are shown in Figure 2f. DINCs without any triggers only released 19% of DOX during the testing period of 24 h. In contrast, cumulative DOX release rates of 75% and 56% were observed for GSH (10 mM) and the NIR laser (785 nm, 2.0 W cm\(^{-2}\)) treatment groups, respectively. In particular, DINCs treated with the combination of GSH and NIR laser exhibited the highest dissolution rate of approximately 88%. To further demonstrate that the combination of GSH and NIR laser irradiation could synergistically promote DOX release, we used TEM to examine changes in the morphology of DINCs. As shown in Figure 2g, after treatment with both GSH and NIR laser, the structure of DINCs exhibited a loose state in comparison to the initial structure, and many free small drug particles were released from the DINCs, suggesting that DOX release of DINCs could be significantly accelerated by the combination of GSH and NIR laser irradiation. Taken together, the studies of DOX release profiles confirmed that DINCs had outstanding reduction-responsive features; additionally, DOX release could also be further accelerated by NIR laser irradiation due to the activation of hyperthermia from coloaded ICG.

**In Vitro Cellular Uptake of DINCs**

The cellular uptake of DINCs was investigated using CLSM and FCM. As shown in Figure 3a, both green (DOX) and red (ICG) fluorescent signals were present and colocalized in the cytoplasm and nucleus of SCC-15 cells treated with DINCs, indicating that DOX and ICG were simultaneously transported into the cells by the PEG-PPS-cRGD nanocapsules. In particular, the cells treated with short-term laser irradiation exhibited significantly increased cellular uptake of DINCs compared with the cells without laser irradiation, as evidenced by the highest red and green fluorescence intensity, explaining that DINCs generated a PCI effect under laser irradiation. Figure S3 shows fluorescence colocalization analysis of SCC-15 cells treated with DINCs plus laser irradiation, and the morphological characteristics of fluorescence curve of both DOX and ICG were near coincidence. Furthermore, the FCM results also validated that the fluorescence intensity was 1.39-times higher in the cells treated with DINCs in the presence compared with the absence of laser irradiation (Figure 3b and 3c). Taken together, PEG-PPS-cRGD nanocapsules were capable of delivering both DOX and ICG into cells simultaneously, and the cellular uptake efficiency could be further enhanced by the NIR laser irradiation-induced PCI effect.

**Detection of Intracellular ROS Generation by DINCs**

Intracellular ROS generation by DINCs under laser irradiation was assessed using DCFH-DA, which can be oxidized in cells by ROS to DCF to emit bright green fluorescence.\(^{39}\) As shown in Figure 4a, it was difficult to observe the green fluorescence in PBS-treated cells with laser irradiation. In free ICG-treated cells, an extremely weak fluorescent signal was detected, which was probably due to the poor intracellular uptake ability of free ICG. After laser irradiation, the cells treated with DINCs exhibited increased ROS generation compared with the PBS and free ICG groups, as evidenced by the highest green fluorescence intensity in the cells. The FCM results also confirmed that after laser irradiation, DINCs-treated cells had the strongest fluorescence intensity of DCF, which was 8.3- and 6.9-times higher than in the PBS and free ICG-treated cells (Figure 4b and 4c). Consequently, PEG-PPS-cRGD nanocapsules efficiently enhanced the intracellular uptake rate of ICG, resulting in higher concentrations of intracellular ROS after laser irradiation, which could be used for PDT.

**In Vitro Cytotoxicity Detection of DINCs**

To investigate the in vitro cytotoxicity of DINCs toward SCC-15 cells, the MTT assay was adopted. As shown in Figure 5a, the PEG-PPS-cRGD branched copolymer presented excellent biocompatibility, as indicated by more than 85% cell survival at the highest concentration of 360 \( \mu \)g/mL. Figure 5b shows the cytotoxicity of the cells treated with PBS with laser irradiation, free DOX, and DINCs with or without laser irradiation. PBS with laser irradiation did not influence cell viability, indicating that laser irradiation at a power density of 2.0 W cm\(^{-2}\) was...
appropriate for the treatment of SCC-15 cells. Of note, DINC-treated cells showed drug concentration-related cytotoxicity, with a gradual decline in cell viability with increasing drug concentration. More importantly, DINC-treated cells with laser irradiation showed 84.5% cell death at the highest drug concentration.

Fig. 6. In vivo imaging and biodistribution of DINCs. (a) In vivo fluorescence imaging of nude mice bearing SCC-15 tumors after intravenous injection with free ICG and DINCs (10 mg/kg ICG) at different times. (b) Ex vivo fluorescence imaging of various organs and tumor tissue after intravenous injection with free ICG and DINCs at 48 h. (c) Average fluorescence intensity of the organs and tumor by quantitative analysis at 48 h post-injection. Data represent means ± SD (n=3). (d) ICG distribution within the tumor tissue at 48 h post-injection. Scale bar: 100 μm. (e) Infrared thermographic images of nude mice bearing SCC-15 tumors after intravenous injection with free ICG and DINCs (10 mg/kg ICG) at different times; the tumor site was exposed under laser irradiation (785 nm, 2.0 W cm⁻²) for 5 min before taking the pictures.
concentration (18 μg/mL of DOX and 72 μg/mL of ICG), demonstrating a 1.96-fold increase in cytotoxicity compared with the 43.2% observed for DINC-treated cells without laser irradiation. These results demonstrated that laser irradiation significantly enhanced the cytotoxicity of DINCs by triggering a synergistic photo-chemo combined activity.

The live-dead assay was performed to visually observe the cytotoxicity of DINCs, and the fluorescence images are shown in Figure 5c. Consistent with the MTT results, the PBS-treated cells with laser irradiation did not show obvious cell death, with only a few cells exhibiting red fluorescence. The cells incubated with DINCs exhibited effective cytotoxicity, as demonstrated by a greater number of cells emitting red fluorescence. Moreover, after laser irradiation, the cytotoxicity of DINC-treated cells was further enhanced, as evidenced by the significantly reduced cell density and highest proportions of red cells, suggesting that the NIR light-triggered photo-chemo combination therapy of DINCs was able to achieve more cell death.

Furthermore, Annexin-V/PI double-staining assay was used to evaluate apoptotic or necrotic cells, and the results were quantified by FCM, shown in Figure 5d. The percentage of total apoptotic cells (including early and late apoptosis) treated with DINCs with laser dramatically increased to 64.5%, which was much higher compared with the cells treated with PBS (0.07%), PBS with laser (6.8%), free DOX (44.8%), and DINCs without laser (25.6%). These results demonstrated that the apoptosis-inducing ability of DINCs could be sharply enhanced in the presence of laser irradiation.

**In Vivo Imaging and Biodistribution of DINCs**

To evaluate the tumor-targeting ability, the BALB/c nude SCC-15-tumor-bearing mice were intravenously injected with free...
IGC and DINCs, and the fluorescence distribution of ICG was subsequently recorded at different time points (0, 1, 3, 6, 12, 24, 36, and 48 h) using an ex/in vivo imaging system. As shown in Figure 6a, fluorescence signals were detected throughout the body of the mice within the first 6 h in both the free ICG and DINC treatment groups. Subsequently, with extended time, the fluorescence intensity at the tumor site was significantly enhanced in DINC-treated mice compared with free ICG-treated mice at every time point. However, the fluorescence signal of free ICG was barely detected at 48 h post-injection. At 48 h post-administration, the mice were sacrificed, and various main organs and tumor were isolated for ex vivo imaging for further detection of the fluorescence distribution of free ICG and DINCs. In contrast to free ICG, a higher fluorescence signal was detected on the tumor of DINC-treated mice (Figure 6b), and the quantitative average fluorescence intensity revealed a 1.9-fold increase (Figure 6c). Furthermore, the fluorescence images of tumor sections confirmed that DINCs were capable of penetrating deeply in the tumor tissue (Figure 6d), and the fluorescence intensity of ICG in DINCs treated group was much higher than that of the group treated with free ICG (Figure S4). Overall, these biodistribution results validated that ICG loading into PEG-PPS-cRGD nanocapsules significantly enhanced its tumor-targeting ability through both the EPR effect and cRGD-integrin binding capability, resulting in higher tumor accumulation.40,41

**In Vivo Photothermal Effect of DINCs**

The SCC-15 tumor-bearing mice were intravenously injected with free ICG (10 mg/kg ICG) and DINCs (2.5 mg/kg DOX and 10 mg/kg ICG), and the photothermal effect on the tumor site after laser irradiation (785 nm, 2 W cm\(^{-2}\), 5 min) was recorded using an infrared thermal imager (Figure 6e). For the mice treated with free ICG, temperatures of 30.3, 44.7 and 40.6 °C were observed at 0, 24 and 48 h post-administration, respectively. Of note, the tumors treated with DINCs showed an enhanced photothermal effect compared with those that received free ICG, as indicated by the temperature increase to above 50 °C at both studied time points, suggesting that DINCs could generate a higher temperature at the tumor site after laser irradiation due to its outstanding tumor-targeting ability, leading to higher tumor accumulation. Moreover, the results demonstrated that DINCs with NIR laser could achieve an efficient photothermal effect that would be capable of irreversibly destroying the tumor.

**In Vivo Photo-Chemo Combination Effect of DINCs**

The in vivo photo-chemo combination effect of DINCs in the treatment of squamous cell carcinoma was investigated using SCC-15 tumor-bearing mice. As shown in Figure 7a, 7b and 7c, the DINC treatment group exhibited stronger tumor growth inhibition compared with the PBS and free DOX treatment groups, with an inhibition rate of 72.6% at day 28, demonstrating that the tumor-targeting ability of PEG-PPS-cRGD nanocapsules could effectively improve the effect of anticancer drugs. Additionally, the tumors treated with PBS plus laser irradiation (785 nm, 2 W cm\(^{-2}\), 5 min) grew rapidly, demonstrating that tumor growth was not inhibited by laser irradiation under these treatment conditions. In particular, the tumors treated with DINCs with laser irradiation had completely disappeared at day 24, and no tumor regrowth was observed even at day 49 (Figure S5), suggesting that NIR laser-triggered photo-chemo combination therapy of DINCs could effectively kill squamous cell carcinoma tumors with no recurrence. To further assess the antitumor effect of DINCs, H&E and TUNEL staining of tumor sections are shown in Figure 7d. In comparison to the PBS and free DOX groups, the DINC group showed more cells with nuclear condensation and fragmentation in the H&E images, demonstrating that DINCs had better antitumor activity. Additionally, the tumors treated with DINCs showed more apoptotic cells than those in PBS and free DOX groups, as evidenced by more green fluorescent cells in the tumor section stained using the TUNEL assay, validating the outstanding tumor growth inhibition ability of DINCs. Overall, the DINCs showed prominent tumor inhibition efficacy, especially in NIR laser-triggered photo-chemo combination therapy.

**Biosafety Evaluation of DINCs**

The in vivo toxicity of DINCs was evaluated by recording body weight changes and by histological analysis of main organs. As shown in Figure S6, there was no obvious body weight loss during the treatment with DINCs with or without laser irradiation. Furthermore, histological analysis of H&E stained sections showed that the mice treated by DINCs with or without laser irradiation had no anomalous histopathological changes in the tested organs, including brain, heart, liver, spleen, lung, and kidney (Figure S7). These results confirmed that the treatment with DINCs with or without laser irradiation would not cause significant toxic side effect in vivo.

**Conclusions**

In summary, we developed a NIR light-triggered synergistic photo-chemo combination therapy platform by combination drug codelivery of DOX and ICG via reduction-responsive self-crosslinked PEG-PPS-cRGD nanocapsules. The dissolution studies demonstrated that the nanocapsules had sensitive reduction-responsive drug release profiles, while the drug release rate could be further accelerated with the assistance of NIR laser irradiation. Owing to the small size, targeting ligand of cRGD and PCI effect, the nanocapsules could effectively be taken up by squamous cells in vitro as well as target the tumor of squamous cell carcinoma in vivo. Additionally, the nanocapsules exhibited an outstanding photothermal effect and ROS generation under laser irradiation, which was also evidenced by the enhanced cytotoxicity in the treated squamous cells. Finally, the in vivo antitumor and biosafety investigations validated that this synergistic photo-chemo combination treatment by DINCs plus NIR laser irradiation could completely eliminate the tumor and significantly prevent tumor recurrence, with a favorable biological safety profile in the SCC-15 tumor-bearing mice model. Altogether, this high-performance NIR-triggered photo-chemo combination...
therapeutic platform may offer a promising strategy for the treatment of various malignant tumors.

Conflicts of interest
There are no conflicts to declare.

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