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PAPER

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In Vitro and In Vivo Photothermally Enhanced Chemotherapy by Single-Walled Carbon Nanohorns as a Drug Delivery System

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Single-walled carbon nanohorns (SWNHs) have exhibited many special advantages in biomedical applications. Herein, doxorubicin-loaded SWNHs (DOX-SWNHs) are prepared and further modified by amphiphilic deoxycholic acid modified-hydropropyl chitosan (DCA-HPCHS) to improve their biocompatibility. The obtained DOX-SWNHs/DCA-HPCHS drug delivery system (DDS) possesses high stability in physiological media and excellent photothermal property when exposed to laser irradiation in near-infrared (NIR) region, which dramatically enhance the chemotherapy of DOX. Cell viability assays show that the growth of 4T1 cells are remarkably inhibitory under the condition of incubation with DOX-SWNHs/DCA-HPCHS and subsequent exposure to 808 nm laser irradiation to produce mild photothermal heating to 43 °C. Further investigation reveals that the photothermally enhanced chemotherapy derived from a promotion of DOX-SWNHs/DCA-HPCHS uptake by the cancer cells rather than a light-triggered release of DOX. DOX-SWNHs/DCA-HPCHS in combination with the use of laser irradiation exhibits much better anticancer effect than the controls. Hence, the DOX-SWNHs/DCA-HPCHS as a multifunctional DDS has been proposed and is hopeful for medical use in the future.

Introduction

The past decades have witnessed the advantages of carbon nanomaterials in biomedical applications in detection, imaging and drug delivery both *in vitro* and *in vivo*.[1-4] As a promising drug delivery system (DDS), carbon nanomaterials have exhibited excellent physical and chemical properties.[5-8] One of the most important advantages of carbonaceous nanomaterials, such as single-walled carbon nanotubes (SWNTs), single-walled carbon nanohorns (SWNHs) and nano graphene, is that they demonstrate strong absorption in near-infared (NIR) region, which makes them good candidates as photothermal therapy (PTT) agents and *in vivo* NIR imaging agents.[9-11] In that, SWNHs demonstrate superior advantages for biomedical applications, such as i) SWNHs aggregates possess relative uniform morphology compared to their counterparts. Nearly 2000 tubules with a diameter of 2-5 nm and a length of 40-50 nm form a conically-shaped aggregate with a diameter of 80-100 nm,[12, 13] which has been proved to be an optimal size for the enhanced permeability and retention (EPR) effect and thus be able to effectively accumulate at the tumor site. ii) SWNHs can be produced in large quantities with high purity simply by laser ablation, and the free of metal catalysts contamination overcomes the inferiority from SWNTs.[14, 15] Importantly, extensive toxicological assessment of SWNHs suggests that SWNHs has quite low toxcity both *in vitro* and *in vivo*.[16-19] iii) Large quantities of holes and functional groups can be easily introduced to SWNHs, which facilitate the further multifunctionality.[20-22] Moreover, both of the surface and the inner part of SWNHs are able to provide accommodation for guest molecules and endow them with huge capacity as a DDS.[23, 24]

Combination therapies are receiving more and more attentions in recent years for cancer treatment.[25-29] Hyperthermia, a mild heating to about 43 °C, is frequently employed as an adjunctive therapy mode of tumor together with other traditional therapeutic method such as chemotherapy, photodynamic therapy (PDT) and radiotherapy.[30-33] It has been reported that the therapeutic efficacy is significantly improved when combined with hyperthermia, since hyperthermia can facilitate the therapeutic agents to reach the targeting site by increasing the perfusion of blood and the vessel permeability in tumor.[34, 35] However, traditional hyperthermia treatment (limb perfusion, incubation chambers and radiowave irradiation) may bring nonspecific heating, which can greatly enhance the toxicity of therapeutic agents to normal tissues, leading to severe side effects.[36, 37] Site-specific photothermal heating in tumor might provide a better choice for the hyperthermia therapy. Local hyperthermia in tumor site can be available by exposing the tumor with high administration of photothermal agents to laser irradiation. The most well investigated photothermal agents are gold nanoparticles and carbon nanomaterials, which can effectively convert NIR laser irradiation to heat.[38-42] For example, Yoo's group has developed RGD-modified half-shelled gold nanoparticles to deliver methotrexate (MTX), the efficacy of

MTX was greatly improved both *in vitro* and *in vivo* as the drug release was triggered by the heat generated by the half shelled gold nanoparticles upon NIR irradiation.[43] Feng and his coworkers have used a polyethylene glycol and polyethylenimine co-modified nanographene oxide for gene delivery, and the transfection efficiency was dramatically increased as a result of a mild photothermal heating when exposed to a low power NIR irradiation.[44] Kumar's group have reported a DDS based on SWNTs for targeted drug delivery in combination with photothermal therapy and an accelerated killing of tumor cells was observed.[45]

Inspired by the concept of photothermally enhanced therapy modes, we herein proposed a photothermally enhanced chemotherapy drug delivery system (DDS), DOX-SWNHs/DCA-HPCHS nanoparticles, with SWNHs as the photothermal agent and DOX as the chemotherapy agent (**Figure 1**). The as-prepared DOX-SWNHs/DCA-HPCHS was highly stable and biocompatible. The *in vitro* experiments revealed that the uptake of DOX-SWNHs was dramatically promoted with a phtotothermal heating to about 43 ^{o}C, leading to enhaced cytotoxicity of DOX to 4T1 model cells. The photothermally enhanced chemotherapy effect was also observed *in vivo* since DOX-SWNHs/DCA-HPCHS demonstrated a striking antitumor effect on the tumor-bearing mice once in combination with the use of laser irradiation.

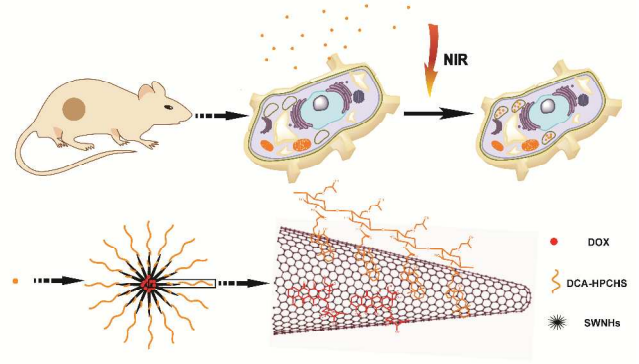

Figure 1. Chemical structure of DOX-SWNHs/DCA-HPCHS and the scheme of photothermally enhanced chemotherapy based on this DDS.

Experimental

Chemicals and materials

Chitosan (M.W.~5.0×10^5) with a 90% degree of de-acetylation was supplied by Jinan Haidebei Biochemical Co. Ltd., China. Deoxycholic acid (DCA) was provided by Alfa Aesar China Co. Ltd., China. N-hydroxysuccinimide (NHS) and 1-ethyl-3 -(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), dimethyl sulfoxide (DMSO) and triethylamine were obtained from Shanghai RichJoint Chemical Reagents Co. Ltd, China. Propylene oxide and toluene were purchased from Sinopharm Chemical Reagent Co. Ltd., China. 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) and doxorubicin hydrochloride (DOX•HCl) were purchased from Sigma Company. Ltd. Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Laboratories (Japan). All commercially available solvents and reagents were analytical grade and were used without further purification.

Synthesis of DCA-HPCHS

DCA-HPCHS was prepared by our previously reported method.[51] Briefly, a mixture of DCA (0.48 g), EDC (0.11 g) and NHS (0.12 g) in 100 mL of methanol was added to 1% (wt) of acetic acid aqueous solution (100 mL) containing chitosan (1 g) under stirring condition. After reacting under room temperature for 22 h, the resulting mixture was poured into a mixed solution of methanol (7 mL) and ammonia (3 mL) to precipitate the final product, deoxycholic acid modified chitosan (DCA-CHS), which was collected by filtration and washed with acetone several times. The as-prepared DCA-CHS was dispersed in a mixture of sodium hydrate (0.8 g) and isopropanol (30 mL) to alkalize for 4 h. Then propylene epoxide (4.4 mL) was added to the above solution to react for 24 h at 45 ^{o}C. The resulting mixture was neutralized by acetic acid to precipitate the desired product. DCA-HPCHS was obtained by filtration and subsequent washing with 75% ethanol and acetone for several times and vacuum drying at 50 ^{o}C.

Preparation and characterization of DOX-SWNHs/DCA-HPCHS

DOX-SWNHs/DCA-HPCHS was prepared with a vacuum assisted solvent evaporation method. Briefly, 5 μL of triethylamine was first added to 1 mL of 1 mg mL^{-1} DOX•HCl aqueous solution, the resulting solution was stirred at room temperature for 2 h to produce neutralized doxorubicin. 1 mg of SWNHs was suspended in 1 mL of toluene and sonicated for 20 min. Then 60 mg of DCA-HPCHS was added to the DOX aqueous solution and mixed with the SWNHs suspension. The mixture was stirred and the toluene was evaporated with the assistance of a vacuum. The product was centrifuged at 12000 rpm for 1 h to remove the large aggregates of SWNHs and was ultra-filtrated at 5000 rpm for 30 min for several times to remove the unloaded DOX. Finally, the solution was redispersed to desired concentrations. The size of samples was characterized using a Malvern Zetasizer Nano ZS 90 at 25 ^{o}C. Transmission electron microscope (TEM) image was carried out on JEM-2011 (JEOL, Japan) operated at 200 kV. NIR absorption was conducted with PE Lambda 750 UV/Vis/NIR spectrophotometer.

Temperature elevation induced by laser irradiation assay

DOX-SWNHs/DCA-HPCHS (SWNHs concentration, 5 μg mL^{-1}; DOX concentration, 5 μg mL^{-1}) in PBS (500 μL) and PBS alone (as control, 500 μL) were irradiated with 808-nm laser light at a power density of 0.6 W cm^{-2} for 10 min. The temperature of the solutions was recorded with an infrared thermometer mini hand-held laser infrared temperature measurement gun (Fluke F59, USA) .

Photothermal treatment and cell growth inhibition assays

Cell viabilities of 4T1 cells incubated with either DOX-SWNHs/DCA-HPCHS or SWNHs/DCA-HPCHS solution with or without 808 nm-laser treatment were determined by CCK-8 assays, respectively. First, 4T1 cells with ca. 5×10^4 cells per well density were seeded into a 96-well culture plate with 200 μL of RPMI-1640 supplemented with 10% FBS and 1% PS in each well, and incubated at 37 ^{o}C under a humidified atmosphere with 5% CO_2 for 24 h. Then the aged culture medium was replaced by freshly prepared culture medium containing either DOX-SWNHs/DCA-HPCHS or SWNHs/DCA-HPCHS solution in a series of gradient concentrations (0, 1, 2, 5, 10 μg mL^{-1}). For the 808 nm laser treatment groups, the 96-well culture plate was

immediately irradiated by an 808 nm laser at a series of power density (0, 0.2, 0.4, 0.6 W cm^{-2}). After that, the culture medium was replaced by fresh RPMI-1640 and incubated for another 24 h. Then the culture medium was removed and 100 μL of DMEM without phenol red and 10 μL of CCK-8 were added into each well. After incubated for another 1 h, the absorbance of each well was measured at 450 nm by a microplate reader.

Confocal laser microscopic characterization

4T1 cells were seeded into confocal dishes with ca. 5×10^4 cells per dish and allowed to grow until 60% confluence. Cells were washed twice with PBS. Subsequently, fresh RPMI-1640 with different concentrations of DOX-SWNHs/DCA-HPCHS was added and was immediately irradiated by an 808 nm laser at a series of power density (0.2, 0.4, 0.6 W cm^{-2}). After that, the culture medium was replaced by fresh RPMI-1640 and incubated for another 3 h. Then the cells were washed twice with ice-cold PBS and then dyed with DAPI for visualizing under a laser scanning confocal microscope (OLYMPUS FV1000-IX81, Japan).

Animal experiments

5-week-old female Balb/c mice were purchased from Nanjing Peng Sheng Biological Technology Co. Ltd and all animal experiments were conducted under protocols approved by Soochow University Laboratory Animal Center. 40 μL of ca. 5×10^5 cells μL^{-1} 4T1 cells suspension was subcutaneously injected into the right flank of each mouse.

NIR thermal imaging

Mice bearing 4T1 tumors in the laser-treated groups were irradiated with an 808 nm laser at a power density of 0.4 W cm^{-2} for 20 min, and simultaneously imaged by an IR thermal camera (Infrared Cameras. Inc.).

Tumor sections for histological examination

For histological examination, tumor tissues in the centre of tumors were fixed in 4% formalin and conducted with paraffin embedded sections for H&E staining. The slices were examined by a digital microscope (Leica QWin).

Results and discussions

SWNHs was loaded with DOX via π-π stacking interaction between the anthraquinone ring of DOX and the large conjugate system of SWNHs. Briefly, DOX was firstly neutralized by reacting with triethylamine to enhance the interaction between SWNHs and DOX. Then DCA-HPCHS was introduced to obtain highly biocompatible and water-dispersible DOX-SWNHs/DCA-HPCHS with a vaccum-assisted evaporation protocol. As Figure 2a depicted, the as-prepared DOX-SWNHs/DCA-HPCHS possessed high stability in physiological media including water, PBS, RPMI-1640 and FBS. The DLS results revealed that the hydrodynamic diameters of DCA-HPCHS, DOX/DCA-HPCHS, SWNHs/DCA-HPCHS, DOX-SWNHs/DCA-HPCHS were ca. 190 nm, 255 nm, 220 nm and 164 nm, respectively (Figure 2b). It was speculated that the DOX/DCA-HPCHS and SWNHs/DCA-HPCHS were in different forms in aqueous solution according to the DLS results. As we previously reported, DCA-HPCHS tended to self-assemble into polymeric micelles and the neutralized DOX could be incorporated into the inner core through hydrophobic-hydrophobic interaction. [50] However, the DCA-HPCHS probably served as an amphiphilic surfactant in SWNHs/DCA-HPCHS, with its hydrophobic moiety anchoring onto SWNHs and the hydrophilic hydropropyl group maintaining

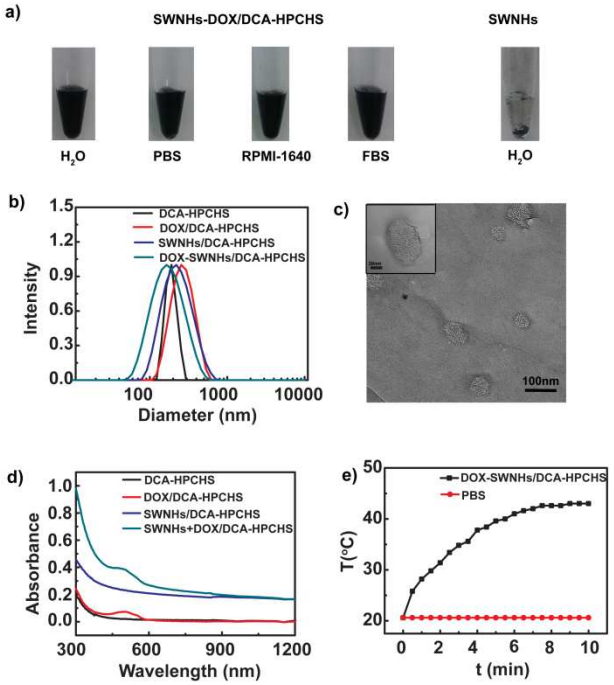

Figure 2. Characterization of DOX-SWNHs/DCA-HPCHS. (a) Stability test of DOX-SWNHs/DCA-HPCHS in water, PBS, RPMI-1640 and FBS. (b) Particle size distribution of DCA-HPCHS, DOX/DCA-HPCHS, SWNHs/DCA-HPCHS and DOX-SWNHs/DCA-HPCHS. (c) TEM image of DOX-SWNHs/DCA-HPCHS. (d) NIR absorption of DCA-HPCHS, DOX/DCA-HPCHS, SWNHs/DCA-HPCHS and DOX-SWNHs/DCA-HPCHS. (e) *In vitro* temperature elevation of DOX-SWNHs/DCA-HPCHS (0.1 mg mL^{-1}).

the stability in aqueous environment, just as the case of the PEG-aptamer conjugates modified SWNHs.[47] Besides, it was reasonable that the DOX-SWNHs/DCA-HPCHS was smaller than SWNHs/DCA-HPCHS, since the most of hydrophobic moieties of SWNHs were occupied by the loaded DOX, the hydrophobic-hydrophobic interaction between SWNHs could dramatically be weakened, resulting in better dispersion in aqueous solution. The transmission electron microscopy (TEM) revealed that each DOX-SWNHs/DCA-HPCHS contained only one SWNHs, which confirmed our previous hypothesis (Figure 2c).

The UV/Vis/NIR absorption spectra of DCA-HPCHS, DOX/DCA-HPCHS, SWNHs/DCA-HPCHS and DOX-SWNHs/DCA-HPCHS were recorded with a PE Lambda 750 UV/Vis/NIR spectrophotometer. As illustrated in Figure 2d, the SWNHs exhibited a strong absorption in the NIR region and DOX demonstrated a prominent absorption at 488 nm, whereas DOX-SWNHs/DCA-HPCHS possessed the characteristic absorption of SWNHs and DOX, suggesting that DOX had been successfully loaded onto SWNHs. Moreover, the content of DOX in the DOX-SWNHs/DCA-HPCHS could be quantified by its absorption at 488 nm over the background of SWNHs/DCA-HPCHS.

As Figure 2e showed, DOX-SWNHs/DCA-HPCHS could lead to obvious temperature elevation, while the PBS alone demonstrated negligible temperature increase when exposed to an 808 nm laser irradiation. Moreover, the photothermal property of DOX-SWNHs/DCA-HPCHS was concentration- and power density- dependent (data not shown). Notably, the temperature of solution could rapidly rise to 43°C within 8 min in the presence of 5 μg mL^{-1} of DOX-SWNHs/DCA-HPCHS under 808 nm laser irradiation at a power density of 0.6 W cm^{-2} and maintain this temperature with prolonged irradiation time, which was desirable since this temperature was not able to cause significant cancer cell damage but could promote the toxicity of DOX in this DDS as discussed thereinafter.

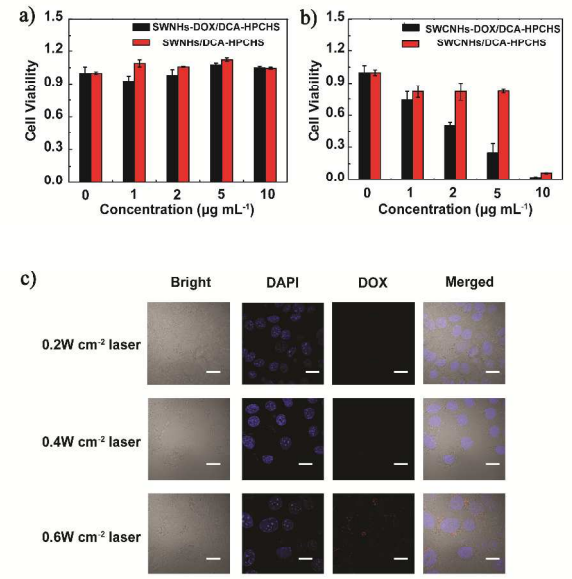

Figure 3. *In vitro* cell viability. 4T1 cells incubated with either DOX-SWNHs/DCA-HPCHS or SWNHs/DCA-HPCHS without (a) and with (b) the 808 nm laser irradiation at a power density of 0.6 W cm^{-2}. (c) Confocal fluorescence images of 4T1 cells incubated with 5 μg mL^{-1} DOX-SWNHs/DCA-HPCHS with the 808 nm laser irradiation at different power densities (0.2 W cm^{-2}, 0.4 W cm^{-2} and 0.6 W cm^{-2}).

Figure 3a-b demonstrated the cell growth viability of 4T1 cells incubated with DOX-SWNHs/DCA-HPCHS and SWNHs/DCA-HPCHS either with or without an 808 nm laser irradiation. For non-irradiated 4T1 cells, DOX-SWNHs/DCA-HPCHS and SWNHs/DCA-HPCHS didn't show significant cytotoxicity at concentrations ranging from 1 to 10 μg mL^{-1}, indicating that DOX and SWNHs below 10 μg mL^{-1} in this DDS were not able to kill cancer cells (Figure S1a). Notably, after a photothermal heating to around 43 °C with the 808 nm laser irradiation at a power density of 0.6 W cm^{-2}, SWNHs/DCA-HPCHs inhibited cell growth merely by ca. 13% while DOX-SWNHs/DCA-HPCHS resulted in a cell growth inhibition of ca. 73% at a concentration of 5 μg mL^{-1}. These results suggested that the photothermal heating to 43°C alone was not able to lead to significant cell death but could dramatically increase the chemotherapy efficacy of DOX. Moreover, the viability of 4T1 cells was closely related to the dose of DOX-SWNHs/DCA-HPCHS and the power density of the 808 nm laser (Figure S1b). For 4T1 cells incubated with 5 μg mL^{-1} DOX-SWNHs/DCA-HPCHS, only those treated with the laser at a power density of 0.6W cm^{-2} could lead to a significant cell growth inhibition and those exposed to a lower power density irradiation hardly caused any cell death. It was probably because the photothermal heating was not high enough to enhance the chemotherapy of DOX-SWNHs/DCA-HPCHS at either lower concentration of SWNHs or lower laser power density. This was in highly accordance with previous report that the significant enhancement of toxicity of DOX could only be realized when the incubation was done at about 43°C.[48, 49]

According to previous literature reported, the photothermally enhanced chemotherapy orginated from either the light triggered DOX release or promotion of cell uptake of DOX-carrier.[50, 51] To explore the underlying mechanism of the photothermally enhanced chemotherapy in this DDS, the DOX released from DOX-SWNHs/DCA-HPCHS with or without a laser irradiation were investigated. As Figure S2 illustrated, the fluorescence intensity of free DOX in DOX-SWNHs/DCA-HPCHS with 0.6 W cm^{-2} and 0.2 W cm^{-2} laser irradiation were 10.4 and 5.6, respectively, whereas that of free DOX itself was 286, which indicated that the DOX released from DOX-SWNHs/DCA-HPCHS with 808 nm laser irradiation was negligible. That is to say, DOX-SWNHs/DCA-HPCHS didn't show a significant light-triggered release of DOX with the 808 nm laser irradiation. Therefore, the efficacy increase of DOX should be ascribed to the increase cellular uptake of DOX-SWNHs/DCA-HPCHS. DOX fluorescence measured by flow cytometry confirmed the increased cellular uptake of DOX-SWNHs/DCA-HPCHS after the 808 nm laser irradiation at a power density of 0.6 W cm^{-2} (Figure S3). While the cells incubated with DOX-SWNHs/DCA-HPCHS at a power density of either 0.2 W cm^{-2} or 0.4 W cm^{-2} didn't show any DOX fluorescence, which was in well agreement with the cell growth inhibition results.

Figure 3c showed the confocal laser microscopy images of 4T1 cells incubated DOX-SWNHs/DCA-HPCHS after the 808 nm laser irradiation at a series of power density. The dose of DOX and SWNHs were 5 μg mL^{-1}. Red fluorescence with different intensities was observed clearly in 4T1 cells incubated with DOX-SWNHs/DCA-HPCHS after a 808 nm laser irradiation at a power density of 0.6 W cm^{-2}, while no fluorescence could be detected in 4T1 cells incubated with DOX-SWNHs/DCA-HPCHS irradiated at a lower power density. This result again confirmed that only a power density above 0.6 W cm^{-2} laser irradiation could lead to a significant cellular uptake of DOX-SWNHs/DCA-HPCHS.

Mice bearing 4T1 tumors were intratumorally injected with 40 μL of 1 mg mL^{-1} either DOX-SWNHs/DCA-HPCHS, or SWCNHs/DCA-HPCHS solution, and the control groups were injected with equivalent amount of saline. Then the mice were treated with the 808 nm laser irradiation at a power density of 0.4 W cm^{-2} for 20 min, and simultaneously imaged by an IR thermal camera (Figure 4a). It was found that the tumors injected with DOX-SWNHs/DCA-HPCHS and SWCNHs/DCA-HPCHS were rapidly heated up to 43 °C and then kept constant at this temperature when exposed to laser irradiation. In contrast, the control groups and DOX/DCA-HPCHS injected groups didn't show significant temperature increase under the same condition (Figure S4). Thus we set the power density as 0.4 W cm^{-2} for the

following photothermally enhanced chemotherapy experiment.

As the *in vivo* environment is complicated and share little in common with the *in vitro* condition, the *in vivo* therapeutic efficacy of DOX-SWNHs/DCA-HPCHS was evaluated. 30 tumor

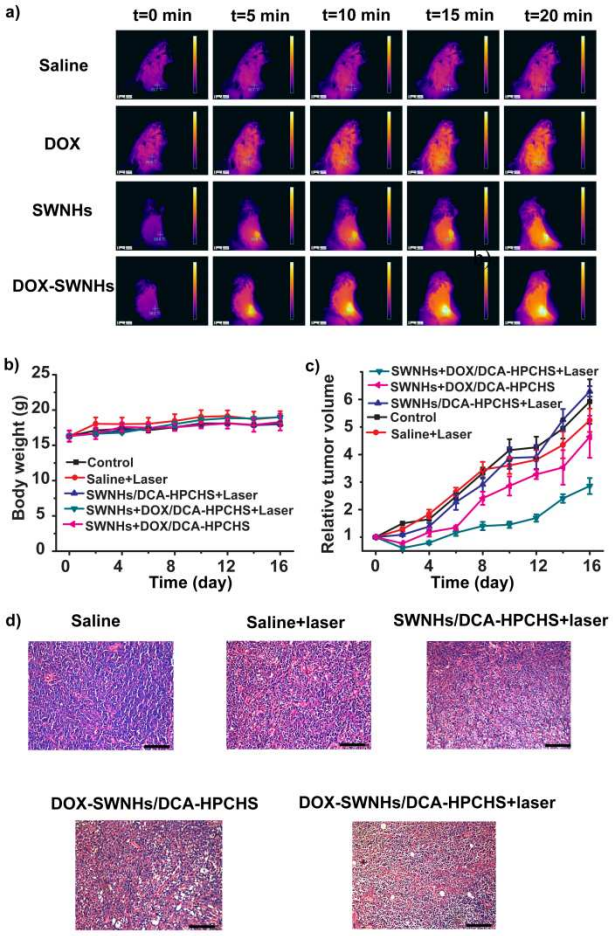

Figure 4. *In vivo* photothermally enhanced chemotherapy. (a) NIR thermal images of 4T1 tumor bearing mice injected with either saline or DOX/DCA-HPCHS, SWNHs/DCA-HPCHS and DOX-SWNHs/DCA-HPCHS (1 mg mL^{-1}, 40 μL). (b) The tumor growth curves of the mice after treatment. (c) The body weight change of the mice after treatment. (d) Tumor slices stained with H&E from different groups.

bearing Balb/c mice were randomly distributed into 5 groups, with 6 mice per group. The experiment groups were intratumorally injected with either 40 μL of 1 mg mL^{-1} DOX-SWNHs/DCA-HPCHS solution or SWNHs/DCA-HPCHS solution. For control groups, mice were treated with the same volume of saline. Then the three laser treated groups were irradiated at the tumor site with the 808 nm laser for 10 min at a power density of 0.4 W cm^{-2}. The tumor sizes were measured with an electronic digital caliper every other day after treatment. At day 17, all the mice were sacrificed. It was found that the DOX-SWCNHs/DCA-HPCHS+laser group demonstrated a significant antitumor effect. By contrast, the tumor sizes of the SWNHs/DCA-HPCHS+laser group didn't exhibit any differences from the control group, which once again confirmed that phothothermal therapy at this condition could not effectively kill the tumor cells (Figure 4c). Moreover, only the tumor size of the mice in the DOX-SWCNHs/DCA-HPCHS+laser group and DOX-SWCNHs/DCA-HPCHS group became smaller in the first 6 days and those in other group continued to grow, which indicated that this anticancer effect should benefit mainly from chemotherapy but not photothermal therapy. Notably, compared with the DOX/DCA-HPCHS+laser group, the antitumor effect of the DOX-SWNHs/DCA-HPCHS+laser group was able to sustain as long as 10 days with a single injection and laser exposure, the tumor volume showed ca. 65% reduction compared with the control group, confirming a photothermally enhance chemotherapy *in vivo*. However, the tumor sizes of the DOX-SWNHs/DCA-HPCHS+laser group began to grow after 12 days, it was probably due to the lack of DOX and the therapeutic effect might be improved simply with additional injection of DOX-SWNHs/DCA-HPCHS or laser exposure.

The body-weight of the mice was also monitored in the experiment (Figure 4b). It was found that the experimental groups did not show any decrease during the treatment compared with the control group, indicating there were no obvious side effects. It should ascribe to the strong interaction between DOX and SWNHs, and DOX was not able to be released from the DDS before DOX-SWNHs/DCA-HPCHS was up-taken by the cancer cells. Since the normal tissue was far from the laser irradiation, DOX-SWNHs/DCA-HPCHS internalized by normal cells was limited, and hence the toxicity of DOX to normal cells was neglegible. Haematoxylin and eosin (H&E) staining of tumor slices was conducted for tumors of the mice in the five groups (Figure 4d). In the saline only group, the cancer cells showed activating proliferation with many blue dyeing nucleus. However, the nucleus of cancer cells became shrinkage and condensation with dark dyeing, and erythrocyte appeared in intercellular substance in the DOX-SWNHs/DCA-HPCHS+laser treated group. For the other 3 groups, the cancer cells proliferated with more intercellular substance compared with the saline only group. This results demonstrated that the DOX-SWNHs/DCA-HPCHS+laser treated group which resulted significant apoptosis and necrosis on cancer cells had better antitumor effect than other 4 groups.

Conclusions

In summary, we developed a combined therapeutic DDS based on SWNHs. DOX was loaded onto SWNHs through a π-π stacking interaction and further modified by DCA-HPCHS to prepare DOX-SWNHs/DCA-HPCHS nanoparticles. The DOX-SWNHs/DCA-HPCHS nanoparticles possesses high stability and excellent biocompatibility in physiological solutions. The DDS combines the photothermal properties from SWNHs and chemotherapeutic properties from DOX organically. The chemo-therapy curative effect is remarkably enhanced by mild photothermal heating through 808nm-laser irradiation both *in vitro* and *in vivo*. Hence, the SWCNHs as a multifunctional drug delivery system (DDS) has been proposed and is hopefully for medicinal use in the future.

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† Electronic Supplementary Information (ESI) available: [Viability of 4T1 cells incubated with either SWNHs/DCA-HPCHS or DOX-SWNHs/DCA-HPCHS with an 808 nm laser irradiation at a series of power density, The fluorescence intensity of water, free DOX and DOX-SWNHs/DCA-HPCHS with an 808 nm laser irradiation at either 0.2 W cm^{-2} or 0.6 W cm^{-2}, Flow cytometry of 4T1 cells incubated with DOX-SWNHs/DCA-HPCHS exposed to 808 nm laser irradiation, The temperature elevation on the surface of tumors of mice, Tumor photographs of the five treated groups]. See DOI: 10.1039/b000000x/

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