Redox-responsive mesoporous silica nanoparticles for chemophotodynamic combination cancer therapy

Liuying Zhu 1,2, Weiwei Zhang 1,3, Ping Song 1, Wanzhen Li 1, Xiaolu Chen 1, Fei Ge 4, Lin Gui 4, Kai Yang 4, Yugui Tao 5,6 and Du Guocheng 7,8

1. Introduction

Nowadays, cancer remains the primary threat to human life [1-4]. Chemotherapy is still the most traditional and widely used method [5] in cancer treatment, inducing tumor cell apoptosis. Due to the small molecule and high toxicity, chemotherapy has many adverse reactions, such as non-target cell uptake and non-specific biological distribution, which can easily cause severe systemic toxicity [1]. Doxorubicin (DOX) is a broad-spectrum anticancer drug that treats malignant tumors by provoking long-term disruption of DNA replication [3]. Like many chemotherapeutics, DOX also has shortcomings, such as non-targeting and poor water solubility [6-8], leading to only a relatively small fraction of drugs delivered and accumulated in tumor tissues through blood circulation [9]. Therefore, traditional chemotherapy shows relatively low targeting effects and high toxicity. It is imperative to exploit new nano-scale drug delivery systems (NDDS) to solve the traditional chemotherapy side effects and improve targeting rates [10-12].

In recent years, nanocarrier materials have received extensive research because they have small particles [13], low toxicity, high biocompatibility [14], and high chemical stability [15]. Among these nanocarriers, the mesoporous silica nanoparticles (MSNs) show a series of significant advantages, such as excellent...
biomaterials [15], easy synthesis [16], large surface area [17], excellent chemical stability [18], unique mesoporosity [19], easy functionalization [20], and chemical inertness [21], attracting widespread attention. Chen et al. [22] developed MSN-DOX-G2 compound Bcl-2 siRNA to inhibit Bcl-2 mRNA expression and enhance the effect of chemotherapeutic drugs. Zhang et al. [23] developed Ru/Gd-Al@MSNs, a multifunctional diagnostic platform used for in vivo fluorescence and magnetic resonance (MR) dual-mode imaging. Therefore, MSN may be one of the most promising carrier materials [24].

Single therapy is hard to provide the expected effect. Therefore, combining nanocarrier materials with different treatment methods may improve cancer treatment effects [6]. Photodynamic therapy (PDT) is a widely studied exogenous stimulation therapy that requires light, photosensitizers, and molecular oxygen [25–28]. Under laser irradiation, the generated ROS induces cell necrosis or apoptosis by destroying cell lipids, proteins, and DNA [29, 30]. However, many photosensitizers show non-specific damage and poor water solubility [31], greatly reducing PDT efficacy and limiting its application potential [28]. Chlorin e6 (Ce6) is an excellent hydrophobic photosensitizer with outstanding stability [32]. The limitations of its application and small particle size lead to a short circulation time and difficulty accumulating at the tumor site [26]. Muddineti et al. [33] reported DP-Ce6-M, which improved the poor solubility of Ce6 and effectively inhibited cell growth under laser irradiation. Hence, MSN combined with DOX and Ce6 may reduce DOX dosage and relieve toxicity. On the other hand, Ce6 exerts PDT function to a greater extent [34, 35], improving the treatment effect. Since MSN-based drug delivery systems may risk uncontrolled drug release or premature leakage during transport, a ‘gatekeeper’ with a stimulatory response could perfectly solve this limitation.

The cancer microenvironment has high glutathione (GSH) content [36, 37], which can react with disulfide bonds. Therefore, it provides a new idea for the construction of nanomaterials. A composite carrier could be designed and developed to consume glutathione at the lesion site, so as when it reaches the vicinity of the tumor with blood circulation, the disulfide bond is broken, achieving passive targeting [38]. Thus, disulfide bonds show application potential in controlled drug release. To avoid the premature leakage of the drug, carboxymethyl chitosan (CMCS) was used to coat the mesoporous silica. CMCS is a derivative of chitosan [39] with good biodegradability [40], excellent biocompatibility [41], and low toxicity. Compared with hyaluronic acid, alginate, and chitosan, the amino group on CMCS can undergo an amide reaction with the carboxyl group on MSN-SS–COOH for drug protection in delivery systems. Drug release was restricted by surface grafting of CMCS. When reaching the vicinity of the tumor, the increase in GSH content leads to the breakage of the disulfide bond, and the concentrated release of the drug after the separation of CMCS from MSN.

Herein, a drug delivery system based on GSH-responsive mesoporous silica nanoparticles was constructed by co-loading the chemotherapy drug doxorubicin and the photosensitizer Ce6 to target the tumor site, achieving chemotherapy and photodynamic combination therapy (Scheme 1). The antitumor activity of DOX/Ce6@MSC in vitro was studied by cytotoxicity, ROS evaluation, and ultra-thin section analysis. The DOX/Ce6@MSC showed to be an effective therapy against MCF-7 cells. The histopathological and TUNEL analyses of tumor-bearing mice exhibited high biocompatibility and excellent anticancer effects of DOX/Ce6@MSC in vivo. Altogether, the results indicate that DOX/Ce6@MSC nanoparticles could effectively target drugs to tumor cells and enhance the anticancer effect through a multi-pronged assault.

2. Experimental section

2.1. Materials
N-Hexadecytrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS), 3-aminopropyl triethoxysilane (APTES), N-hydroxysuccinimide (NHS), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from Aladdin (Shanghai, China). Carboxymethyl chitosan, chlorin e6, amylin hydrochloride, and reduced glutathione were obtained from Shanghai Macklin Biochemical Co., Ltd. DMEM high glucose culture, fetal bovine serum, and penicillin-streptomycin double-antibody were purchased from Gibco (USA). Phosphate-buffered saline (PBS), Cell Counting Kit-8 (CCK-8), annexin-fluorescein isothiocyanate (Annexin-FITC), propidium diiodide (PI), calcein-AM, 1,3-Diphenylisobenzofuran (DPBF) and 2′,7′-Dichlorodihydrofluorescein diacetate (DCFH-DA) were acquired from Nanjing KeyGen Biotech Co., Ltd.

2.2. MSN synthesis and amination
The MSN was synthesized as previously reported [30, 42]. 0.5 g of CTAB and 0.14 g of NaOH (molar ratio = 0.39:1) were placed into deionized water (250 ml). The solution was stirred and heated to 80 °C. 4.2 ml of TEOS was added within 2 min and stirred vigorously for 2 h. After cooling down, the product was centrifuged at 12000 rpm for 5 min to collect. Finally, the CTAB was removed by calcination at 550 °C.
To prepare amino-functionalized monodisperse silica microspheres, 50 mg of MSN was added to 20 ml of toluene and mixed well, then 700 μl of APTES was added. The temperature was raised to 90 °C and stirred for 24 h, and subsequently centrifuged at 12000 rpm for 5 min to obtain MSN-NH₂.

### 2.3. MSN-SS-COOH synthesis

200 mg of MSN-NH₂ was thoroughly mixed with a PBS pH 7.4 solution, then 270 mg 3,3-dithiodipropionic acid, 270 mg EDC, and 163 mg NHS (molar ratio = 1:1.24:1.25) were dissolved in the solution. The mixture was reacted for 24 h, and washed 3 times with deionized water. Then the MSN-SS-COOH was collected by drying at 60 °C.

### 2.4. Drug loading and synthesis of DOX/Ce6@MSC

200 mg of MSN-SS-COOH was added to 20 ml of deionized water and dispersed by ultrasound. Then, 20 mg of DOX and 20 mg of Ce6 (molar ratio = 1.1:1) were thoroughly mixed into the solution and reacted for 24 h. Then, washed 3 times with deionized water by centrifugation (12000 rpm, 5 min) to obtain DOX/Ce6@MSN-SS-COOH (DOX/Ce6@M).

100 mg of DOX/Ce6@M was dispersed in 30 ml of PBS with pH = 5.8. EDC and NHS (molar ratio = 1:1) were placed into the solution and reacted for 1 h to activate the carboxyl. Finally, 20 ml of 5 mg ml⁻¹ CMCS solution was added and stirred for 12 h, washed 3 times with deionized water, and collected DOX/Ce6@MSN-SS-CMCS (DOX/Ce6@MSC).

### 2.5. Characterization of DOX/Ce6@MSC

Scanning electron microscopy (S-4800, Hitachi, Japan) examined the DOX/Ce6@MSC morphology. UV–vis spectral analysis was performed with a UV–visible spectrophotometer (Shimadzu, UV-3600). Infrared analysis was performed with a Fourier transform infrared (FT-IR) spectrometer (Shimadzu, IR Prestige-21). Particle size and Zeta potential were measured by Brookhaven Zeta Pals. The thermogravimetric analysis (TGA) was performed on an automatic differential thermogravimetric analyzer (Shimadzu DSC-60A). X-ray powder diffraction (XRD) was tested by an x-ray diffractometer (German, Bruker D8).
2.6. DOX/Ce6@MSC release behavior
Three GSH solutions (pH 7.4) with 0.02 mM, 2 mM, and 10 mM concentrations were prepared. A parallel group of DOX/Ce6@MSC solution (1 mg ml⁻¹) was dispersed in dialysis bags, immersed in 20 ml of release buffer solution containing 0.02 mM, 2 mM, and 10 mM GSH, and placed in a shaker at 37 °C. The samples were at intervals to measure UV absorption value of DOX at 480 nm.

2.7. Cell culture
MCF-7 cells were provided from the Institute of Basic Medicine, Chinese Academy of Medical Sciences (Beijing, China). The cells were cultured in DMEM containing 10% fetal bovine serum at 37 °C with 5% CO₂.

2.8. Cell uptake test in vitro
The DOX fluorescence excitation wavelength is 488 nm, and the emission wavelength is 560–620 nm. Therefore, it can be used for fluorescence detection of cell uptake at different times. The 1 × 10⁵ MCF-7 cells seeded in 6-well plates were treated with DOX/Ce6@MSC for 2 h, 6 h, and 12 h and washed with PBS 3 times. Then, they were harvested at each predetermined period. The DOX fluorescence intensity was examined by flow cytometry to characterize the phagocytosis of nanoparticles by cells.

The intracellular drug delivery behavior and the distribution of DOX/Ce6@MSC in MCF-7 cells were monitored by a fluorescence microscope. Firstly, 1 × 10⁵ MCF-7 cells were inoculated in 96-well plates and treated with DOX/Ce6@MSC for 1, 2, 6, and 12 h. Then, cells were washed with PBS, fixed with 4% paraformaldehyde (1 ml) for 10 min, washed 3 times with PBS, and stained with DAPI (400 μl) for 15 min. Ultimately, the samples were examined and photographed with a fluorescence microscope.

2.9. Cytotoxicity and apoptosis assay in vitro
To explore the function of nanoparticles on cell viability, a distinction was made between the laser-irradiated and non-irradiated groups. In the non-irradiated group, MSN, MSN-SS-COOH, MSN-SS-CMCS, DOX, Ce6, and DOX/Ce6@MSC were co-cultured with MCF-7 cells (1 × 10⁵) in 96-well plates for 24 h. The blank group was treated with a pure DMEM medium. Subsequently, CCK-8 was added and incubated for 2 h; the cell viability of samples was measured. In the laser-irradiated group, 24 h after the DOX/Ce6@MSC and Ce6 treatment, the plate was irradiated 5 min with a 660 nm laser, followed by CCK-8 incubation and cell viability measures.

The apoptosis test assessed the antitumor ability of DOX/Ce6@MSC. MCF-7 cells (1 × 10⁵) were cultured on 6-well plates and incubated with different DOX/Ce6@MSC concentrations ([DOX] = 0.5, 1, 3, 5 μg ml⁻¹). The blank group was treated with a pure DMEM medium, and the control groups were treated with free DOX (5 μg ml⁻¹), Ce6 (5 μg ml⁻¹), and Ce6 (5 μg ml⁻¹) plus laser. After 24 h, the cells were collected and co-stained with PI and Annexin-V FITC (5 μl each) for 10 min. Ultimately, the cells were investigated by flow cytometry.

We performed a cell live-death staining experiment to observe the apoptosis of MCF-7 cells after combined treatment. Firstly, 1 × 10⁵ MCF-7 cells were inoculated in 6-well plates and treated with free DOX, Ce6 and different DOX/Ce6@MSC concentrations ([DOX] = 5, 10, 20 μg ml⁻¹) for 24 h. The light group was only irradiated with a 660 nm laser for 5 min. Then, the cells were stained with calcine-AM and PI for 30 min, washed 3 times with PBS, and then observed with a fluorescence microscope.

2.10. Detection of singlet oxygen
Through laser irradiation, the photosensitizer can produce highly toxic ROS, an important photodynamic therapy feature [28]. DPBF can irreversibly react with ROS. Therefore, UV-Vis spectroscopy could record the absorption reduction of DPBF at 450 nm. Based on this, the ROS release was monitored. DOX/Ce6@MSC ([Ce6] = 5 μg ml⁻¹) was added to the DPBF solution and irradiated with a 660 nm 100 mW cm⁻² laser. The light absorption curves of the solution were noted every 2 min.

The PDT therapeutic effect in cells was investigated with a DCFH-DA probe. However, the probe itself is non-fluorescent, and it can be converted to high fluorescence intensity 2',7'-Dichlorofluorescin (DCF) after reacting with ROS [6]. The probe solution was diluted at a 1:1000 ratio, and the prepared probe solution was kept away from light and placed at 4 °C for later use. 1 × 10⁵ MCF-7 cells were seeded in 6-well plates and treated with DOX/Ce6@MSC ([Ce6] = 5 μg ml⁻¹) for 24 h. Then the cells were treated with DCFH-DA (10 mM) for 30 min, washed with DMEM without serum, and irradiated with a 660 nm laser for 2, 4, 6, and 8 min, respectively. The samples were examined with a fluorescence microscope. The amount of ROS in cells was determined from the intensity of green fluorescence produced by the combined probe and ROS. Alternatively, the cells were collected and immediately measured the fluorescence intensity by flow cytometry.
2.11. Ultra-thin cell section analysis
The 1 × 10³ MCF-7 cells in 6-well plates were treated with DOX/Ce6@MSC for 24 h, the blank group was treated with a pure DMEM medium, and the combination treatment group received laser irradiation for PDT treatment. The cells were collected, fixed with 2.5% glutaraldehyde at 4 °C overnight, and washed with PBS. Finally, the samples were fixed, sliced, stained and observed under transmission electron microscopy [26].

2.12. Animal and tumor models
BALB/c female nude mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. Subcutaneous xenografts of MCF-7 cells were generated by injecting 50 μl PBS containing 1 × 10⁶ cells onto the forelimb. When the tumor volume reached 50 mm³, it was used for the follow-up experiments. All animal procedures were performed following the guidelines for care and use of Laboratory Animals of Soochow University and approved by the Soochow University Laboratory Animal Center (Approval Number:202108A507).

2.13. In vivo antitumor efficacy
To study the nanoparticles' influence on the vital signs of mice, the mice were weighed at a fixed time after injection and drew a weight change curve. Then the mice inoculated with MCF-7 cells were randomly divided into 3 groups: blank (PBS-treated), DOX/Ce6@MSC, and the DOX/Ce6@MSC + laser. The DOX/Ce6@MSC doses were 2 mg kg⁻¹. 24 h after DOX/Ce6@MSC injection, the plus laser group was irradiated for 5 min at the tumor site, while other conditions remained unchanged. The sizes and volume of the tumor were measured and recorded daily during the 14 consecutive days of treatment. Tumors were photographed and recorded. On the 14th day, mice were euthanized to collect solid tumors for hematoxylin-eosin (H&E) and TUNEL staining analyses.

2.14. DOX/Ce6@MSC safety evaluation
To examine the DOX/Ce6@MSC toxicity in vivo, mice were divided into 4 groups and treated with PBS, 660 nm laser, DOX/Ce6@MSC, and DOX/Ce6@MSC + laser, respectively. The plus laser group was irradiated with a 660 nm laser for 5 min. The mice were euthanized after being treated for 30 days. The major organs were obtained for H&E staining, which detected the toxicity in vivo.

3. Results and discussion

3.1. Characterization of DOX/Ce6@MSC
The MSN was synthesized as previously reported [30, 42]. Figure 1(a) is a representative scanning electron microscope (SEM) image of MSN, showing the synthesized MSN as spherical particles with uniform size, smooth surface, and good monodispersity. After the disulfide bond was linked on the MSN surface, DOX and Ce6 were loaded, and coated with carboxymethyl chitosan, the DOX/Ce6@MSC surface became unsmooth, and the monodispersity and arrangement worsened (figure 1(b)). The MSN and DOX/Ce6@MSC average diameters were measured by a laser particle size analyzer. The average particle size of MSN was 158.07 nm (figure 1(c)), while that of DOX/Ce6@MSC increased to 202.35 nm (figure 1(d)), proving the successfully grafting of the functionalized molecules to the MSN.

The successful construction of DOX/Ce6@MSC was detected by FT-IR. According to figure 2(a), MSN showed characteristic peaks at 3431 cm⁻¹ and 960 cm⁻¹ due to the –OH group. The Si–O–Si group peak was revealed at 1046 cm⁻¹, meaning that the mesoporous silica nanoparticles were successfully synthesized. An additional MSN-NH₂ peak was identified at 1563 cm⁻¹ due to the N–H bending. The MSN-SS-COOH peaks at 1701 cm⁻¹ indicate the presence of carboxy. The typical carboxymethyl group peaks were revealed at 1414 and 1320 cm⁻¹. After loading the drugs, DOX/Ce6@M and DOX/Ce6@MSC showed the absorption bands at 1717, 1436, and 1406 cm⁻¹ assigned to COO¹, C=C, and =C–H bonds from Ce6 and DOX. Therefore, indicating successful drug loading. In the UV–vis spectrum, the free Ce6 showed a sharp absorption peak at 600–700 nm (figure 2(b)). The bathochromic shift was observed when Ce6 was loaded into MSN, indicating successful Ce6 loading. As shown in figure 2(c), DOX/Ce6@MSC retained a reduced peak at approximately 660 nm. Therefore, it was inferred that DOX/Ce6@MSC might still be suitable for photodynamic therapy. The bathochromic shift of DOX was also observed, showing that DOX was successfully loaded into MSN. According to our drug loading rate determination shown in table S1 (available online at stacks.iop.org/MRX/9/045401/mmedia), the drug loading rate of DOX was 9.8 ± 0.43% and that of Ce6 was 10.1 ± 0.74%.

Different chemical groups have different charge characteristics, and introducing different functional groups would change the MSN surface potential. As illustrated in figure 2(d), the Zeta potential of MSN was −37 mV, attributed to the abundance of −OH groups. After functionalization with the amino group, the MSN-NH₂
Figure 1. SEM photographs of MSN (a) and DOX/Ce6@MSC (b); Size distribution of MSN, PDI: 0.215 (c) and DOX/Ce6@MSC, PDI: 0.198 (d).

Figure 2. FT-IR spectra of MSN, MSN-NH2, MSN-SS-COOH, DOX, Ce6, DOX/Ce6@M, DOX/Ce6@MSC (a); UV–vis spectrum of MSN, DOX, Ce6, DOX/Ce6@MSC (b); fluorescence spectra of Ce6, DOX/Ce6@MSC (c); and Zeta potentials of MSN, MSN-NH2, MSN-SS-COOH, and DOX/Ce6@MSC (d).
potential rose to 19.1 mV. In contrast, the potential of MSN-SS-COOH was reversed from positive to −58.1 mV due to the carboxy groups. Finally, when DOX and Ce6 were loaded, and the CMCS was grafted, the DOX/Ce6@MSC potential was converted to −32.3 mV. This series of charge changes further proved that the MSN surface was successfully modified.

A specific surface area porosity analyzer characterized the specific surface area and pore size distribution of different functionalized MSNs. MSN showed a typical type IV isotherm, a porous, uniform, and ordered mesoporous structure (figure 3(a)). MSN showed a high specific surface area and large pore volume, reaching 799.12 m² g⁻¹ (figure 3(a)) and 0.27 cm³ g⁻¹ (figure 3(b)). Compared to MSN, the DOX/Ce6@MSC were only 91.72 m² g⁻¹ (figure 3(a)) and 0.09 cm³ g⁻¹ (figure 3(b)), respectively. The specific surface area and pore volume of MSN were significantly reduced after modification, indicating that DOX and Ce6 had loaded into the pores of MSN at this time. Meanwhile, the XRD result showed the typical MSN diffraction peaks. After drug loading, the diffraction peak became broader and weaker, and the order degree decreased. The result also showed that most drug molecules could enter the mesoporous channels (figure 3(c)).

The thermogravimetric analysis is shown in figure 3(d). When the temperature reached 800 °C, the thermogravimetric loss of MSN was 16.31%. After the disulfide bond was modified and the drug was loaded, the thermogravimetric loss rate increased to 40.91%, proving successful drug loading. Finally, the thermogravimetric loss of DOX/Ce6@MSC increased to 64.31%. The thermogravimetric loss increased significantly with the increased functionalization steps, successfully processing the modification.

3.2. The drug release test
Under sink conditions (figure S1), we examined the drug release behavior of DOX/Ce6@MSC at different GSH concentrations, and the results are shown in figure 4(a). The increase of GSH concentration broke the disulfide bond, increasing the drug release rate. When the GSH concentration was 0.02 mM, only 21.6% of the drugs were released. In contrast, when the GSH concentration was 2 mM, the drug release rate increased to 44.4%. Finally, at 10 mM GSH, 59.7% of the drugs were released.

3.3. In vitro cellular uptake
The DOX/Ce6@MSC uptake by MCF-7 cells was evaluated by flow cytometry technology to monitor the time-dependent intracellular behavior of the drug delivery system. Figure 4(b) shows that relative fluorescence intensity increased with the uptake time. The DOX/Ce6@MSC nanoparticles effectively enter MCF-7 cells reaching maximum uptake at 12 h. DOX can produce red fluorescence when excited, so intracellular distribution is easy to observe. When the cells were incubated with DOX/Ce6@MSC, the red fluorescence of

![Figure 3. Nitrogen adsorption-desorption isotherms of MSN and DOX/Ce6@MSC (a); Pore size distribution of MSN and DOX/Ce6@MSC (b); XRD patterns of MSN and DOX/Ce6@MSC (c); TGA of MSN, DOX/Ce6@M, and DOX/Ce6@MSC (d).](image-url)
DOX gathered near the blue cell nucleus stained with DAPI (figure 4(c)). The results suggested that the loaded DOX could be quickly released to exert therapeutic effects. The increasing red fluorescence accumulated in cells with extended uptake time, proving that MCF-7 cells effectively phagocytosed DOX/Ce6@MSC.

3.4. In vitro anticancer activity
DOX/Ce6@MSC cytotoxicity against MCF-7 cells was measured by the CCK-8 reagent. As shown in figure 5(a), MSN, MSN-SS-COOH, and MSN-SS-CMCS nanoparticles showed low cytotoxicity at 24 h post-treatment. Therefore, MSN, MSN-SS-COOH, and MSN-SS-CMCS reveal significant biocompatibility and safety. Moreover, cell viability decreased with increasing drug concentration in all treatment groups, indicating that DOX/Ce6@MSC had a significant antitumor effect. When the concentration was 20 μg ml⁻¹, the cell viability of
the DOX/Ce6@MSC + Laser group was lower than that of DOX/Ce6@MSC, DOX/Ce6@MSN-SS-COOH, and free DOX groups, indicating that the PDT effect of Ce6 is indeed helpful for tumor cell therapy. The apoptosis-inducing ability of DOX/Ce6@MSC was verified by flow cytometry. The proportion of apoptotic cells increased with DOX concentration (figure 5(c)). Notably, the cell survival rate with DOX/Ce6@MSC ([DOX] = 5 μg ml)⁻¹ was 58.32%, while the same concentration plus laser group was only 39.59%. Compared with the 55.30% cell survival rate of free DOX-treated cells, it can be concluded that chemotherapy combined with photodynamic therapy showed an excellent antitumor effect on MCF-7 cancer cells.

The live-death cell staining experiment was used to explore the effect of DOX/Ce6@MSC on MCF-7 cells viability and the result in figure 6. The number of dead cells increased with DOX/Ce6@MSC concentration, while the number of live cells decreased, as shown by PI and Calcein-AM staining. The results were entirely consistent with the cytotoxicity results, indicating that the ability of DOX to induce apoptosis was concentration-dependent. Finally, the plus laser group appeared more dead cells than the no laser at the same DOX/Ce6@MSC concentration. The cells treated with Ce6 plus laser showed anti-proliferation ability, but the combination of DOX induction could play a greater role in the antitumor effect. Therefore, the DOX/
Ce6@MSC nanoparticles combined with chemotherapy and photodynamic therapy were feasible and effective, revealing superior cytotoxicity.

3.5. Cellular ROS evaluation
Photodynamic therapy is the basic principle of photodynamic therapy, especially singlet oxygen ($^1$O$_2$), activating photosensitizers under laser conditions to produce highly cytotoxic ROS [6]. As a detection agent, the fluorescent probe DCFH-DA was used to detect the intracellular ROS levels of MCF-7 cells by fluorescence microscopy and flow cytometry. DPBF was used to evaluate the ROS levels of DOX/Ce6@MSC under 660 nm laser irradiation. The UV absorption peak intensity of DPBF decreased significantly with irradiation time (figure 7(a)), confirming ROS increase, and the DOX/Ce6@MSC photodynamic effects. Fluorescence microscopy images (figure 7(c)) showed that the green fluorescence gradually increased with the irradiation time, revealing substantial ROS production consistent with flow cytometry results (figure 7(b)). It was not controversial that ROS could damage tumor cells, leading to apoptosis or necrosis. Thus, photodynamic therapy offers a new therapeutic effect for treating tumors.

Figure 6. Live-Dead cell assays. After treatment, cells were stained with PI and Calcein-AM and observed by fluorescence microscope. The blank group was treated with DMEM medium, and the concentration of DOX/Ce6@MSC in the figure was calculated by DOX.
3.6. Ultrastructural and morphological features of cells

To deeply understand the anti-proliferative effect of DOX/Ce6@MSC, ultra-thin cell section analysis was performed to study ultrastructure and morphological changes. The morphology of cells treated with DMEM was complete, the cell boundaries were clean, and the overall shape was stretched polygonal (figure 8(a)). However, there was no significant change in MSN-SS-CMCS-treated cells, and the cell membranes and organelles were relatively intact (figure 8(b)). Some apoptosis characteristics appeared in the DOX/Ce6@MSC-treated cells (figure 8(c)). Finally, the DOX/Ce6@MSC plus laser-treated cells began to dissolve, and the structure was destroyed (figure 8(d)). These results confirmed that DOX/Ce6@MSC smoothly transported drugs to tumor cells, and chemotherapy combined with photodynamic therapy could kill tumor cells more effectively, showing strong anticancer ability on MCF-7 cells.

3.7. In vivo anticancer activity

Subcutaneous xenografts of MCF-7 were generated by injection to evaluate the antitumor activity in mice. Figure 9(b) shows that the mice’s weight changed slightly and remained stable during the treatment period, suggesting no apparent side effects on mice growth. The change in tumor volume during treatment in mice in the blank group (PBS-treated) in figure 9(a) increased significantly over 14 days. In comparison, the DOX/Ce6@MSC group tumor volume was inhibited and even decreased with treatment time, indicating a therapeutic effect on mice. Simultaneously, the tumor volume in the DOX/Ce6@MSC + laser group was smaller than the DOX/Ce6@MSC group, confirming the superiority of the combination therapy. The statistical analysis of tumor weight (figure 9(c)) also proved the antitumor effects in vivo. TUNEL staining results of mice are arranged in figure 9(e); blue are the nuclei of normal cells, and red represent apoptotic cells. Apart from the blank group, the DOX/Ce6@MSC and DOX/Ce6@MSC + Laser groups could induce apoptosis, and the anticancer effect of the DOX/Ce6@MSC+ laser group was superior. Moreover, prominent apoptosis was observed by H & E.
staining analysis in the mice tumor sections after treatment (figure 9(d)). Furthermore, the H & E staining results showed no apparent abnormalities or damages in the major organ sections, confirming that DOX/Ce6@MSC had good biocompatibility. Taken together, the above results indicate the excellent antitumor effects of DOX/Ce6@MSC in vivo.

3.8. Safety evaluation in vivo
Toxicity assessment of drugs is indispensable in biosafety evaluation. After successfully injecting DOX/Ce6@MSC into the healthy mice for 30 days, the main organs, including heart, liver, spleen, lung, and kidney, were collected and analyzed by H & E staining, as shown in figure 10. The histopathological analysis revealed no obvious tissue damages or cell necrosis in mice treated with PBS, 660 nm laser, DOX/Ce6@MSC, or DOX/Ce6@MSC + laser group. Therefore, it could be inferred that the DOX/Ce6@MSC was non-toxic with good biosafety. Meanwhile, in vivo biodistribution in mice (figure S2), DOX/Ce6@MSCs were mainly enriched in the liver. The silicon content in major organs decreased significantly after 14 days compared to 1 day of administration. The results showed that DOX/Ce6@MSC could be efficiently cleared or degraded in major organs and safely excreted.

4. Conclusions
We successfully synthesized a nano-drug loading platform of MSN with modified disulfide bonds and carboxymethyl chitosan on their surface. Finally, we obtained composites with good monodispersity, uniform size, and good biocompatibility. At the same time, composites were endowed with fluorescence and photodynamic properties to achieve combined treatment after co-loading of DOX and Ce6. Compared with non-targeted drugs, DOX/Ce6@MSC enhanced the targeting ability and avoided the early release of drugs. In vitro cytotoxicity and uptake experiments showed that MCF-7 cells could effectively phagocytose the DOX/
Ce6@MSC nanoparticles with a significant antitumor effect. Under 660 nm laser irradiation, ROS were triggered to strengthen the efficacy of chemotherapy in vivo and in vitro. Notably, DOX/Ce6@MSC injections showed an excellent inhibitory effect on tumor cells without noticeable side effects on the main organs of mice. Meanwhile,

**Figure 9.** Representative images of tumors at 0, 3, 7, and 14 days (a); Weight change curve of mice during treatment (b); tumor weight at 14 days (c); H & E of mice main organs and tumor tissues of (d); TUNEL analysis of mice tumor tissues (e). Data are means ± SD (n = 6–8), compared to the blank group, **p < 0.01.
in vivo biosafety experiments showed that DOX/Ce6@MSC was not physiological toxicity to mice. Based on the above results, we could conclude that chemotherapy combined with photodynamic therapy exhibited a significant enhancement effect on MCF-7 cells. Therefore, the combination therapy on the MSN platform based on GSH stimulus-response may be a promising and highly effective anticancer treatment.

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Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

Author contributions

All authors contributed to the data analysis and manuscript revision.

Competing interests

The authors have declared that no competing interest exists.

ORCID iDs

Weiwei Zhang @ https://orcid.org/0000-0001-6141-9153
Fei Ge @ https://orcid.org/0000-0002-6308-3224
Yugui Tao @ https://orcid.org/0000-0002-0144-4872
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