Biodegradable flower-like manganese for synergistic photothermal and photodynamic therapy applications

Wei Chen¹ · Yuyan Yan¹ · Renlu Han¹ · Jun Hu¹ · Yafei Hou² · Keqi Tang¹

Received: 21 September 2020 / Accepted: 21 December 2020 / Published online: 20 January 2021
© The Author(s), under exclusive licence to European Photochemistry Association, European Society for Photobiology 2021

Abstract

A flower-like nanostructured MnO_2 with near-infrared (NIR) light-triggered high photothermal conversion capability of 30% and reactive oxygen species (ROS) generation ability was successfully developed. Different from the reported MnO_2 nanomaterials those were used in the nanomedicine field for only relieving tumor hypoxia and/or imaging, the flower-like MnO_2 inherently acts as a competent agent for simultaneously enhanced photothermal and photodynamic therapy.

Graphic abstract

A flower-like nanostructured MnO_2 with near-infrared (NIR) light triggered high photothermal conversion capability of 30% and reactive oxygen species (ROS) generation ability was successfully developed.

Keywords Biodegradable · Flower-like manganese · Photothermal therapy · Photodynamic therapy

1 Introduction

Cancer has seriously threatened human health. For the treatment of cancer, it usually faces a few challenges, especially the nonspecificity which inevitably causes damage to normal tissues. As a developing specific method of cancer treatment, photothermal therapy (PTT) has attracted intensive interest. PTT relies on the irradiation of PTT agent to convert light energy into hyperthermia and kill cancer cells [1]. Ideal PTT agent exhibits specific light window for deep tissue penetration, high light-heat conversion capability, photostability and low toxicity. To date, diverse organic or inorganic nanomaterials such as Au [2], Prussian [3], Cu_{2-x}S [4], graphene [5], transition-metal dichalcogenides [6], black phosphorus [7], MXenes [8], and soon have been widely used as the efficient PTT

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s43630-020-00010-w>.

✉ Renlu Han
hanrenlu@nbu.edu.cn

✉ Keqi Tang
tangkeqi@nbu.edu.cn

¹ Institute of Mass Spectrometry, State Key Laboratory Base of Novel Functional Materials and Preparation Science, School of Materials Science and Chemical Engineering, Ningbo University, Ningbo 315211, People's Republic of China

² Department of Microelectronic Science and Engineering, Ningbo University, Ningbo 315211, People's Republic of China

agents. However, the non-degradability of these PTT agents would hinder them further toward clinical application because of the risk of chronic toxicity, and thus it is of great significance and challenge to develop biodegradable PTT agent for cancer therapy. Although exciting, when PTT was employed alone, a non-uniform heat distribution tends to be induced, leading to a poor therapeutic efficacy. Therefore, combination of PTT with other treatment strategies to enhance the efficacy of cancer treatment is necessary.

Photodynamic therapy (PDT), mainly relying on the irradiation of organic photosensitizers to generate reactive oxygen species (ROS) for cancer cells killing, has attracted great attentions due to its minimal invasiveness, site-specific activation, low toxic side effects, and negligible drug resistance [9]. Recently, it has been reported that the integration of PTT and PDT (PTT/PDT) would be a promising strategy for cancer therapy even using low-dose reagents and moderate light irradiation [10]. However, the efficiency of PDT is always weakened by hypoxic tumour microenvironment (TME) because of the strict dependence of PDT on molecular oxygen [11]. Furthermore, glutathione (GSH), which is an antioxidant and overexpressed in cancer cells, can scavenge ROS, and thus reduce the efficiency of PDT [12]. To date, various strategies employing nanocarriers to deliver oxygen or deplete GSH inside the tumours have been explored for enhancing the efficiency of PTT/PDT. For example, MnO_2 -based nanomaterials [13], PLGA-catalase-based nanoparticle [14], metal–organic framework [15] and GSH-cleavable nanoparticle [16] have been synthesized for oxygen supply or GSH depletion. However, of these studies, to endow the nanocarrier with PTT, O_2 -evolving, GSH depletion and PDT functions, a complex all-in-one structure needs to be fabricated laboriously with PTT agent, organic photosensitizer, oxygen donor and so on. Therefore, it is of great significance to develop a structure-simplified one-for-all system to implement oxygen supply, GSH depletion and synergic PTT/PDT.

MnO_2 has attracted a great deal of attention as a TME-responsive nanomaterial. As reported, MnO_2 nanomaterials could react with H_2O_2 and GSH in TME to release Mn^{2+} for magnetic resonance imaging [17]. Especially, MnO_2 shows high biosecurity because Mn is an important element involved in physiological metabolism of human body. However, the ability of MnO_2 as therapeutic agent for cancer treatment has been neglected. Recently, oxygen vacancies have been proven to serve as trap sites for photo-generated electrons, and tend to induce the appearance of intermediate energy levels, leading to improved performance of nanomaterials. For example, Zhou et al. fabricated ultrathin MnO_2 nanosheets for photothermal therapy driven by oxygen vacancy [18]. Liu et al. fabricated MoO_{3-x} QDs that enabled NIR light induce photothermal conversion and

ROS generation [19]. Although this theory has drawn lots of attentions in many fields, its potentials in the field of biomedicine has been hardly reported.

Herein, we developed a one-for-all multifunctional therapeutic nanoplatform just using a single flower-like MnO_2 , which can realize PTT and enhance PDT by self-supply oxygen and GSH depletion for cancer treatment in vitro. The flower-like MnO_2 was facilely prepared via a simple one-step method using formamide to reduce potassium permanganate (Fig. 1a). Benefiting from the oxygen vacancies of the flower-like nanostructure, MnO_2 shows high efficiency of photothermal conversion and ROS generation in near-infrared (NIR) light region. Based on the unique performances, the flower-like MnO_2 enables to simultaneously execute PTT and PDT for cancer cells killing under 808 nm light irradiation (Fig. 1b). Significantly, once taken up by cancer cells, the MnO_2 degrades rapidly within the TME by decomposing endogenous H_2O_2 into oxygen and reducing over expressed GSH in cancer cells, thus enhancing PDT through self-supply oxygen and GSH depletion. Our work highlighted a facilely prepared, one-for-all, biodegradable, and high-efficiency phototherapy platform with the capability of self-enhancing efficiency, which is more promising for clinical trials.

2 Experimental section

2.1 Materials

Potassium permanganate (KMnO_4), methanamide, hydrogen peroxide (H_2O_2), 1-Ethyl-3-(3-dimethylaminopropyl)

Fig. 1 Synthesis of flower-like MnO_2 and its application in cancer cell killing

carbodiimide hydrochloride (EDC) and hydroxysuccinimide (NHS) were purchased from Aladdin Reagent Co., Ltd (Shanghai, China). Dulbecco's modified eagle's medium (DMEM), trypsin–EDTA solution (0.25%) and penicillin/streptomycin were obtained from Gibco. Phosphate-buffered saline (PBS) was purchased from HyClone Laboratories, Inc. Propidium iodide (PI), calcein acetoxymethyl ester (calcein-AM), glutathione (GSH), fluorescein 5(6)-isothiocyanate, 9,10-anthracenediyl-bis(methylene)-dimalonic acid (ABDA), 2,7-Dichlorofluorescein diacetate (DCFH-DA) and Hoechst 33,258 were supplied by Sigma-Aldrich.

2.2 Synthesis of the MnO_2 nanoflowers

First, KMnO_4 (2 mmol) was dissolved in 160 mL of deionized (DI) water, and the mixture was adequately stirred for 30 min. Then, 5 mL of methanamide was added dropwise into the above solution, and the system was kept static at 40 °C for 8 h. Finally, after the product was centrifuged at 14,000 r for 5 min, the MnO_2 nanoflowers were washed with water and ethanol for twice and dried under vacuum.

2.3 Synthesis of the MnO_2 -FITC

First, the MnO_2 nanoflowers (200 mg) were dispersed into anhydrous toluene (50 mL), (3-aminopropyl)triethoxysilane (APTES, 100 μL) was added into the solution under stirring and the mixture was refluxed for 24 h. The obtained product (named as $\text{MnO}_2\text{-NH}_2$) was collected by centrifugation (14,000 r, 5 min), washed with ethanol and dried under vacuum. Then, $\text{MnO}_2\text{-NH}_2$ (20 mg), hydroxysuccinimide (NHS, 5 mg), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 5 mg) and 3',6'-dihydroxy-5-isothiocyanato-3H-spiro[isobenzofuran-1,9'-xanthen]-3-one (FITC 1 mg) were dissolved into water (10 mL), and the mixture was stirred for 12 h at room temperature. Finally, the product (named as $\text{MnO}_2\text{-FITC}$) was collected by centrifuge (12,000 r, 5 min), washed with water for four times and dried under vacuum at 30 °C.

2.4 Measurement of photothermal effect of MnO_2

A series concentration of MnO_2 nanoflower aqueous dispersions (0, 62.5, 125, 250 and 500 $\mu\text{g mL}^{-1}$) was placed in a transparent quartz cuvette. The bottom of the cuvette was irradiated with 808 nm light. During this process, a thermocouple microprobe was inserted into the aqueous dispersion to monitor the temperature change.

2.5 In vitro degradation Test

MnO_2 nanoflowers were dispersed in PBS solution with GSH (1 mM) and H_2O_2 (100 mM) to modulate tumor

microenvironments and then transferred into a dialysis bag and dialyzed. At given time points, the released Mn was measured by ICP-MS, and the morphology change of the samples was observed by TEM.

2.6 In vitro cytotoxicity assays

Methylthiazolyl tetrazolium (MTT) assay was performed to investigate the cytotoxicity of MnO_2 nanoflowers using HeLa as model cells. HeLa cells were, respectively, seeded in a 96-well plate for 24 h incubation to attach the bottom. A cell culture medium with a gradient concentration of MnO_2 nanoflowers (0, 6.25, 12.5, 25 and 50 $\mu\text{g mL}^{-1}$) was then added to each well to displace the original cell culture medium and co-cultured for 24 h. After that, 10 μL of MTT was added into each well for further 4-h incubation. Last, DMSO was added to dissolve the formazan crystals, and the relative cell viability was calculated on basis of the absorbance of formazan at 565 nm by microplate reader.

2.7 Detection of ROS

ABDA dispersion containing MnO_2 nanoflowers (1.5 mg mL^{-1}) was irradiated with 808 nm light for 5 min, and the absorption peak at 380 nm of ABDA was detected. DCFH-DA was used to detect the intracellular ROS generation. HeLa cells were seeded in a 96-well plate to attach the bottom, and 10 μL of DCFH-DA (60 $\mu\text{g mL}^{-1}$) was added for 30-min incubation. A cell culture medium with MnO_2 nanoflowers (50 $\mu\text{g mL}^{-1}$) was then added to displace the original cell culture medium and co-cultured for 4 h. After that, the cells were washed with PBS to remove the residual nanoflowers. Last, the cells were exposed to 808 nm light for different time (0, 5 and 10 min) and imaged using a fluorescent microscope.

2.8 In vitro phototherapy assay

HeLa cells were seeded in a 96-well plate for 24 h incubation. A cell culture medium with a gradient concentration of MnO_2 nanoflowers (0, 6.25, 12.5, 25 and 50 $\mu\text{g mL}^{-1}$) was then added to each well to displace the original cell culture medium and co-cultured for 4 h. After that, the cells were exposed to 808 nm light (1.5 W cm^{-2}) for 10 min. After 24 h incubation, 10 μL of MTT was added into each well for further 4 h incubation. Last, DMSO was added to dissolve the formazan crystals, and the relative cell viability was calculated on the basis of the absorbance of formazan at 565 nm by microplate reader.

In order to further verify the MTT assay results, the phototherapy effect was further observed by live/dead cells staining. The experiment process was consistent with the MTT assay. Briefly, the cells after various treatments

were cultured for another 24 h, a mixture of Calcein-AM ($5 \mu\text{g mL}^{-1}$) and PI ($10 \mu\text{g mL}^{-1}$) was added. After 30-min incubation, the cells were observed and imaged via an inverted fluorescence microscope.

2.9 Characterization

Scanning electron microscopy (SEM) images were obtained from a Hitachi SU-70 field emission SEM. Transmission electron microscopy (TEM) images were obtained on an electron microscope (JEM 2100). X-ray diffraction (XRD) patterns were recorded with a Bruker D8. Ultraviolet–visible (UV–Vis) spectra were measured on a UV–Vis spectrometer (SHIMADZU UV-1900). Fourier transform infrared (FTIR) spectra were recorded on a Thermo Fisher NICOLET Is10. N_2 adsorption–desorption isotherms were obtained with ASAP2020HD88. Fluorescence microscopy images were observed using a fluorescence microscope (LEICA DMi8). Dynamic light scattering (DLS) and zeta potential were observed using Malvern Zetasizer Nano ZS90.

3 Results and discussion

3.1 Synthesis and characterization of MnO_2 nanoparticles

The SEM (Fig. 2a) and TEM (Fig. 2b) images show the as-prepared MnO_2 exhibits a three-dimensional flower-like nanostructure. The size distribution of MnO_2 nanoflower is about 45 nm, which agreed with that of dynamic light scattering measurement (Fig. S1). Moreover, the magnified TEM image reveals that the nanoflowers may be formed by self-assembly of nanosheets, which would provide lots of anchoring sites for drug loading and functional modification (Fig. S2). The interplanar distance of about 0.14 nm which corresponds to the (412) lattice of MnO_2 (Inset in Fig. 2b) and the two main broad reflections at $2\theta = 37.4^\circ$ and 65.6° in X-ray diffraction (XRD) pattern (Fig. S3) indicate the MnO_2 nanoflower was crystalline (JCPDS42-1169). The X-ray photoelectron spectroscopy (XPS) further proves the presence of Mn and O in MnO_2 nanoflower. The two peaks at 635 and 660 eV assigned to $\text{Mn } 2p_{3/2}$ and $\text{Mn } 2p_{1/2}$ (Fig. S4) are shifted to lower energy compared with the reported ML- MnO_2 nanomaterials [18], which might be attributed to the presence of lower valence state caused by the oxygen vacancy. In the peak fitting plot of the two main peaks in MnO_2 , the peaks at 654.4 and 643.19 eV are assigned to

Fig. 2 **a** SEM image of MnO_2 , **b** TEM and HRTEM (inset) image of MnO_2 , **c** XPS spectra of MnO_2 , **d** Nitrogen adsorption–desorption isotherm and pore size distribution (inset) of MnO_2

Mn^{4+} , the peaks at 653.4 and 641.77 eV are ascribed to Mn^{4+} near the OV (Fig. 2c). The N_2 adsorption–desorption isotherms revealed the MnO_2 nanoflower possesses a large surface area (about $156 \text{ m}^2 \text{ g}^{-1}$) and macroporosity structure ($\sim 35 \text{ nm}$) (Fig. 2d), which would be very favourable for enhancing drug loading capacity and chemical reaction sites in design and fabrication cancer therapeutic systems. In the Fourier transform infrared (FTIR) spectrum of MnO_2 (Fig. S5), the characteristic bands at 1632 and 1402 cm^{-1} may be attributed to O–H bending vibration combined with Mn atom, implying the existence of numerous hydroxyl groups. The large number of functional groups can improve the hydrophilicity of MnO_2 , making it well dispersed in water, PBS and cell culture (Fig. S6), and thus avoiding the subsequent modification of biocompatible biomolecules.

3.2 Photothermal effect and ROS generation of the flower-like MnO_2

Optical absorption and photothermal conversion are two essential prerequisites for the site-specific activated cancer phototherapy. Hence, the optical properties of as-prepared flower-like MnO_2 were first investigated. As displayed in Fig. S7, the broad absorption of MnO_2 in the Vis–NIR range suggests MnO_2 can be used as a PTT agent. The digital image of MnO_2 dispersions with various concentrations demonstrates the good dispersion of MnO_2 in water (The

inset in Fig. S7). The temperature increase of MnO_2 dispersion implies strong photothermal effect which depends on both concentration (Fig. 3a) and NIR light intensity (Fig. S8). Moreover, the photothermal conversion efficiency of MnO_2 nanoflower is evaluated to be 30% according to the reported method (Figs. 3b and S9), which is quite promising compared with the reported $\text{Cu}_2\text{-xS}$ (11%) [4], $\text{MoO}_{3\text{-x}}$ (26%) [19] and black phosphorus quantum dots (28%) [7]. Additionally, negligible change was observed in photothermal effects after three cycles of heating and cooling processes under a high irradiation intensity of 1.0 W cm^{-2} (Fig. 3c), strongly implying the high photostability of MnO_2 nanoflower. Moreover, the photothermal effect was hardly affected even if the MnO_2 was incubated with water for a month (Fig. S10), further demonstrating the high stability of the flower-like MnO_2 . All these results suggest that the MnO_2 as prepared here could be employed as a potential and stable PTT agent.

It is well known that MnO_2 nanomaterials are mainly used as for relieving tumour hypoxia and bioimaging; however, its capability as photocatalyst to generate ROS for PDT has not been reported. Herein, 9, 10-anthracenediyl-bis(methylene)-dimalonic acid (ABDA) was used as a molecular probe to monitor the ROS production from MnO_2 . The characteristic absorption peak of ABDA at 380 nm significantly decreased after reacting with ROS. Figure 3d shows the absorption intensity of ABDA gradually decreased when the MnO_2

Fig. 3 **a** Photothermal heating curves of MnO_2 dispersion with various concentrations upon 808 nm light irradiation (1.0 W cm^{-2}), **b** Photothermal profile of MnO_2 dispersion ($250 \mu\text{g mL}^{-1}$) exposed to 808 nm light for 1500 s, followed by natural cooling to room temperature, **c** Photothermal stability of MnO_2 for three NIR light ON/OFF cycles, **d** The ROS generation of MnO_2 under different conditions

was exposed to 808 nm light, implying MnO_2 could generate ROS triggered by NIR light. Meanwhile, the negligible change was observed in the absorption intensity of ABDA for the laser only or MnO_2 only group. Moreover, given the TME shows the higher GSH and H_2O_2 content compared with normal tissues [17], ROS generation of MnO_2 in various solution containing GSH (1 mM), H_2O_2 (100 mM), or GSH + H_2O_2 (1 mM, 100 mM) were also studied, respectively. Compared with the MnO_2 + light group, the enhanced ROS production in MnO_2 + H_2O_2 + light group suggests MnO_2 could indeed increase ROS by decomposing H_2O_2 into oxygen. While with the GSH addition, the ROS production in MnO_2 + GSH + light and MnO_2 + H_2O_2 + light groups indicate GSH might scavenge the ROS produced in PDT. These results demonstrate the ability of the prepared MnO_2 nanoflower as photocatalyst to generate ROS and the potential application in the enhancement of PDT by self-oxygen supply and GSH depletion (Fig. 3d). It is worth mentioning that the ROS generating ability of MnO_2 was little affected after three cycles of NIR light irradiation (Fig. S11), suggesting the high photostability of MnO_2 as a photocatalyst for PDT application, which is superior to many reported organic photosensitizer molecules that would be easily decomposed by light.

3.3 Cellular uptake and intracellular ROS generation

Based on super photothermal conversion and ROS generation performances of MnO_2 nanoflower in aqueous solution, the photothermal and ROS-mediated cancer cell killing application was evaluated using HeLa cells as cancer cell models. The cellular uptake of MnO_2 nanoflower was first investigated considering that cell uptake is a prerequisite for effective treatment. As shown in Fig. S12, the green signals emitted by MnO_2 -FITC were only observed in the cytoplasm and not in the extracellular area, suggesting the MnO_2 -FITC nanoflowers were ingested by HeLa cells rather than adhesion on the cell surfaces. Additionally, 2, 7-dichlorodihydrofluorescein diacetate (DCFH-DA) was employed

as fluorescent probe to investigate the ROS production inside the cells, because DCFH-DA would emit green fluorescent after being oxidized by ROS. As expected, the distinct green fluorescence was observed inside the HeLa cells treated with MnO_2 and NIR light, while no green signal was observed for the cells without NIR light irradiation (Fig. S13), implying MnO_2 could generate ROS inside cells with 808 nm light illumination. By the way, the green fluorescence becomes stronger with the longer time of light irradiation; therefore, an on-demand treatment may be achieved by adjusting the irradiation time.

3.4 In vitro phototherapy studies

To verify the biomedical application potential of obtained MnO_2 nanoflower, the photothermal and ROS-mediated cancer cell killing ability was quantitatively evaluated according to the standard MTT assay. First, a NIR light irradiation of up to 15 min does not cause the cells death (Fig. S14), demonstrating that the laser intensity employed in experiments is safe for the cells. Further, the MnO_2 nanoflowers do not cause significant cell death even when the concentration was up to $50 \mu\text{g mL}^{-1}$ (Fig. 4a), indicating its biosafety for in vitro applications. Excitingly, when treated with both MnO_2 and NIR light irradiation, the cell survival rate is significantly reduced (Fig. 4b), confirming the photothermal and ROS-mediated cancer cell killing ability of MnO_2 nanoflowers. Moreover, the larger the dose of MnO_2 nanoflowers, the more the efficiency of the cell killing in vitro. Figure 4b exhibits more than 60% of HeLa cells incubated with $50 \mu\text{g mL}^{-1}$ of MnO_2 are killed upon 808 nm laser irradiation. To intuitively display the antitumor performance of MnO_2 , the HeLa cells after various treatments were stained by Calcein-AM/PI. As shown in Fig. S15, the green ones are living cells and the red ones represent dead cells. Obviously, the group for NIR only or MnO_2 only has negligible red fluorescence, indicating NIR light or MnO_2 poses no toxicity to cell. In contrast, the red fluorescence is found in the groups of cells treated with MnO_2 and NIR light, especially the red fluorescence in higher concentration groups remarkably

Fig. 4 Cell viability of HeLa cells after incubation with MnO_2 at various concentrations **a** without and **b** with NIR light irradiation

increased and almost no green fluorescence was observed for the highest concentration group. This treatment effect is almost consistent with the MTT results.

3.5 In vitro degradation studies

The biggest challenge of nanomaterials for biomedical applications is the non-biodegradability, which may result in long-term toxicity. Fortunately, it has been reported that MnO_2 nanoflowers can be degraded after exposure to GSH or H_2O_2 . Thus, we predict MnO_2 nanoflowers would serve as biodegradable PTT/PDT agent because the tumour tissue is characterized by over-expressed GSH and H_2O_2 . To confirm this hypothesis, the biodegradation behaviour of MnO_2 in simulated normal (without H_2O_2 and GSH) and tumour tissues (GSH 1 mM, H_2O_2 100 mM) conditions were investigated, respectively. The TEM images (Fig. S16) show that most MnO_2 nanoflowers were significantly collapsed after 24 h, specially the framework of MnO_2 was almost completely degraded after 70 h. The Mn^{2+} released from MnO_2 was also monitored by inductively coupled plasma mass spectrometry (ICP-MS) (Fig. S17). As expected, the GSH and H_2O_2 significantly induce MnO_2 degradation to release Mn^{2+} , while ignorable release of Mn^{2+} was observed in PBS (pH 7.4). In addition, the absorption at 380 nm of MnO_2 decreased greatly with the time increase, where it is almost disappeared after 70 h under mimic TME (Fig. S18a). In contrast, the absorption band exhibited negligible change in simulated normal tissue condition (Fig. S18b). These results suggested that the MnO_2 nanoflowers can be degraded in the tumour microenvironment, which may make the as-prepared flower-like MnO_2 have higher clinical prospects than traditional nanomaterials.

4 Conclusion

In summary, we reported a flower-like nanostructured MnO_2 with high photothermal conversion and ROS generation capability upon 808 nm NIR light irradiation for the first time. Benefiting from the performance, we show in this proof-of-concept demonstration that the MnO_2 can act as a light absorbing therapeutic agent of synergetic PTT/PDT for cancer therapy. Significantly, the MnO_2 degrades rapidly within the simulated TME by decomposing endogenous H_2O_2 into oxygen and reducing over expressed GSH in cancer cells, leading to the enhancement of PDT. Different from the reported MnO_2 nanomaterials that are employed for only relieving tumour hypoxia and imaging, the MnO_2 nanoflowers inherently act as a competent agent for cancer therapy. Our work highlighted a facilely prepared, one-for-all, biodegradable, and high-efficiency phototherapy platform with the capability of self-enhancing efficiency, which is promising

for possible biomedical applications and further clinical trials. The preliminary results may motivate extensive follow-on studies on the preparation of one-for-all multifunctional nanoplatfrom and provide reasonable design strategy to turn more metal-oxide semiconductor into excellent phototherapy agents.

Acknowledgements We are grateful to the Natural Science Foundation of Zhejiang Province (LY21H180001, LQ18A040001), the Natural Science Foundation of Ningbo (2019A610198), and K. C. Wong Magna Fund in Ningbo University (xkzwl1507).

Compliance with ethical standards

Conflict of interest There are no conflicts to declare.

References

1. Liu, Y. J., Bhattarai, P., Dai, Z. F., & Chen, X. Y. (2017). Photothermal therapy and photoacoustic imaging via nanotheranostics in fighting cancer. *Chemical Society Reviews*, 48, 2053–2108.
2. Yang, D., Yang, G. X., Yang, P. P., Lv, R. C., Gai, S. L., Li, X. C., et al. (2017). Assembly of Au plasmonic photothermal agent and iron oxide nanoparticles on ultrathin black phosphorus for targeted photothermal and photodynamic cancer therapy. *Advanced Functional Materials*, 27, 1700371.
3. Zhu, W. W., Liu, K., Sun, X. Q., Wang, X., Li, Y. G., Cheng, L., & Liu, Z. (2015). Mn^{2+} -doped prussian blue nanocubes for bimodal imaging and photothermal therapy with enhanced performance. *ACS Applied Materials and Interfaces*, 7, 11575–11582.
4. Xiang, H. J., Xue, F. F., Yi, T., Tham, H. P., Liu, J. G., & Zhao, Y. L. (2018). Cu_2S nanocrystals cross-linked with chlorin e6-functionalized polyethylenimine for synergistic photodynamic and photothermal therapy of cancer. *ACS Applied Materials and Interfaces*, 10, 16344–16351.
5. Yang, K., Feng, L. Z., Shi, X. Z., & Liu, Z. (2013). Nano-graphene in biomedicine: theranostic applications. *Chemical Society Reviews*, 42, 530–547.
6. Xu, J. T., Gulzar, A., Liu, Y. H., Bi, H. T., Gai, S. L., Liu, B., et al. (2017). Integration of IR-808 sensitized upconversion nanostructure and MoS_2 nanosheet for 808 nm NIR light triggered phototherapy and bioimaging. *Small (Weinheim an der Bergstrasse, Germany)*, 13, 1701841.
7. Sun, Z. B., Xie, H. H., Tang, S. Y., Yu, X. F., Guo, Z. N., Shao, J. D., et al. (2015). Ultrasmall black phosphorus quantum dots: synthesis and use as photothermal agents. *Angewandte Chemie International Edition*, 54, 11526–11530.
8. Dai, C., Chen, Y., Jing, X. X., Xiang, L. H., Yang, D. Y., Lin, H., et al. (2017). Two-dimensional tantalum carbide (MXenes) composite nanosheets for multiple imaging-guided photothermal tumor ablation. *ACS Nano*, 11, 12696–12712.
9. Lucky, S. S., Soo, K. C., & Zhang, Y. (2015). Nanoparticles in photodynamic therapy. *Chemical Reviews*, 115, 1990–2042.
10. Deng, K. R., Hou, Z. Y., Deng, X. R., Yang, P. P., Li, C. X., & Lin, J. (2015). Enhanced antitumor efficacy by 808 nm laser-induced synergistic photothermal and photodynamic therapy based on a indocyanine-green-attached $\text{W}_{18}\text{O}_{49}$ nanostructure. *Advanced Functional Materials*, 25, 7280–7290.

11. Jung, H. S., Han, J., Shi, H., Koo, S., Singh, H., Kim, H. J., et al. (2017). Overcoming the limits of hypoxia in photodynamic therapy: a carbonic anhydrase IX-targeted approach. *Journal of the American Chemical Society*, 139, 7595–7602.
12. Ju, E. G., Dong, K., Chen, Z. W., Liu, Z., Liu, C. Q., Huang, Y. Y., et al. (2016). Copper(II)-graphitic carbon nitride triggered synergy: improved ROS generation and reduced glutathione levels for enhanced photodynamic therapy. *Angewandte Chemie International Edition*, 55, 11467–11471.
13. He, D. G., Hai, L., He, X., Yang, X., & Li, H. W. (2017). Glutathione-activatable and O_2/Mn^{2+} -evolving nanocomposite for highly efficient and selective photodynamic and gene-silencing dual therapy. *Advanced Functional Materials*, 27, 1704089.
14. Phua, S. Z. F., Yang, G. B., Lim, W. Q., Verma, A., Chen, H. Z., Thanabalu, T., & Zhao, Y. L. (2019). Catalase-integrated hyaluronic acid as nanocarriers for enhanced photodynamic therapy in solid tumor. *ACS Nano*, 13, 4742–4751.
15. Zhang, W., Lu, J., Gao, X. N., Li, P., Zhang, W., Ma, Y., et al. (2018). Enhanced photodynamic therapy by reduced levels of intracellular glutathione obtained by employing a nano-MOF with Cu-II as the active center. *Angewandte Chemie International Edition*, 57, 4891–4896.
16. Shi, J. H., Wang, T. R., You, Y. Q., Akhtar, M. L., Liu, Z. J., Han, F., et al. (2019). Enhancement of ultralow-intensity NIR light-triggered photodynamic therapy based on exo-and endogenous synergistic effects through combined glutathione-depletion chemotherapy. *Nanoscale*, 11, 13078–13088.
17. Fan, W. P., Bu, W. B., Shen, B., He, Q. J., Cui, Z. W., Liu, Y. Y., et al. (2015). Intelligent MnO_2 nanosheets anchored with upconversion nanoprobe for concurrent pH-/H₂O₂-responsive UCL imaging and oxygen-elevated synergetic therapy. *Advanced Materials*, 27, 4155–4161.
18. Wang, L., Guan, S. Y., Weng, Y. Z. W., Xu, S. M., Lu, H., Meng, X. M., & Zhou, S. Y. (2019). Highly efficient vacancy-driven photothermal therapy mediated by ultrathin MnO_2 nanosheets. *ACS Applied Materials and Interfaces*, 11, 6267–6275.
19. Ding, D. D., Guo, W., Guo, C. S., Sun, J. Z., Zheng, N. N., Wang, F., et al. (2017). MoO_{3-x} quantum dots for photoacoustic imaging guided photothermal/photodynamic cancer treatment. *Nanoscale*, 9, 2020–2029.