Tumor Microenvironment-Triggered Supramolecular System as an In Situ Nanotheranostic Generator for Cancer Phototherapy

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The efficacy of photosensitizers in cancer phototherapy is often limited by photobleaching, low tumor selectivity, and tumor hypoxia. Assembling photosensitizers into nanostructures can improve photodynamic therapy efficacy and the safety profile of photosensitizers. Herein by employing supramolecular assembly, enhanced theranostic capability of Mn²⁺-assisted assembly of a photosensitizer (sinoporphyrin sodium, DVDMS) is demonstrated. A tumor environment-triggered coassembly strategy is further developed to form Mn/DVDMS nanotheranostics (nanoDVD) for cancer phototherapy. MnO₂ nanosheets serve as a highly effective DVDMS carrier and in situ oxygen and nanoDVD generator. In MCF-7 cells and xenograft tumors, MnO₂/DVDMS is reduced by glutathione (GSH) and H₂O₂ and reassembled into nanoDVD, which can be monitored by activated magnetic resonance/fluorescence/photoacoustic signals. Intriguingly, the decrease of GSH, the production of O₂, and the formation of nanoDVD are shown to be synergistic with phototherapy to improve antitumor efficacy in vitro and in vivo, offering a new avenue for cancer theranostics.
significantly different nanostructures (Figure S1, Supporting Information). Interestingly, given an increase in the [Mn$^{2+}$]/[DVDMS] ratio, DVDMS could be organized to form irregular fragments, nanospheres, and space grids. These observations imply that appropriate intermolecular interactions between Mn$^{2+}$ and DVDMS molecules are necessary to form well-defined nanoDVD, which might be due to the competition of the interactions of the Mn$^{2+}$ and porphyrin ring/carboxylate radicals from DVDMS, and DVDMS π–π stacking.\[7b,9\] As expected, dynamic light scattering (DLS) data illustrated an increase in the average hydrodynamic size of nanoDVD with an increase in the [Mn$^{2+}$]/[DVDMS] ratio (Figure S1H, Supporting Information), but the electronegativity of the nanoDVD was reduced as the amount of Mn$^{2+}$ increased (Figure S1I, Supporting Information). Meanwhile, X-ray photoelectron spectroscopy (XPS) data further confirmed the nanoDVD supramolecular assemblies (Figure S2, Supporting Information).

We subsequently investigated the potential of nanoDVD to serve as probes for in vivo imaging. T$\text{\textsubscript{1}}$-weighted magnetic resonance (MR) images of Mn$^{2+}$ and nanoDVD demonstrated concentration-dependent contrast enhancement under a 7T MR scanner (Figure S3A,B, Supporting Information), which is expected since Mn$^{2+}$ with its five unpaired 3d electrons is an effective contrast agent in MR imaging. In addition, the fluorescence of DVDMS (Ex 630 nm) with the addition of Mn$^{2+}$ was quenched (Figure S3C,D, Supporting Information) as a result of self-quenching of the nanoDVD nanostructure.\[10\] Therefore, the Mn$^{2+}$-assisted self-assembly of DVDMS in phototherapy treatment could be monitored via fluorescence imaging.

Prior to studying the phototherapy property of nanoDVD, we investigated the UV–vis absorption of a DVDMS solution mixed with Mn$^{2+}$ with its five unpaired 3d electrons is an effective contrast agent in MR imaging. As expected, the Mn$^{2+}$-assisted self-assembly of DVDMS in phototherapy treatment could be monitored via fluorescence imaging.
To demonstrate the photochemical properties of nanoDVD, we compared the photoactivity of DVDMS mixed with different amounts of Mn²⁺ under light irradiation at 630 nm by detecting the fluorescence of 2',7'-dichlorodihydrofluorescein (DCFH).[11] We found that Mn²⁺ obviously enhanced the PDT property of DVDMS (Figure 1B), regardless of fluorescence self-quenching. These observations indicate that, in the nanoDVD nanostructure, Mn²⁺ may enhance intersystem crossing and increase the triplet state of DVDMS, followed by efficient singlet state O₂ (¹O₂) production when encountering triplet state O₂.[12] We also examined the photothermal therapy (PTT) effect by measuring the temperature increase of various concentrations of DVDMS under laser radiation.NanoDVD exhibited a larger temperature increase than DVDMS (Figure 1C). The improved PTT effect of nanoDVD may be due to a high density of porphyrin, similar to what was reported by Zheng co-workers.[10,13] Overall, our results suggest that Mn²⁺ could significantly enhance the PDT and PTT properties of DVDMS and that nanoDVD nanoassemblies have the potential for imaging-guided PDT/PTT combination therapy.

In order to further study Mn²⁺-assisted self-assembly in cells, we sequentially incubated DVDMS and Mn²⁺ with MCF-7 cells (Figure 1A(c)). The fluorescence microscopy images of MCF-7 cells after incubation with DVDMS showed strong red fluorescence, which was quenched when Mn²⁺ was added (Figure 1D). Mn²⁺-induced fluorescence quenching of DVDMS was further confirmed by flow cytometry (Figure 1E). Cellular transmission electron microscope (TEM) data revealed large numbers of nanoassemblies in the cytosol (Figure 1F). The results indicate that Mn²⁺ could interact with DVDMS in cells. 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay also demonstrated that Mn²⁺ could obviously enhance the NIR therapy properties of DVDMS (Figure 1G).

Notably, tumor hypoxia and oxygen consumption during PDT often hamper the efficacy of photodynamic therapy.[14] Based on the unique properties of nanoDVD, we further explored the use of tumor stimuli-responsive drug carriers that have the potential to improve cancer phototherapy by providing a rational material design approach necessary for in situ generation of both nanoDVD and O₂. We reasoned that using manganese dioxide (MnO₂) nanosheets[15] would offer such a “smart” DVDMS delivery system: (1) excellent efficiency for DVDMS loading, (2) targeted release of Mn²⁺ ions in the tumor microenvironment, (3) sustainable production of nanoDVD and O₂ within the tumor tissue, (4) activatable MR/fluorescence/photoacoustic (PA) imaging, (5) enhanced photochemical properties, and (6) biocompatibility for clinical translation.

As a proof-of-concept experiment, we first assembled DVDMS molecules on the surface of MnO₂ nanosheets with a high loading efficiency (~95%, MnO₂: DVDMS (wt: wt) = 1:1); their strong physisorption resulted from the large specific surface area and the Mn-N coordinate bonding (Figure 2A).

**Figure 2.** A) Schematic illustration showing the fabrication process of MnO₂/DVDMS and reaction in GSH solution or H₂O₂ in PBS (pH 5.5). TEM of B) MnO₂ nanosheet, C) MnO₂/DVDMS + GSH, D) and MnO₂/DVDMS + H₂O₂/H⁺. E) DLS of the MnO₂ nanosheet (a), MnO₂/DVDMS (b), MnO₂/DVDMS + GSH (c), and MnO₂/DVDMS + H₂O₂/H⁺ (d). F) Zeta potential of the MnO₂ nanosheet (a), MnO₂/DVDMS (b), MnO₂/DVDMS + GSH (c), and MnO₂/DVDMS + H₂O₂/H⁺ (d). G) O₂ production of MnO₂ (a) and MnO₂/DVDMS (b) in H₂O₂. H) MR imaging study of the MnO₂/DVDMS: 1/T₁ versus Mn concentration for the MnO₂/DVDMS solution (black line), the MnO₂/DVDMS + GSH solution (red line), and MnO₂/DVDMS + H₂O₂/H⁺ (blue line). I) 1/T₁-weighted MR imaging phantom images obtained from MnO₂/DVDMS, MnO₂/DVDMS + GSH, and MnO₂/DVDMS + H₂O₂/H⁺.
The prepared MnO₂ nanosheets exhibited a typical 2D sheet-like morphology (Figure 2B) with a thickness of roughly 0.6 nm (Figure S5A, Supporting Information). After loading DVDMS, the MnO₂/DVDMS size changed to 91.2 nm, smaller than that of pure MnO₂ (128.4 nm). This change could be attributed to the additional ultrasonic treatment that was applied after MnO₂ was modified with DVDMS. In addition, both UV–vis absorption and FT-IR studies implied that the nanosheet surface was coated with DVDMS (Figure S5B,C, Supporting Information).

Interestingly, due to the surface modification with DVDMS, MnO₂/DVDMS exhibited better stability than MnO₂ in both phosphate buffer saline (PBS) and complex biological environments (i.e., dulbecco's modified eagle medium (DMEM) and fetal bovine serum (FBS)) (Figure S6A, Supporting Information).

As expected, both MnO₂ and MnO₂/DVDMS could be reduced to Mn²⁺ ions by intracellular glutathione (GSH). Alternatively, they could be reduced into Mn³⁺ with the production of O₂ via intracellular H₂O₂ (Figure 2A and Figure S6B,C (Supporting Information)). TEM data revealed that in GSH or H₂O₂ the typical 2D sheet-like nanostructure of MnO₂/DVDMS (Figure 2B) changed into nanoDVD-like irregular nanoparticles (PBS, pH 5.5) (Figure 2C,D and the Supporting Information) with an average hydrodynamic size of 197.2 nm (GSH) or 122.4 nm (H₂O₂) (Figure 2E). Similarly, the zeta potential of MnO₂/DVDMS + GSH or MnO₂/DVDMS + H₂O₂/H⁺ was higher than that of MnO₂ or MnO₂/DVDMS (Figure 2F). Combined with the XPS study of MnO₂/DVDMS + GSH and MnO₂/DVDMS + H₂O₂/H⁺ (Figure S7, Supporting Information), we could conclude that after the reduction of MnO₂/DVDMS in the GSH solution or H₂O₂/H⁺ solution, the released Mn²⁺ connected with the carboxyl and porphyrin ring of DVDMS reassembled into nanoDVD, leading to enhanced photochemical effects (Figure S8A,B, Supporting Information).

Furthermore, the fluorescence intensity of the DVDMS was quenched to only 13.8% that of pure DVDMS after loading onto the surface of the MnO₂ nanosheet. On the contrary, when GSH or H₂O₂/H⁺ was added, the fluorescence was partially recovered (Figure S8C,D, Supporting Information).

Because GSH and H₂O₂/H⁺ could reduce MnO₂ into Mn²⁺, MnO₂/DVDMS + GSH, and MnO₂/DVDMS + H₂O₂/H⁺ in T₁-weighted MR imaging exhibited much stronger enhancement than MnO₂/DVDMS (Figure S2G–J, Supporting Information). The r₁ value of MnO₂/DVDMS was enhanced by 34.7-fold after being reduced by GSH, and by 32.5-fold in the presence of H₂O₂/H⁺, suggesting the potential of MR imaging guided drug release in the tumor area. Both the MnO₂ nanosheet and MnO₂/DVDMS possessed concentration-dependent PA properties (Figure S9A,B, Supporting Information). However, DVDMS and nanoDVD exhibited weaker PA signals than the MnO₂ nanosheet (Figure S9C,D, Supporting Information). Taken together, such activated multimodal imaging can be readily integrated into the in situ self-assembly approach for cancer theranostic application.

Based on the promising photochemical results described above, we further explored the bioreactions that the MnO₂/DVDMS nanoparticles carried out in the tumor cells (Figure 3A). Both MnO₂/DVDMS and MnO₂ were effectively internalized by MCF-7 cells (Figure 3B), and the cellular GSH

![Figure 3](https://www.advancedsciencenews.com/doi/10.1002/adma.201705928.suppinfo)
and H₂O₂ levels were reduced with an increasing amount of MnO₂ or MnO₂/DVDMS (Figure 3B). From the thin-section cell TEM images (Figure S10, Supporting Information), we found differences in the cells incubated with MnO₂ or DVDMS. The cells incubated with MnO₂/DVDMS had irregular nanoparticles that could be easily distinguished from the cells themselves. In addition, the energy dispersive spectrometer (EDS) element line scanning of Mn confirmed that the nanostructure in the cells incubated with MnO₂/DVDMS was Mn based. Similar findings were also observed for nanoDVD nanoassemblies, with nanoggregates distributed in the cell cytosol (Figure 3C).

We additionally evaluated the biocompatibility and PDT/PTT effects of MnO₂/DVDMS on MCF-7 cells using the standard MTT assay and calcein acetoxymethyl ester/propidium iodide (calcein-AM/PI) experiment. As shown in Figure S10B (Supporting Information), after incubation with MCF-7 cells for 12 h, MnO₂/DVDMS did not exhibit noticeable cytotoxicity. When the cells were treated with MnO₂, DVDMS, and MnO₂/DVDMS plus 630 nm laser irradiation, DVDMS and MnO₂/DVDMS demonstrated significant cytotoxicity (Figure 3D,E and Figure S10C (Supporting Information)). MnO₂/DVDMS exhibited more effective PDT/PTT killing of MCF-7 cells than DVDMS, presumably due to several advantages of the in situ self-assembly strategy: (i) MnO₂/DVDMS could react with GSH in the MCF-7 cells, therefore the consumption of ROS by endogenous GSH was reduced;[16] (ii) MnO₂/DVDMS could react with H₂O₂ in the MCF-7 cells and downregulate the expression of hypoxia-inducible factor 1α (HIF-1α) (Figure S10D, Supporting Information), ameliorating hypoxia and providing more O₂ for PDT;[17] (iii) after the release of Mn²⁺ and DVDMS from.
the reduction of MnO$_2$/DVDMS, nanoDVD was formed and both the efficacies of PDT and PTT were enhanced.

The ability to monitor anticancer treatments in real time can yield invaluable predictive information regarding drug delivery and therapeutic efficacy. Notably, the integration of fluorescence imaging, MR imaging, and PA into our MnO$_2$/DVDMS system could offer high optical sensitivity and good spatial resolution for in vivo monitoring of various biochemical processes. As expected, after the injection of DVDMS, MnO$_2$, and MnO$_2$/DVDMS, the PA images and region of interest (ROI) analysis demonstrated that MnO$_2$ in the MnO$_2$/DVDMS reduced over time and that most of the MnO$_2$ was gone within 24 h (Figure 4A,B and Figure S11A (Supporting Information)). On the contrary, the fluorescence signal increased quickly after MnO$_2$/DVDMS injection (Figure 4C,D). In addition, an obvious $T_1$ contrast enhancement could be observed in the tumor region after the injection of MnO$_2$/DVDMS (Figure 4E,F), further evidence for the stimuli-responsiveness of MnO$_2$/DVDMS with the released Mn$^{2+}$. Consistent with the in vivo imaging results, TEM images of tumor slices confirmed that a large number of nanoDVD nanoassemblies were produced in the tumor area injected intratumorally (i.t.) with MnO$_2$/DVDMS, similar to that injected with DVDMS and Mn$^{2+}$ (Figure S11B, Supporting Information), but not with DVDMS or MnO$_2$ (Figure 4G).

Encouraged by the imaging data that revealed the well-controlled tumor environment responsiveness of MnO$_2$/DVDMS, we continued in vivo phototherapy by injecting MnO$_2$/DVDMS into different groups of MCF-7 tumor-bearing mice, and in vivo tumor inhibition effect was evaluated upon 630 nm laser illumination. The temperature change during irradiation was monitored by an IR thermal camera (Figure 4H). The tumor temperature of mice with MnO$_2$/DVDMS injections (DVDMS: i.v. 4.73 mg kg$^{-1}$; i.t. 1.18 mg kg$^{-1}$) showed rapid increase and maintained at 45 °C (i.v.) or 50 °C (i.t.) during laser irradiation. In contrast, the tumor temperature showed little change for mice injected with MnO$_2$ or DVDMS under irradiation with the same parameters (Figure 4H,I). DVDMS (i.t.) with laser irradiation was associated with an initial delay of tumor growth, but the tumor succumbed later. However, treatment with MnO$_2$/DVDMS (i.t.) and DVDMS (i.t.) combined with Mn$^{2+}$ (i.t.) reduced tumor growth significantly, and the group treated with the MnO$_2$/DVDMS (i.t.) experienced complete tumor regression within 14 d (Figure 4J). More importantly, tumors receiving MnO$_2$/DVDMS (i.t.) exhibited a more significant reduction in growth compared with tumors receiving DVDMS (i.t.) combined with Mn$^{2+}$ (i.t.). In addition, the tumors treated with MnO$_2$/DVDMS (i.v.) showed greater therapeutic effect than those treated with an i.v. injection of DVDMS. These results clearly indicate that multifunctional MnO$_2$/DVDMS considerably improves tumor therapeutic efficacy.

Besides the promising phototherapy effect in vivo, there were no changes in the relative body weights of any of the mice that we studied (Figure S12A, Supporting Information), indicating the low acute toxicity during the treatment. In addition, the biosafety assessment of the MnO$_2$/DVDMS including the alanine aminotransferase, aspartate transaminase, and hematoxylin and eosin stained organ slices showed no obvious in vivo toxicity of MnO$_2$/DVDMS (Figure S12B,C, Supporting Information).

In conclusion, we have reported the design and synthesis of a novel tumor environment-triggered supramolecular assembly of nanostructure. This supramolecular engineering nanoplatform integrates a variety of functions that include imaging (fluorescent imaging, MR imaging, and PA imaging) as well as synergistic combination of phototherapies (PTT and PDT). The MnO$_2$ nanosheet served as a highly effective carrier for photosensitizer DVDMS and as an in situ oxygen and nanoDVD generator. In the tumor environment, MnO$_2$/DVDMS could be reduced by GSH and H$_2$O$_2$, leading to the release of Mn$^{2+}$, DVDMS, and O$_2$. The nanoDVD assembled within the tumor cells/tissues features enhanced theranostic capability, as demonstrated by the Mn$^{2+}$-assisted nanoassembly process of DVDMS. We found that the drug delivery and treatment effect could be monitored by activated fluorescence/MR/PA imaging. Furthermore, the consumption of GSH, the production of O$_2$, and the formation of nanoDVD demonstrated overall improved phototherapy efficacy in vitro and in vivo. We believe that this report not only represents a simple approach to construct stimuli-responsive supramolecular nanomaterials but also provides a unique theranostic nanoplatform for cancer phototherapy through cancer-specific delivery and image-guided evaluation of therapy.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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