1. Introduction

Until now, treatment of cancer is still a great challenge for human beings. The conventional cancer treatment options such as chemotherapy, radiotherapy, and surgery are often associated with systemic side effects, high recurrence rate, and low therapy efficiency. Photodynamic therapy (PDT) is an alternative tumor treatment with minimal invasiveness, low cumulative toxicity, and reduced long-term mortality. Usually, it employs cytotoxic reactive oxygen species (ROS) such as singlet oxygen that is produced by an excited photosensitizer (PS) under local illumination for the ablation of tumor cells. However, the hydrophobicity of PS and the lack of tumor selectivity severely hamper the application of PDT. Conjugation of nanoparticles with PS has substantially increased the solubility of PS in aqueous media and tumor accumulation, taking advantage of the enhanced permeability and retention (EPR) effect of nanoparticles. However, the premature leakage of PS from nanocarriers significantly reduces the accumulation of PS within a tumor, thereby enhancing nonspecific accumulation in normal tissues, which inevitably leads to a limited efficacy for photodynamic therapy (PDT) and the enhanced systematic phototoxicity. Moreover, local hypoxia of the tumor tissue also seriously hinders the PDT. To overcome these limitations, an acidic H$_2$O$_2$-responsive and O$_2$-evolving core–shell PDT nanoplatform is developed by using MnO$_2$ shell as a switchable shield to prevent the premature release of loaded PS in core and elevate the O$_2$ concentration within tumor tissue. The inner core SiO$_2$-methylene blue obtained by co-condensation has a high PS payload and the outer MnO$_2$ shell shields PS from leaking into blood after intravenous injection until reaching tumor tissue. Moreover, the shell MnO$_2$ simultaneously endows the theranostic nanocomposite with redox activity toward H$_2$O$_2$ in the acidic microenvironment of tumor tissue to generate O$_2$ and thus overcomes the hypoxia of cancer cells. More importantly, the Mn(II) ion reduced from Mn(IV) is capable of in vivo magnetic resonance imaging selectively in response to overexpressed acidic H$_2$O$_2$. The facile incorporation of the switchable MnO$_2$ shell into one multifunctional diagnostic and therapeutic nanoplatform has great potential for future clinical application.

MnO$_2$ Gatekeeper: An Intelligent and O$_2$-Evolving Shell for Preventing Premature Release of High Cargo Payload Core, Overcoming Tumor Hypoxia, and Acidic H$_2$O$_2$-Sensitive MRI

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drawback, several nanoplatforms have been developed to modify the hypoxic tumor microenvironment through elevating the oxygen concentration within tumors,[8] such as red blood cells-enhanced PDT that uses erythrocytes to transport oxygen into the tumor and in situ O2-evolving PDT in response to H2O2 that is excessively produced in malignant cancerous cells compared to normal cells.[6b,9] Due to the high O2-generation efficiency of MnO2 nanostructures in acidic and H2O2-rich environment in solid tumors, MnO2-based nanostructures have attracted considerable interests in PDT as PS carrier including MnO2 sheet loaded with upconversion nanocomposites and poly(allylamine hydrochloride)-stabilized MnO2 covalently conjugated with the photosensitizer (chlorine e6). Despite those inspiring results, there are still great demands to explore new MnO2-based nanostructures to extend their application in enhancing the in vivo PDT performance. Owing to the pH/redox-responsive properties, MnO2 nanostructures can be simultaneously reduced to Mn2+ by acidic H2O2 in the production of oxygen, resulting in decomposition of nanostructures. Therefore, the exploration of MnO2-based nanostructures as intelligent “gatekeeper” in response to acidic H2O2 in solid tumors may be highly attractive to the next generation of multifunctional stimuli-responsive nanoagent for efficient cancer diagnostic and therapy.

Herein, we have designed and fabricated an intelligent core–shell theranostic platform based on SiO2 and MnO2 for avoiding leakage of the payload, stimuli-responsive, H2O2-activatable and O2-evolving PDT, and simultaneous in vivo acidic H2O2-sensitive magnetic resonance imaging (MRI). As shown in Scheme 1, the SiO2-methylene blue (MB) core was produced by co-condensation of tetraethoxysilane (TEOS) and MB in the presence of NH3·H2O to obtain a high MB loading amount. To prevent the escape of MB in a physiological condition that is induced by the concentration gradient between the complex and the solvent, a MnO2 shell was coated onto the SiO2-MB core through in situ reduction of KMnO4 by hydroxylated polyethylene glycol (PEG) at room temperature (SiO2-MB@MnO2). Coating of MnO2 effectively avoids the premature release of MB molecules due to the stability of MnO2 at neutral pH, reducing the system distribution of MB and thus enhancing the accumulation of SiO2-MB@MnO2 within tumor tissue via EPR.

Scheme 1. Schematic representation for synthetic process and therapeutic mechanism of SiO2-MB@MnO2.
effect. After endocytosis by tumor cells, the MnO₂ shell will be reduced to Mn²⁺ in respond to the overexpressed H₂O₂ at an acidic pH, triggering the generation of O₂ and the release of MB in the acidic microenvironment of tumor cells. The O₂ evolving can overcome the hypoxia of cancer cells and then promotes the kinetics of ¹O₂ generation, enhancing the PDT efficacy. Meanwhile, the released Mn²⁺ ions have excellent T₁-MRI performances for tumor imaging and detection. Our work presents a facile design to incorporate different functionalities into single theranostic nanoplatform based on core/shell SiO₂/MnO₂ nano-system, which is promising for future biomedical applications.

2. Results and Discussion

2.1. Synthesis and Characterization of SiO₂-MB@MnO₂

The core of SiO₂-MB was synthesized by condensation of TEOS in the presence of MB molecules and a base catalyst (SiO₂-MB). The MnO₂ layer was introduced as a “gatekeeper” for pH/redox-triggered controlled release of MB via the reduction of KMnO₄ with PEG, resulting in a carboxyl-functionalized MnO₂-coated SiO₂-MB nanocomposites (SiO₂-MB@MnO₂). Transmission electron microscopy (TEM) images indicate that the as-prepared SiO₂-MB has a spherical structure with smooth surface and an average diameter of 220 nm (Figure 1A). However, the introduction of MnO₂ results in a rough surface (Figure 1B) and the color of particles suspension is changed from blue to dark cyan (Figure S1, Supporting Information). In addition, there were two typical binding-energy peaks at 654 and 642 eV in the X-ray photoelectron spectroscopy of SiO₂-MB@MnO₂, which corresponds to Mn(IV)2p₁/₂ and Mn(IV)2p₃/₂, further confirming the generation of MnO₂ (Figure S2, Supporting Information). Furthermore, we can observe the MnO₂ shell clearly from the enlarged TEM images of SiO₂-MB@MnO₂ (Figure 1C,D). Meanwhile, the high resolution TEM (HRTEM) image (Figure 1E) taken from SiO₂-MB@MnO₂ shows well-resolved lattice fringes with inter-planar distance of 0.24 nm indexed to the (100) plane of MnO₂ sheet along the [001] direction. The thickness of the outer MnO₂ shell is ~5 nm. The diffraction ring in the selected area electron diffraction (SAED) pattern (Figure 1F) recorded from SiO₂-MB@MnO₂ can be also identified as the (100) plane of the layered MnO₂. The corresponding energy-dispersive X-ray spectroscopy (EDX) elemental mapping images (Figure 1G–K) of carbon, oxygen, silica, sulfur, and manganese further reveal that the loaded MB and the coated MnO₂ shell are homogeneously distributed in the whole SiO₂-MB@MnO₂.

The emerging of the characteristic peaks at 383 and 610 nm in the UV–vis spectrum of the SiO₂-MB@MnO₂ matches well with those of MnO₂ and MB dimer, respectively, further

![Figure 1. Typical TEM images of A) SiO₂-MB and B) SiO₂-MB@MnO₂. The enlarged TEM images from C–E) HRTEM, F) the corresponding SAED patterns, and G–K) EDX elemental mapping of C, O, Si, S, and Mn of SiO₂-MB@MnO₂.](image-url)
verifying the successful loading of MB and the introduction of the MnO₂ layer (Figure S3A, Supporting Information). The SiO₂-MB@MnO₂ nanoparticles are stable and dispersed well in water with an average dynamic light scatter (DLS) diameter of 300 nm and negative charge (zeta potential, −38 mV) (Figure S3B,C, Supporting Information). The negative charge is probably resulted from the −COOH from oxidation of −OH by KMnO₄ (Figure S4, Supporting Information). The percentage of MB in SiO₂-MB@MnO₂ was determined by thermal gravimetric analysis (TGA), which indicates a decrease of 24% in the weight at 250–600 °C due to the evaporation of the MB molecules (Figure S5, Supporting Information).

2.2. Acidic H₂O₂-Enhanced Generation of O₂ and ¹O₂ by SiO₂-MB@MnO₂

In acidic H₂O₂, MnO₂ is reduced into Mn(II) ions upon the oxidation of H₂O₂ into O₂: 2H₂O₂ + 2H⁺ +MnO₂ = 4H₂O + O₂ +Mn²⁺.[60] We first quantified the generation of O₂ upon response of SiO₂-MB@MnO₂ to acidic H₂O₂ using an O₂ probe [Ru(dpp)₃]Cl₂ (RDPP), whose fluorescence can be quenched by O₂. After addition of H₂O₂ (100 × 10⁻⁶ m) to SiO₂-MB@MnO₂ suspension (in phosphate-buffered saline (PBS) of pH 5.5), the fluorescence intensity of RDPP is quenched immediately within 4 min and constantly kept at a lower level in the following 8 min (Figure 2A), indicating the elevation of O₂ concentration in the solution. For the groups of SiO₂-MB with H₂O₂ and SiO₂-MB@MnO₂ without H₂O₂, there is no decrease in the fluorescence intensity, further confirming that the generation of O₂ is resulted from the oxidation of H₂O₂ by MnO₂ shell. Furthermore, H₂O₂ enhanced generation of ¹O₂ was also assessed with 1,3-diphenylisobenzofuran (DPBF), a chemical trapping reagent of ¹O₂ that can be oxidized by ¹O₂ irreversibly, resulting in the decrease of its characteristic peak at 410 nm. As shown in Figure 2B, the absorbance of DPBF in SiO₂-MB@MnO₂ suspension gradually decreases to 87% within the incubation time under irradiation of a 650 nm laser at 100 mW cm⁻². Further addition of 20 × 10⁻⁶, 40 × 10⁻⁶, or 80 × 10⁻⁶ m H₂O₂ results in a more obvious decrease of the absorbance of DPBF. These
results validate that the SiO2-MB@MnO2 can much enhance the generation of 1O2 in response to the addition of H2O2. It is acknowledged that the oxidation of H2O2 by MnO2 is a H+-consumed process in an acidic microenvironment, which can increase the pH of the local environment (Figure S6, Supporting Information). Therefore, three typical pH micro-environments were established to imitate the normal tissues (pH = 7.4) and tumor acidic conditions (pH = 6.0 and 5.0) to investigate the pH dependence of the 1O2 generation kinetic. As shown in Figure 2C, the absorbance of DPBF in neutral buffer solution is only decreased to 80% in 10 min. Comparatively, the decreasing is significantly accelerated in mild acidic buffer solutions to 73% (pH = 6.0) and 64% (pH = 5.0) during 10 min. It indicates that the 1O2 generation from SiO2-MB@MnO2 is an ultrasensitive pH-responsive behavior, which is very favorable in cancer treatment with PDT because the 1O2 generation under illumination is rather low when it is circulating within the neutral blood vessels and normal tissues, which can significantly mitigate the side effects of PDT based on SiO2-MB@MnO2. But when SiO2-MB@MnO2 accumulates within tumor tissues, the acidic environment of tumors substantially elevates the 1O2 generation and correspondingly enhances the efficacy of PDT. Then the O2 and 1O2 production within HeLa cells were examined by confocal laser fluorescence scanning microscope (CLSM) with O2 probe RDPP and ROS probe 2,7′-dichlorodihydrofluorescein diacetate (H2DCFDA), respectively. After preincubation with RDPP (5 × 10^{-6} M) for 4 h, the cells were further co-incubated with SiO2-MB@MnO2 for 4, 12, and 24 h and then the fluorescence of RDPP within cells was observed by CLSM. Obviously, the green fluorescence gradually diminishes with incubation time compared with that of control cells (Figure 2D), indicating the increase of intracellular O2 resulted from the cleavage of intracellular H2O2 by SiO2-MB@MnO2, which overcomes the hypoxia of cancer cells significantly. As shown in Figure 2E, upon laser irradiation, the stronger fluorescence intensity of H2DCFDA can be observed in the cells treated with SiO2-MB@MnO2 compared with the cells treated with MB and SiO2-MB. These results illustrate that the introduction of the MnO2 shell can trigger the generation of O2 from H2O2 oxidation within tumor cells, further enhancing the generation of 1O2 under irradiation, which could increase the efficacy of PDT in the treatment of cancer cells.

2.3. SiO2-MB@MnO2 Enhanced PDT Efficacy in Tumor Cells

The cytotoxicity of SiO2-MB@MnO2 under the dark and light irradiation condition was further evaluated in HeLa cells. According to standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Figure 3A), no obvious cytotoxicity is displayed by HeLa cells after being treated with SiO2-MB@MnO2 at the concentration of 0–50 µg mL^{-1} for 24 h under dark, providing a biocompatibility concentration for further biological applications. The PDT efficacy of SiO2-MB@MnO2 was evaluated by comparatively measuring cell viabilities after incubating HeLa cells with culture medium, MB, SiO2-MB, and SiO2-MB@MnO2 under irradiation (Figure 3B). Treatment of SiO2-MB@MnO2 results in 92% cell death, whereas MB or SiO2-MB treatment only results in 42% or 64% cell death, respectively, which indicates that the coating of MnO2 shell much enhances the photodynamic therapy effects. To intuitively display the therapeutic efficiency, live-dead cell staining was performed by co-staining cells with calcein acetoxyethyl ester (calcein AM, green fluorescence) and propidium iodide (PI, red fluorescence) and was observed with CLSM (Figure 3C). Under irradiation for 15 min, the cells treated with free MB or SiO2-MB show slight fluorescence change, indicating the negligible cell death compared with negative control and laser alone group. However, the cells treated with SiO2-MB@MnO2 show apparent fluorescence change with a dramatic decrease in green color and drastic increase in red color, indicating a significant destruction of the treated cells. These illustrate that the synergy between MnO2 shell and MB is essential for H2O2 enhanced phototoxicity of SiO2-MB@MnO2. Suggesting that SiO2-MB@MnO2 is an efficient photodynamic agent for hypoxic cancer cells under specific laser irradiation.

2.4. Tumor Microenvironment-Boosted MB Release and Stability of SiO2-MB@MnO2 in Physiological Condition

After reduced to Mn^{2+}, MnO2 shell gradually dissolves into the solvent and the SiO2-MB nanoparticles will be reexposed, leading to the selectively escape of MB in tumor. To observe the MB release from the SiO2-MB@MnO2 at tumor pH and over-expressed H2O2 microenvironment, aqueous buffer solutions of pH 7.4 and 5.5 with or without H2O2 were used to partially mimic the microenvironments of healthy tissue and tumor tissue, and the release profiles of MB in these solutions were observed, respectively. As shown in Figure 4A, the MB molecules within SiO2-MB were gradually released with the cumulative release amount of 82% at pH 7.4, meanwhile resulting in a cavity in the center of nanoparticles (Figure S7, Supporting Information), but the entrapped MB in the SiO2-MB@MnO2 exhibits extremely low leakage at pH 7.4 in the absence of H2O2. It has been reported that the concentration of H2O2 in human serum is less than 0.25 × 10^{-6} M,^{19} so we further investigated the MB release from SiO2-MB@MnO2 in PBS (pH 7.4) with 0.25 × 10^{-6} M H2O2. The entrapped MB displays a negligible release below 10% within 24 h. The results demonstrate the high stability of SiO2-MB@MnO2 in a physiological environment. In contrast, in the acidic H2O2 solution (pH 5.5), the SiO2-MB@MnO2 nanocomposites exhibit burst and nearly 71% of the entrapped MB is released during the initial 0.5 h. After that, the release of MB turns slowly, only achieving 87% release amount until 5 h, and then keeps constant. The corresponding morphology evolution of SiO2-MB@MnO2 in acidic H2O2 solution (pH 5.5) was characterized using TEM (Figure 4B). The obvious cavity appears in the center of SiO2-MB@MnO2 and the particle edge is blurry at 0.5 h, resulting from the release of the entrapped MB and the partial dissolution of the MnO2 shell (Figure 4B(a)). More interestingly, the outer MnO2 shell totally vanishes at 1 h, leaving the spherical shell of SiO2, but the center cavity continues to enlarge in the following 4 h with thinner and thinner shell (Figure 4B(b–d)). At 24 h, the shells almost completely collapse and cleavage into scattered fragments at 48 h (Figure 4B(e,f)). Two processes are probably involved in self-decomposing of the core–shell structure.
of SiO₂-MB@MnO₂ in the acidic H₂O₂ solution. The MnO₂ shell is first dissolved during its reduction to Mn²⁺ by H₂O₂ in acidic condition, and then MB molecules escape from the center of the SiO₂-MB nanoparticles by concentration gradient-driven diffusion, simultaneously triggering the decomposition of nanocarriers. Particularly, the spilled O₂ resulted from the oxidation of H₂O₂ can accelerate the mass transport in the solution, which further promotes the release of MB.[14] These results suggest the key role of the introduced MnO₂ shell in preventing premature release of MB from SiO₂-MB@MnO₂ in physiological environment, such as blood, and selective release of MB upon intelligent dissolution in response to acidic H₂O₂ in tumor tissue. In the microenvironment of the tumor, the MnO₂ shell still can prevent the release of MB from the inner SiO₂-MB core and thus efficiently increase the accumulation of MB molecules in cancer cells compared with its counterpart without the MnO₂ layer (Figure S8, Supporting Information).

The high stability of the nanoparticles in blood could prolong their residence time in the bloodstream, thereby enhancing the accumulation within the tumor tissues via EPR effect. To explore whether the switching effect of the MnO₂ shell still works in vivo, we measured the circulation time of MB within the SiO₂-MB and SiO₂-MB@MnO₂ in blood and their accumulation within the tumor tissues by quantifying the MB concentration in blood and tumor versus time after the both materials were injected into tumor bearing mice via the tail vein. As shown in Figure 4C, the time-dependent MB concentration in blood for both materials can be fitted well by a one-compartment model with nonlinear elimination. The blood circulation half-lives \( t_{1/2} \) for MB in SiO₂-MB@MnO₂ and SiO₂-MB are 6.2 ± 1.1 and 1.6 ± 0.9 h, respectively, indicating that the blood circulation time of MB can be significantly prolonged by coating the MnO₂ shell. In addition, the time-dependent MB distribution in tumor tissues reveals a higher accumulation of MB from SiO₂-MB@MnO₂ than that from SiO₂-MB during the whole experiment time (Figure 4D). The MnO₂ shell dramatically alters the in vivo behaviors of the SiO₂-MB@MnO₂. In blood, the stable MnO₂ shell finely protects the release of MB, whereas the SiO₂-MB without the protective layer is inclined to release MB molecules, which probably leads to the elimination.

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Figure 3. A) Viability of the HeLa cells treated with SiO₂-MB@MnO₂ at various concentrations (0, 5, 10, 20, 30, 40, and 50 µg mL⁻¹) under dark. Photodynamic destruction of HeLa cells in the presence of culture medium, laser only, free MB, SiO₂-MB, and SiO₂-MB@MnO₂ (50 µg mL⁻¹, equal to 12 µg mL⁻¹ MB) with laser irradiation evaluated by B) MTT assay and C) CLSM (viable cells were stained with calcein-AM, and dead cells were stained with PI). Scale bars: 50 µm.
of MB from mice body. These results indicate that the intact structure and the coated PEG layer significantly prolong blood circulation time of SiO$_2$-MB@MnO$_2$, and thus to enhance their accumulation within tumor tissue, which will be beneficial to the following PDT performance in vivo.

### 2.5. MRI Properties Measurements

Several nano contrast agents (CAs) that respond to tumor microenvironment, such as pH and redox potential, have been reported for tumor-specific MRI.[8b,15] During the reduction of MnO$_2$ in acidic H$_2$O$_2$, the released Mn(II) ions in the tumor microenvironment will highly improve the T$_1$-MRI performance for tumor imaging and detection. We then examined the relaxation properties of SiO$_2$-MB@MnO$_2$ to investigate its capacity serving as contrast agents for MRI using a 3.0 T clinical MRI scanner. The MR images of the aqueous solution of SiO$_2$-MB@MnO$_2$ in different PBS (pH 5.0, 6.0, and 7.4) containing H$_2$O$_2$ were displayed in Figure 5. Compared with that in neutral PBS (pH 7.4), SiO$_2$-MB@MnO$_2$ dispersed in acidic H$_2$O$_2$ (pH 6.0 and 5.0) for 2 h shows the concentration-dependent brightness and darkness effects (Figure 5A) and the longitudinal ($r_1$) relaxivity value is also increased from 0.006 $\times$ 10$^{-3}$ M$^{-1}$ s$^{-1}$ at pH 7.4 to 3.1 $\times$ 10$^{-3}$ M$^{-1}$ s$^{-1}$ ($r_1^{(10pH 7.4} \times 10^{-3}$ M$^{-1}$ s$^{-1}$ ($r_1^{(10pH 7.4})$), respectively, at pH 6.0 magnitude increase of the $r_1$ value after changing the pH value from 7.4 to 5.0.

Then the T$_1$-weighted MRI of the tumor was characterized after intravenous injection of SiO$_2$-MB@MnO$_2$ into the mice bearing U14 tumor at 0, 4, 12, and 24 h (Figure 5C). Prior to injection, there is no significant difference between the tumor tissue and normal tissue, while the signal intensity in the tumor area increases obviously after injection for 4 h with the relative signal intensity (RSI) of 1.9 (Figure S9, Supporting Information). The tumor area turns brighter after 12 h post-injection but the RSI was decreased to 1.6, which is caused possibly by the diffusion of Mn(II) ions to the normal tissues. The bright signal of tumor lasts for more than 24 h after the injection with a RSI of 2.4, although the distribution of element Mn in kidney and liver is higher at this time (Figure S10, Supporting Information), which provides an instructive therapy time and further suggests that the brightness of normal tissues could come from the diffused Mn(II) ions from tumor. The EPR effect might extend the residence time of Mn(II) ions in the tumor and thus result in a higher RSI value at 24 h. These results indicate that SiO$_2$-MB@MnO$_2$ can accumulate via EPR effect in the tumor area where the overexpressed acidic H$_2$O$_2$ will disintegrate the MnO$_2$ shell to release Mn(II) ions that make it more accessible to water molecules and shorten the relaxation time of water protons to improve the visibility.[15b,16] The ultrasensitivity to the tumor microenvironment endows SiO$_2$-MB@MnO$_2$ with greater selectivity to enhance the MRI contrast of solid tumors, suggesting the great potential for tumor diagnosis.
2.6. In Vivo PDT Tumor Inhibition Efficacy of SiO$_2$-MB@MnO$_2$

Further in vivo experiments were performed to investigate the inhibiting tumor effectiveness of SiO$_2$-MB@MnO$_2$ by PDT. The murine cervix cell line U14 was chosen and subcutaneously injected to the hind limb of each female Kunming mouse to establish xenograft tumors model. When the mean diameter of tumors reaches $\approx 100 \text{ mm}^3$, the mice were divided into five groups and intravenously injected with: (i) saline, (ii) free MB, (iii) SiO$_2$-MB, and (iv) SiO$_2$-MB@MnO$_2$. After 24 h, the tumor-bearing mice were irradiated at the tumor site for 15 min (650 nm, 100 mW cm$^{-2}$) under continuous anesthesia. Following the treatments, tumor volumes and body weights of the mice were recorded every other day for a period of 15 d and the relative tumor volumes ($V/V_0$) and body weights ($W/W_0$) were then plotted as a function of time. As shown in Figure 6A–E, the tumors intravenously injected with either saline or free MB grow rapidly with six and ninefold increase of the average tumor volumes compared to their original volumes, respectively. On the other hand, the growth of tumors is slightly slowed down by SiO$_2$-MB treatment, indicative of the limited anticancer efficacy. Pronounced tumor inhibition effect can be observed in the SiO$_2$-MB@MnO$_2$ treatment group compared with the intact membrane and plump tumor cell nucleus in the control group. Moreover, the nucleuses in both free MB and SiO$_2$-MB treatment groups are partially dwindled with an irregular morphologic change. These results indicate that the pH/redox-responsive and H$_2$O$_2$-triggered PDT based on SiO$_2$-MB@MnO$_2$ is remarkably effective for tumor inhibition but has no toxicity to the other organs (Figure S11, Supporting Information), and thus has great potential for in vivo applications.

In addition, hypoxia inducible factor (HIF)-1$\alpha$ staining assay was carried out to evaluate hypoxic conditions in the tumor after 15 d PDT treatment with or without SiO$_2$-MB@MnO$_2$. As shown in Figure 6G, the tumor tissues of the saline, MB, and SiO$_2$-MB treatment groups were extensively stained dark brown, indicating the accumulation of HIF-1$\alpha$ under hypoxic conditions. In contrast, for the SiO$_2$-MB@MnO$_2$ treatment group, the tumor tissues were largely stained blue, indicating the decreased expression of HIF-1$\alpha$, which suggests that the SiO$_2$-MB@MnO$_2$ can overcome the hypoxia within tumor tissues by oxidation of H$_2$O$_2$ at acidic conditions in vivo, breaking the major limitation of PDT.

3. Conclusions

In summary, we successfully developed a high MB-loading, low-leaking, and O$_2$-evolving PDT agent based on SiO$_2$-MB@MnO$_2$ core–shell nanostructure to achieve intelligent and H$_2$O$_2$-enhanced PDT and MRI for cancer treatment. Co-condensation of SiO$_2$ and MB leads to a high MB loading efficiency with a
radial concentration gradient, resulting in diffusion-driven release of MB. Introduction of the MnO\textsubscript{2} shell finely prevents the escape of MB from the SiO\textsubscript{2}-MB core in blood, and thus avoids the premature release of MB molecules and decomposition of the SiO\textsubscript{2}-MB, which leads to prolonged residence time of the SiO\textsubscript{2}-MB@MnO\textsubscript{2} in the bloodstream and the enhanced accumulation of MB within the tumor tissue. After entering into the tumor tissue by EPR effect, the SiO\textsubscript{2}-MB@MnO\textsubscript{2} will be reduced by overexpressed acidic H\textsubscript{2}O\textsubscript{2}, which generates O\textsubscript{2} and thus elevates the O\textsubscript{2} pressure, overcoming the hypoxia of the tumor tissue and further enhancing the therapeutic efficiency of PDT. In addition, the Mn(II) reduced from Mn(IV) by acidic H\textsubscript{2}O\textsubscript{2} is capable of MRI by shortening the relaxation time of water protons to improve the visibility. For the first time, we have demonstrated the use of MnO\textsubscript{2} as an H\textsubscript{2}O\textsubscript{2}-sensitive shield, O\textsubscript{2}-generation agent, and MRI contrast agent to simultaneously prevent the premature release of loading cargo and increase the O\textsubscript{2} concentration within tumor tissue for the in vivo imaging-guided PDT. This proof of concept might provide a novel route to develop the new tumor microenvironment-sensitive nanoplatform for imaging-guided cancer therapy.

4. Experimental Section

**Materials and Reagents:** KMnO\textsubscript{4}, H\textsubscript{2}O\textsubscript{2} (30%), ethanol, PEG (MW 600), and ammonia hydroxide were obtained from Beijing Chemical Reagents Company (Beijing, China). TEOS and MB were purchased from Aladdin Reagents Company (Shanghai, China). RDPP was provided by Alfa Aesar (MA, USA). DPBF, calcein acetoxymethyl ester (Calcein AM), and PI were purchased from Sigma-Aldrich (MO, USA). N-hydroxysuccinimide

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**Figure 6.** A) Representative photos of mice bearing U14 tumor treated with saline, free MB, SiO\textsubscript{2}-MB, and SiO\textsubscript{2}-MB@MnO\textsubscript{2} on the 15th day. B) Photos of excised tumors, C) the relative tumor volumes curves, D) weights of excised tumors, and E) body weight evolution curve. F) H&E staining of tumor sections harvested from mice on the 15th day. Scale bars are 50 µm. G) HIF-1α staining of tumor slides from U14 tumor bearing mice treated with saline, free MB, SiO\textsubscript{2}-MB, and SiO\textsubscript{2}-MB@MnO\textsubscript{2} on the 15th day. Scale bars are 100 µm.
and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride were purchased from J&K Scientific Co., Ltd. (Beijing, China). The deionized (DI) water was generated using a Millipore Milli-Q system (Billerica, MA). Calcium- and magnesium-free Dulbecco’s PBS and penicillin-streptomycin (100x) was obtained from Life Technologies Corporation (Grand Island, NY). Mitochondrial membrane potential assay kit, intracellular ROS assay kit, and Dulbecco’s Modified Eagle’s Medium were purchased from Thermo Scientific (Beijing, China). Standard fetal bovine serum was purchased from Tianjin Haoyang Biological Manufacturing Co., Ltd. (Tianjin, China). All chemicals were used without additional purification.

**Preparation of SiO₂-MB Nanocomposites**: The SiO₂-MB nanocomposite was prepared according to reported method with some modifications.[1][2] Typically, ammonia hydroxide (3 mL, 28%) was mixed with 75 mL of ethanol (45%). A flask with a mixture was stirred vigorously for 30 min. After that, MB (10 mg) was added to the mixture and the solution was continually stirred for 30 min. Then, TEOS (40 mL) dissolved in 1 mL of ethanol was dropped into the mixture. After stirring for 12 h, SiO₂-MB complex was collected by centrifugation at 10 000 × g for 10 min and was washed with ethanol and water for three times, respectively. TEM was performed on an H-600 electron microscope (Hitachi, Japan) operated at 75 Kev and UV–vis absorption spectra were measured on LAMBDA 25 spectrometer (Perkin-Elmer).

**Synthesis of SiO₂-MB@MnO₂ Core/Shell Nanocomposites**: In a typical synthesis, SiO₂-MB nanocomposite (2 mg) was first dispersed into 10 mL of water and the dispersion was sonicated for 2 min to ensure the SiO₂-MB particles were suspended well in the solution. Then, aqueous PEG (Mₙ: 600, 200 µL, 0.5 mg mL⁻¹) was added into the solution and mixed for 30 min at room temperature under stirring. Subsequently, aqueous KMnO₄ (200 µg mL⁻¹, 250 µL) was added slowly under vigorously stirring and then the mixture was stirred for 30 min at room temperature. The obtained product was centrifuged at 10 000 g for 10 min and was washed with ethanol and water for three times. The coating of MnO₂ layer was confirmed by TEM and UV–vis absorption spectra. The loading amount of MB molecules in SiO₂-MB@MnO₂ was determined by TGA. Zeta potential and hydrodynamic diameter were measured by DLS using Malvern Zetasizer Nano ZS90 (Malvern Instruments Ltd., UK).

**Extracellular O₂ Evolution in Response to H₂O₂**: The extracellular generation of O₂ was monitored with RDPF, an O₂ sensing probe whose fluorescence can be strongly quenched by O₂.[1][2][3][4] Typically, 50 µL of ethanol solution of RDPF (10 × 10⁻⁶ M) was added into 1 mL of SiO₂-MB and SiO₂-MB@MnO₂ (20 µg mL⁻¹) solution suspended in PBS solution (pH 5.5) and the mixture was transferred into a 10 mm cuvette. The fluorescence intensity of RDPF (λₑₓ: 455 nm) at 615 nm was recorded every 2 min after the H₂O₂ (40 × 10⁻⁶ M) was added. In *In Vitro Acidic H₂O₂-Enhanced O₂ Generation at Various pH*: For extracellular O₂ detection, a commercial chemical probe DPBF is used to confirm the O₂ generation by detection its absorption intensity at 500 nm via UV–vis spectrophotometer. First, the generation of O₂ in response to a (USA). Mitochondrial membrane potential assay kit, intracellular ROS assay kit, and Dulbecco’s Modified Eagle’s Medium were purchased from Thermo Scientific (Beijing, China). Standard fetal bovine serum was purchased from Tianjin Haoyang Biological Manufacturing Co., Ltd. (Tianjin, China). All chemicals were used without additional purification.

**Acidic H₂O₂-Boosted SiO₂-MB@MnO₂ Decomposition**: In vitro release profiles of MB from SiO₂-MB@MnO₂ were examined in PBS (pH 7.4 and 5.5). SiO₂-MB@MnO₂ (1 mg mL⁻¹) was dispersed in PBS (10 mL) containing H₂O₂ (0.25 × 10⁻⁶ or 80 × 10⁻⁶ M) and was incubated at 37 °C for 15 min. Then the cells were washed with PBS for three times and were irradiated with the 650 nm laser at 100 mW cm⁻² for 15 min. The cells without particles treatment served as controls. Fluorescence images in the irradiation region were immediately captured by the CLSM using an excitation of 488 nm and an emission of 510–560 nm.

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**Biocompatibility of SiO₂-MB@MnO₂**: HeLa cells (5 × 10⁴ cells per well) seeded into 96-well plates were incubated in 5% CO₂ at 37 °C. After 24 h, different concentrations of SiO₂-MB@MnO₂ (0, 5, 10, 20, 30, 40, and 50 µg mL⁻¹) were added into each well of the 96-well plate and the cells were continued to be cultured for 24 h. Then the cells were washed twice with PBS and then 100 µL of MTT solution (0.5 mg mL⁻¹) was added into each well. After 4 h, the cells were incubated in 5% CO₂ at 37 °C for another 4 h and then the PBS was discarded. The intracellular formazan crystals were dissolved into 100 µL of dimethyl sulphoxide (DMSO). The absorbance at 570 nm was recorded by a plate reader, and the percentage of cell viability was determined by comparing cells with the untreated control.

**In Vitro Photodynamic Therapy Effect**: HeLa cells (cells per well) seeded into 96-well plates and incubated in 5% CO₂ at 37 °C for 24 h. Then the cells were treated with free MB, SiO₂-MB, and SiO₂-MB@MnO₂. All formulations were at 50 µg mL⁻¹ with equal MB amount (12 µg mL⁻¹). After 24 h, the cells were washed with PBS...
three times. Fresh medium (200 µL) was added into each well and then cells were irradiated with the 650 nm laser at 100 mW cm⁻² for 15 min. The cells without particles treatment served as controls. After further incubated for 24 h, the cell viability was detected by MTT assay and observed on a CLSM after co-staining with Calcein AM (40 × 10⁻³ α) and PI (4.5 × 10⁻³ µ) for 10 min.

In Vivo Therapy and Distribution of Different NPs: The in vivo study was performed with protocols approved by Jilin University Laboratory Animal Center and China-Japan Union Hospital of Jilin University. Female Kunming mice (6 weeks old, weighing 20–25 g) were selected from the Animal Center and China-Japan Union Hospital of Jilin University. For each group, 1 mL of (i) saline, (ii) free MB, (iii) SiO₂ -MB, and (iv) SiO₂-MB@MnO₂ (100 µg mL⁻¹, equal to 24 µg mL⁻¹ MB except saline group) were, respectively, injected via the tail vein of each mouse every two days. At 24 h postinjection, tumors on mice of treatment group were exposed with 650 nm laser at 100 mW cm⁻² for 15 min. The tumor size was measured as a function of time with digital calipers in two dimensions and tumor volumes were calculated by the formula: volume = (tumor length × tumor width)²/2. The relative tumor volume was normalized to their initial volumes (V/V₀, V₀ was the tumor volume when the treatment was initiated). On the 15th day, all the animals were sacrificed, and the tumors were dissected and weighted to evaluate the therapeutic efficacy of the different groups.

Histology and HIF-1α Staining: At the eighth day, some of the SiO₂-MB@MnO₂ treated mice were sacrificed and the heart, liver, spleen, lung, and kidney tissues were dissected and fixed in 4% neutral buffered formalin and processed routinely into paraffin. Then the organ tissues were sliced to 4 µm thickness for hematoxylin and eosin (H&E) staining and the slices were observed by a digital microscope (Leica QWin). On the other hand, after the mice were sacrificed on the 15th day, the tumor from those mice was harvested and fixed in 4% neutral buffered formalin and processed routinely into paraffin. The tumors were sliced to 4 µm thickness for H&E and HIF-1α staining and the slices were observed by a digital microscope (Leica QWin).

In Vivo MRI: MRI was conducted with a 3.0 T clinical MRI scanner (Philips, Netherlands). The longitudinal (T₁) phantom images as well as relaxation time (T₂) of SiO₂-MB@MnO₂ in acidic H₂O were recorded at different Mn concentrations in aqueous medium (0.012 × 10⁻³, 0.025 × 10⁻³, 0.05 × 10⁻³, 0.1 × 10⁻³, and 0.2 × 10⁻³ µ). In vivo MRI was performed at various time points (0, 4, 12, and 24 h) after intravenous injection of SiO₂-MB@MnO₂ (100 µg mL⁻¹, 1 mL). T₁-Weighted MR images were acquired using a fast spin three echo sequence with the following parameters: TR/TE = 5310/99 ms, 256 × 256 matrices, repetition time 1. The RSI was calculated from the ratio between signal intensity of SiO₂-MB@MnO₂ treated mice and saline group. The distribution of Mn was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Thermo Scientific Xseries 2, Thermo Fisher Scientific, USA).

Supporting Information

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

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