Right Cu$_{2-x}$S@MnS Core–Shell Nanoparticles as a Photo/H$_2$O$_2$-Responsive Platform for Effective Cancer Theranostics

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1. Introduction

Phototherapy has been heavily researched as a promising treatment option for cancer treatment due to its minimal invasiveness, high selectivity, and lower systemic toxicity.[1] Until now, various nanoagents made of noble metals,[2,3] carbon-based materials,[4,5] organic compounds,[6,7] and inorganic semiconductor compounds[8,9] have been proposed for cancer treatment upon photo-activation. In particular, tumor microenvironment (TME)-triggered nanostructures based on various manganese compounds conjugated with photosensitizers[10–12] have attracted substantial attention. These nanostructures offer a unique type of nanotheranostics, which function in magnetic resonance imaging (MRI) and on-demand toxic reactive oxygen species (ROS) production for photodynamic (PD) therapy upon photo-excitation.

Stimuli-responsive nanomedicines have become a recent research focus as a candidate for cancer treatment because of their effectiveness, sensitivity, and minimal invasiveness. In this work, a novel nanosystem is developed based on Cu$_{2-x}$S@MnS core–shell nanoparticles (CSNPs) in which the Cu$_{2-x}$S core serves as a photosensitizer to generate hyperthermia and reactive oxygen species (ROS), and the MnS shell is used in H$_2$O$_2$-responsive O$_2$ production. Cu$_{2-x}$S@MnS CSNPs with an independent core and shell ratio are synthesized by a controllable hot-injection method, resulting in an optimal photothermal (PT) effect with a PT conversion efficiency of up to 47.9%. An enhanced photodynamic (PD) effect also occurs in an H$_2$O$_2$ environment. More significantly, in vivo experiments demonstrate that Cu$_{2-x}$S@MnS CSNPs can mediate tumor shrinkage in both HeLa tumor cell line-derived xenograft (CDX) and head and neck squamous cell carcinoma (HNSCC) patient-derived xenograft (PDX) models, with the capability of being used as a T1-enhanced magnetic resonance (MR) contrast agent. These results suggest the great potential of as-prepared Cu$_{2-x}$S@MnS CSNPs as photo/H$_2$O$_2$-responsive therapeutic-agents against tumors, even in a complicated and heterogeneous environment, thus promoting the clinical translation of nanomedicine.
modulation of hypoxic TMEs to enhance the cancer therapy via \( \text{H}_2\text{O}_2 \)-response, thus resulting in comprehensive antitumor effects. However, most reported nanotheranostics are manganese-supporting nanostructures with organic photosensitizers loaded and might not be ideal for realizing precisely controlled release of therapeutic payloads or supply of a sufficiently effective antitumor effect with mono-therapy.

Similar to PD therapy, photothermal (PT) therapy is also a novel modality that operates upon appropriate laser irradiation, with negligible attenuation into biological tissues and minimal photodamage to cells of adjacent tissues.\(^{13,14}\) The generated hyperthermia in PT therapy can selectively kill cancer cells via apoptosis/necrosis and alter the TME through increased vascular permeability and improved oxygen pressure levels, thus enhancing the tumor sensitivity to PD therapy.\(^{15,16}\)

The inhibition of DNA-damage repair induced by ROS following hyperthermia further improves the PD treatment effect.\(^{17,18}\) Copper sulphide, with numerous copper-deficient stoichiometries (\( \text{Cu}_{2-x}\text{S} \)), is a promising PT agent due to its stoichiometry-dependent near-infrared region (NIR) localized surface plasmon resonance (LSPR) absorption derived from the copper vacancy.\(^{8,19,20}\) Moreover, copper sulphide also has the ability to mediate PT therapy in which laser irradiation stimulates the generation of ROS to ablate cancer cells,\(^{9,21}\) thus improving the therapeutic effect with the potential to benefit from the advantages of each treatment mode. Therefore, integration of plasmonic \( \text{Cu}_{2-x}\text{S} \) and magnetic manganese compounds into a single unit could be advantageous for efficient PT applications and a hyperthermia/O\(_2\)-enhanced PD effect, as well as avoidance of photobleaching and uncontrolled release of organic photosensitizers.

Even though with the goal of improving tumor oxygenation and enhancing cancer treatment, performance evaluation of these proposed nanomaterials is mostly based on tumor cell line-derived xenograft (CDX) mouse models.\(^{32,23}\) However, these results cannot accurately reflect the clinical application value of the nanomedicine because the CDX models have adapted to the in vitro environment and undergone irreversible genetic and biological changes.\(^{24}\) Indeed, the delivery, accumulation, and penetration of nanomedicines and the sensitivity, tolerability, and distribution of heat and ROS generated in the phototherapy process are likely to be affected by the complexity, heterogeneity, and especially TME in the primary human tumor tissue (e.g., interstitial pressure, extracellular matrix barrier).\(^{25-27}\) Compared with the CDX model, the patient-derived xenograft (PDX) model preserves the intratumoral heterogeneity and histology from the primary human cancer tissues, as well as the regenerative TME, such as blood vessels, tumor stroma, fibroblasts, macrophages, and extracellular matrices, thus making it more reliable for preclinical assessments of nanomedicines.\(^{59,30}\)

In the current work, we prepared multifunctional \( \text{Cu}_{2-x}\text{S} \@\text{MnS} \) core–shell nanoparticles (CSNPs) as a photo/\( \text{H}_2\text{O}_2 \)-responsive platform for enhanced cancer theranostics. The \( \text{Cu}_{2-x}\text{S} \@\text{MnS} \) CSNPs were synthesized in a one-pot hot-injection method and were subsequently surface modified. The incorporation of Mn in the CSNP was found to vary in distribution and transfer of charge carriers, resulting in tunable absorption spectra. The intense optical absorption from the CSNPs in the NIR led to the perfect PT conversion and IR thermal imaging property. At the same time, the MnS shell endows the \( \text{Cu}_{2-x}\text{S} \@\text{MnS} \) CSNPs with the ability to be used as a T1-MR contrast agent. Furthermore, the \( \text{Cu}_{2-x}\text{S} \@\text{MnS} \) CSNPs possess a catalase-like activity to function as an \( \text{H}_2\text{O}_2 \)-responsive platform for enhancing the PD effect in which the \( \text{Cu}_{2-x}\text{S} \) serves as a photosensitizer. Above all, the catalase-like \( \text{Cu}_{2-x}\text{S} \@\text{MnS} \) CSNPs integrate PT therapy, enhanced PD therapy and MRI as a whole, thus demonstrating their potential against tumors in both CDX and PDX models and promoting further applications of nanomedicines based on these nanostructures.

2. Results and Discussion

2.1. Preparation and Characterization of \( \text{Cu}_{2-x}\text{S} \@\text{MnS} \) CSNPs

\( \text{Cu}_{2-x}\text{S} \@\text{MnS} \) CSNPs were synthesized via a one-pot hot-injection method in an oleyamine environment under an \( \text{N}_2 \) atmosphere. Figure 1a offers a brief illustration of the synthetic process for the nanocomposites. First, copper acetate (\( \text{Cu} (\text{CH}_3\text{COO})_2 \)) was selected as the copper source to create the \( \text{Cu}_{2-x}\text{S} \) nanoseeds with the aid of 1-dodecanethiol (\( \text{C}_{12}\text{H}_{25}\text{SH} \)).\(^{31,32}\) The import process of Mn and formation of the \( \text{Cu}_{2-x}\text{S} @\text{MnS} \) CSNPs were conducted by injecting an oleylamine dispersion of manganese acetate (\( \text{Mn} (\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O} \)) into the vessel and reacting at 200–220 °C for 20 min.

As shown in Figure 1b, transmission electron microscopy (TEM) revealed that the final products demonstrated a uniform core–shell structure and a mono-disperse state, and their phase and composition constituents were assessed using X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS). As shown in Figure S1 in the Supporting Information, the XRD patterns of the final products match well with the standard power diffraction pattern of monoclinic \( \text{Cu}_{2-x}\text{S} \) (JCPDS No. 23-0959) and hexagonal MnS (JCPDS No. 03-1062). In parallel, the high-resolution transmission electron microscopy (HRTEM) image confirmed the lattice fringes from the core corresponding to the (804) and (226) planes of the \( \text{Cu}_{2-x}\text{S} \) crystal, with spacings of 2.34 and 2.14 Å, respectively. The lattice spacing of 3.22 Å in the shell is associated with the (002) plane of the MnS crystal. In the XPS data (Figure 1d, e, and Figure S2, Supporting Information), the peaks located at 952.5 and 932.6 eV respectively correspond to Cu 2p1/2 and Cu 2p3/2 levels, certifying the coexistence of Cu\( ^+ \) and Cu\( ^{2+} \).\(^{33,34}\) The characteristic peaks of 653.6 and 641.9 eV respectively correspond to Mn(II) 2p1/2 and Mn(II) 2p3/2 levels.\(^{15} \) Moreover, we conducted composition analysis of the CSNPs via TEM elemental mapping with copper and manganese windows (Figure 1c, sulphur window mapping in Figure S3 in the Supporting Information), further demonstrating that the MnS shell is independently located outside of the \( \text{Cu}_{2-x}\text{S} \) core.

The high chalcocite \( \text{Cu}_{2-x}\text{S} \) could be viewed as a solid–liquid hybrid phase with Cu ions diffusing between different sites, similar to a liquid,\(^{36}\) making the cation exchange reaction possible. Therefore, the growth of the \( \text{Cu}_{2-x}\text{S} \@\text{MnS} \) CSNPs in our work includes nucleation of \( \text{Cu}_{2-x}\text{S} \) through the reaction of Cu and S sources, followed by partial transformation from \( \text{Cu}_{2-x}\text{S} \) to MnS via cation exchange.\(^{37,38}\) To further understand the mechanism,
we carefully studied the formation process by adjusting the reaction conditions after the manganese source injection. Initially, pure Cu$_{2-x}$S NPs (Figure 2a) were formed without the manganese source. After Mn(CH$_3$COO)$_2$·4H$_2$O in oleylamine was injected, a portion of the Cu in the Cu$_{2-x}$S nanoseeds was replaced by Mn, and the Cu$_{2-x}$S@MnS CSNPs with various shell thickness formed at different rates of temperature increase in the reaction process. As shown in Figure 2b, when the rate of temperature increase was maintained at 0.25 °C min$^{-1}$, a thin layer of MnS shell was coated outside the Cu$_{2-x}$S core (thin-shell Cu$_{2-x}$S@MnS). With the rates increased up to 0.55 and 1.00 °C min$^{-1}$, intermediate-shell Cu$_{2-x}$S@MnS (Figure 2c) and thick-shell Cu$_{2-x}$S@MnS (Figure 2d) were formed, respectively, accompanied by diminishing Cu$_{2-x}$S size. The molar ratios of Mn to Cu in the above four NPs were 0:1, 0.23:1, 0.5:1, and 1.18:1, respectively, as determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Moreover, as the cation exchange proceeded, a void emerged between the MnS shell and the Cu$_{2-x}$S nucleus. It is believed that this unique phenomenon can be attributed to the unequal ion diffusion between Cu$^+$ and Mn$^{2+}$, where the more rapidly exported Cu$^+$ diffusion process induced the nanoscale Kirkendall effect.$^{[39,40]}$

2.2. PT Properties of Cu$_{2-x}$S@MnS CSNPs

El-Sayed and coworkers reported that an optimal PT conversion effect could be reached when the LSPR from the plasmonic...
NPs resonates with the incident light, which means that a stronger absorption of the incident light leads to a higher PT activity\[20,41\]. Therefore, the vis–NIR absorption spectra of the Cu\textsubscript{2−x}S@MnS NPs with different MnS shell thicknesses were examined. The pure Cu\textsubscript{2−x}S NPs have an optical absorption band centered at 1700 nm (Figure S4, Supporting Information), consistent with the high chalcocite Cu\textsubscript{2−x}S material. After partial transformation to a MnS shell, the absorption band gradually blueshifted with an obvious intensification and moved to 950 nm for the thick-shell Cu\textsubscript{2−x}S@MnS CSNPs, indicating the strong interaction between the Cu\textsubscript{2−x}S core and the MnS shell.

Before comparison of their PT properties, the four as-synthesized Cu\textsubscript{2−x}S@MnS samples were first transferred from the organic phase into water using an amphiphilic poly(maleic anhydride)-based polymer as a protecting group for the hydrophilic carboxylic acid groups and hydrophobic oleylamine sidechains. The Fourier transform infrared spectroscopy (FTIR) patterns (Figure S5, Supporting Information) showed that selected new peaks (at 1697, 1638, and 1210 cm\textsuperscript{-1}) obviously appeared after material modification, representing the characteristic absorption peaks of C=O and C–O, which indicated successful hydrophilic modification. The PT conversion performances of these Cu\textsubscript{2−x}S@MnS NPs were measured in aqueous dispersion under irradiation with an 808 nm laser for 5 min at an intensity of 0.72 W cm\textsuperscript{-2}. Figure 3a shows the temperature change curves. The temperature of the pure Cu\textsubscript{2−x}S NPs suspension increased from 28.1 to 38.1 °C within 5 min. The thin-shell Cu\textsubscript{2−x}S@MnS and intermediate-shell Cu\textsubscript{2−x}S@MnS CSNPs suspensions had final temperatures of 44 and 47.9 °C, respectively, under the same conditions. The highest temperature of 49.4 °C after irradiation was stimulated by the thick-shell Cu\textsubscript{2−x}S@MnS CSNPs aqueous dispersion. In other words, the thick-shell Cu\textsubscript{2−x}S@MnS CSNPs obtained an optimal PT conversion efficiency upon photo-excitation at 808 nm, and therefore, they were selected as the object for the following study.

To further investigate the PT property of our selected Cu\textsubscript{2−x}S@MnS CSNPs, aqueous dispersions with various concentrations (0, 100, 200, 400, and 500 ppm) were excited by an 808 nm laser. The temperature elevation of these dispersions was found to be proportional to the Cu\textsubscript{2−x}S@MnS concentration (Figure 3b,c). A concentration of 100 ppm showed a temperature increase of 14.3 °C after 5 min of irradiation. As the concentration increased to 500 ppm, the temperature increased by 28.2 °C. In comparison, the temperature of distilled water increased by only 2 °C. The PT conversion efficiency was calculated as 47.9% (Figure S6, Supporting Information) using a modified method, according to previous studies\[20,42\]. In addition, the laser on-off circulation tests displayed in Figure 3d demonstrate the stability of the thick-shell Cu\textsubscript{2−x}S@MnS CSNPs under photo-excitation.

2.3 In Vitro O\textsubscript{2} and ROS Generation of Cu\textsubscript{2−x}S@MnS CSNPs

As elements with multiple redox states, both copper and manganese show striking redox properties, similar to iron, which decomposes H\textsubscript{2}O\textsubscript{2} through Fenton-like pathways\[43\]. Thus, substantial attention is focused on the peroxidase-like activity of Cu\textsubscript{2−x}S and the modulation of the hypoxic TME induced...
by manganese-supporting nanostructures. Herein, we quantified the generation of \( \text{O}_2 \) triggered by the thick-shell \( \text{Cu}_{2-x}\text{S}@\text{MnS} \) CSNPs when exposed to \( \text{H}_2\text{O}_2 \) using a dissolved oxygen meter. As shown in Figure 4a, without \( \text{H}_2\text{O}_2 \), the \( \text{Cu}_{2-x}\text{S}@\text{MnS} \) CSNPs suspension (71 ppm) maintained a low dissolved oxygen level. After addition of \( 28 \times 10^{-3} \text{ m} \) to the \( \text{Cu}_{2-x}\text{S}@\text{MnS} \) CSNPs suspension, a large amount of bubbles was observed, and the dissolved oxygen concentration increased from 7.6 to 13.7 mg L\(^{-1} \) within 12.5 min. Moreover, the dissolved oxygen concentration increased more rapidly and appeared to be higher post-\( \text{H}_2\text{O}_2 \) addition with comparatively more \( \text{H}_2\text{O}_2 \) (56, 85, and 113 \( \times 10^{-3} \text{ m} \), in Figure 4a) added or a higher concentration of \( \text{Cu}_{2-x}\text{S}@\text{MnS} \) CSNPs (107 and 143 ppm, in Figure 4b), indicating that additional \( \text{O}_2 \) is generated from the oxidation of \( \text{H}_2\text{O}_2 \) induced by the \( \text{Cu}_{2-x}\text{S}@\text{MnS} \) CSNPs. However, for dispersions lacking \( \text{Cu}_{2-x}\text{S}@\text{MnS} \) CSNPs (\( \text{C}_{\text{CSNPs}} = 0 \), in Figure 4b), the dissolved oxygen concentrations remained constantly at a lower level, further confirming the necessity of the \( \text{Cu}_{2-x}\text{S}@\text{MnS} \) CSNPs for generation of \( \text{O}_2 \).

Apart from their role as a PT agent, copper sulphides can also stimulate generation of ROS under laser irradiation.\cite{9,44} Therefore, we assessed the generation of ROS induced by the thick-shell \( \text{Cu}_{2-x}\text{S}@\text{MnS} \) CSNPs using singlet oxygen sensor green (SOSG), a fluorescence indicator for \( \text{O}_2 \). As shown in Figure 4c, the aqueous dispersion containing SOSG exhibited weak fluorescence. The fluorescence intensity of SOSG at 525 nm obviously increased with \( \text{Cu}_{2-x}\text{S}@\text{MnS} \) CSNPs incubation under irradiation using an 808 nm laser. A larger concentration of the \( \text{Cu}_{2-x}\text{S}@\text{MnS} \) CSNPs suspension resulted in an obvious increase in the fluorescence intensity of SOSG. Furthermore, considering the dependence of ROS generation on \( \text{O}_2 \), the generation of ROS upon \( \text{H}_2\text{O}_2 \) treatment was also assessed (Figure 4d). The fluorescence intensity of SOSG in the \( \text{Cu}_{2-x}\text{S}@\text{MnS} \) CSNPs suspension with \( \text{H}_2\text{O}_2 \) at 525 nm increased to 305.22% of that in control group after laser irradiation and was much larger than that with only \( \text{Cu}_{2-x}\text{S}@\text{MnS} \) CSNPs or \( \text{H}_2\text{O}_2 \) added under the same conditions. These results show that \( \text{Cu}_{2-x}\text{S}@\text{MnS} \) CSNPs can enhance the generation of ROS in response to the addition of \( \text{H}_2\text{O}_2 \) upon laser irradiation.

2.4. In Vitro Cytotoxicity of \( \text{Cu}_{2-x}\text{S}@\text{MnS} \) CSNPs

Prior to assessing the capacity of the \( \text{Cu}_{2-x}\text{S}@\text{MnS} \) CSNPs to mediate cytotoxicity, we first analyzed their cellular uptake and internalization using HeLa cells. For visualization, the \( \text{Cu}_{2-x}\text{S}@\text{MnS} \) CSNPs were labeled with fluorescein isothiocyanate (FITC, excitation/emission at 494/525 nm) via covalent bonds. Cells were stained with 4,6-diamidino-2-phenylindole (DAPI) and imaged with a confocal laser scanning microscope (CLSM) after incubation with the \( \text{Cu}_{2-x}\text{S}@\text{MnS} \) CSNPs. As shown in Figure S7 in the Supporting Information, a green signal appeared around the domains covered by DAPI after 20 min, suggesting that the CSNPs were taken up by cellular endocytosis and delivered to the cytoplasm. Moreover, we used ICP-AES to quantize the NPs in the cells after incubation with \( \text{Cu}_{2-x}\text{S}@\text{MnS} \) CSNPs at different concentrations.
With the increasing of CSNPs' concentration (Figure 5a), the cellular uptake expressed by the copper content increased, and the internalized CSNPs had a higher amount at 12 h than that at 6 h.

The CCK-8 assay was performed to evaluate the potential cytotoxicity of the Cu$_{2-x}$S@MnS material after HeLa cells were incubated with the CSNPs for 24 h (Figure 5b). Without NIR irradiation, the Cu$_{2-x}$S@MnS CSNPs suspension with concentrations ranging from 0 to 200 ppm showed negligible cytotoxicity and exhibited excellent biocompatibility, i.e., all cells retained greater than 90% viability. After 808 nm laser irradiation for 7 min, the Cu$_{2-x}$S@MnS CSNPs showed significant dose-dependent cytotoxicity. Cells treated with only laser irradiation had a cell viability of nearly 100%. However, enhanced cytotoxicity occurred with Cu$_{2-x}$S@MnS CSNPs suspension concentrations of up to 100 and 200 ppm, with the cell viability decreasing to 61.39% and 43.82%, respectively. Additionally, similar results were achieved via live/dead cell staining performed with calcein acetoxymethyl ester (calcein AM, green fluorescence) and propidium iodide (PI, red fluorescence). As shown in Figure 5c, no fluorescence changes were observed despite treatment of cells with laser irradiation or CSNPs at various concentrations. In contrast, obvious changes with a dramatic increase in red color and decrease in green color, were observed in groups with both CSNPs and laser irradiation treatment, indicating significant dead cells. The cell-killing effects of the Cu$_{2-x}$S@MnS CSNPs were greater when the suspension concentration increased from 100 to 200 ppm, confirming the photodamage of the Cu$_{2-x}$S@MnS CSNPs on cancer cells under 808 nm laser irradiation.

As verified above, the nanoplatforms based on Cu$_{2-x}$S@MnS CSNPs could mediate an efficient PT therapy effect under laser irradiation and generate O$_2$ and ROS in response to H$_2$O$_2$. Moreover, it is known that the cancer cells inside solid tumors are able to constitutively produce H$_2$O$_2$ (from $\approx 50 \times 10^{-6}$ to $100 \times 10^{-6}$ m) by relying on the decreased antioxidant enzyme level. Therefore, Cu$_{2-x}$S@MnS CSNPs are anticipated to guide an enhanced phototherapy effect in response to H$_2$O$_2$ by generating O$_2$ and more ROS after internalization by tumor cells. To confirm this, the in vitro cell cytotoxicity of Cu$_{2-x}$S@MnS CSNPs in an H$_2$O$_2$ environment was also evaluated via the standard CCK-8 assay. HeLa cells were first incubated with Cu$_{2-x}$S@MnS CSNPs suspension for 24 h and subsequently treated with exogenous H$_2$O$_2$ ($100 \times 10^{-3}$ m). H$_2$O$_2$ did not influence cell viability under laser irradiation when the added CSNPs concentration was 0 (Figure 5b). Groups treated with both Cu$_{2-x}$S@MnS CSNPs and H$_2$O$_2$ exhibited comparatively more remarkable cell killing upon laser irradiation, with a cell viability (21.44%) lower than that in normoxic conditions at a CSNPs concentration of 200 ppm. These results indicated that extra H$_2$O$_2$ was helpful in enhancing the phototriggered cancer cell-ablation efficiency of the Cu$_{2-x}$S@MnS CSNPs. Taken together, these results showed that Cu$_{2-x}$S@MnS CSNPs could mediate an improved therapeutic effect in response to H$_2$O$_2$ and photo-irradiation.

![Figure 4. a) Dissolved oxygen concentration in aqueous dispersions containing Cu$_{2-x}$S@MnS CSNPs (71 ppm) with different H$_2$O$_2$ concentrations versus time. Insert: final dissolved oxygen concentration. b) Dissolved oxygen concentration in H$_2$O$_2$ solutions (71 $\times$ 10$^{-3}$ m) with different Cu$_{2-x}$S@MnS CSNPs concentrations versus time. Insert: final dissolved oxygen concentration. c) Normalized fluorescence emission spectrum of SOSG (2.5 $\times$ 10$^{-6}$ m) incubated with Cu$_{2-x}$S@MnS CSNPs suspensions (i.e., 0, 4.17, 8.33, 12.50, and 16.67 ppm) after 808 nm laser irradiation (0.72 W cm$^{-2}$). d) Normalized fluorescence emission spectrum of SOSG with different treatments after laser irradiation.](image-url)
2.5. MR Properties of Cu₂−xS@MnS CSNPs

Considering that manganese-based nanomaterials are one of the most current T1-enhanced MR contrast agents, we explored the feasibility of using our thick-shell Cu₂−xS@MnS CSNPs as an MR contrast agent. First, we recorded the MR images and relaxation times of aqueous dispersions containing Cu₂−xS@MnS CSNPs using a 0.5 T MR animal scanner (MesoMR23-060H-I) at varied Mn concentrations. As shown in Figure 6a, the T1-weighted MR images showed a brightening effect that was correlated with the Mn concentration and was further shown to be highly linear. According to the relaxation rate as a function of Mn concentration, the corresponding longitudinal relaxivity (r₁, effectiveness as a contrast agent) of these CSNPs was calculated to be 1.243 mM⁻¹ s⁻¹, suggesting that our material is suitable for use as a contrast agent in MRI. The contrast-enhancing effect was evaluated in nude mice bearing HeLa tumors. Figure 6c shows the T1-weighted MR images of the mouse before and after intratumoral injection of Cu₂−xS@MnS CSNPs dispersed in a saline solution. The tumor region has a contrast-enhanced signal after CSNPs injection compared with that before, and the relative MR signal intensity in the tumor region shows an increase from 23 046 to 36 516 (Figure S8, Supporting Information). This result suggests the potential application of thick-shell Cu₂−xS@MnS CSNPs as a promising MRI agent for tumor diagnosis.

2.6. In Vivo Tumor Shrinkage Efficacy of Cu₂−xS@MnS CSNPs in CDX Models

Based on the in vitro cytotoxicity of Cu₂−xS@MnS CSNPs upon laser irradiation, we assessed the in vivo phototherapy effect. CDX mice bearing HeLa tumors were first intratumorally injected with CSNPs suspension (100 µL, 200 ppm) and subsequently laser irradiated (808 nm, 0.72 W cm⁻²). The control groups were treated with PBS injection (100 µL) and the same laser exposure. A thermal infrared camera was used...
2.7. Antitumor Efficacy of Cu$_{2-x}$S@MnS CSNPs in PDX Models

The PDX model not only preserves the intratumoral heterogeneity and histology of the primary human cancer tissues but also preserves the regenerative TME, such as blood vessels, tumor stroma, fibroblasts, macrophages, and extracellular matrices, thus making it more significant for preclinical assessments of nanomedicines compared with CDX. Therefore, to further explore the clinical potential of phototherapy based on our thick-shell Cu$_{2-x}$S@MnS CSNPs, a PDX model was constructed based on head and neck squamous cell carcinoma (HNSCC), which belongs to superficial tumor that is preferable for photo-illumination and photo-penetration. The 4th generation of PDX animals was used in this work due to its stabilization (Figure S10 in the Supporting Information shows the tumor volume change curves of mice in the first three generations). Similar to the experiments on the HeLa tumour CDX model (as shown in Figure S11 in the Supporting Information), mice inoculated with the PDX model were intratumorally injected with the CSNPs suspension (100 µL, 200 ppm) followed by laser irradiation (808 nm, 0.72 W cm$^{-2}$) (Group I: CSNPs + laser, n = 6). Controls in these experiments included mice lacking CSNPs injection (Group II: PBS + laser, n = 6), mice lacking laser irradiation (Group III: CSNPs only, n = 6), and mice lacking both treatments (Group IV: untreated, n = 6).

Over these treatments, we evaluated the therapeutic efficacy using haematoxylin and eosin (H&E) staining (Figure S8a). Little damage was found in the tumor tissues from the groups treated with PBS + laser or CSNPs only compared with the untreated group, whereas evident cellular damage such as deformation, nuclei condensation, and destruction of membrane integrity was observed in the CSNPs + laser group. Moreover, to tentatively identify which cellular mechanisms are responsible for the cell death in tumors, we performed a terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay for apoptotic cell death and conducted immunofluorescence labeling using antibodies against a cell proliferation marker (Ki-67). The TUNEL assay demonstrated that Cu$_{2-x}$S@MnS CSNPs induced...
the highest cell apoptosis rate in tumors upon laser excitation. A decrease in the number of Ki-67-positive proliferating cells was observed in tumors treated with CSNPs + laser (10% positive) compared with the other three groups (40% positive for blank control, 39% positive for PBS + laser control, and 25% positive for CSNP only control). These results suggested that Cu$_{2-x}$S@MnS CSNPs had an inhibitory effect on tumor cell proliferation and pro-apoptosis. The decrease in positive proliferating cells in the CSNPs control might be due to the Fenton effect induced by the Cu$_{2-x}$S@MnS material within the special TME with a high H$_2$O$_2$ level. In addition, the tumor growth curves (Figure 8c) further confirmed the tumor shrinkage efficacy of Cu$_{2-x}$S@MnS material within the special TME with a high H$_2$O$_2$ level. In addition, the tumor growth curves (Figure 8c) further confirmed the tumor shrinkage efficacy of Cu$_{2-x}$S@MnS material in the PDX models with the laser irradiation, and the bodyweight plots of mice (Figure S12, Supporting Information) proved the safety of phototherapy stimulated by CSNPs, thus reinforcing that our Cu$_{2-x}$S@MnS CSNPs can be used as an efficient therapeutic agent for cancer therapies.

2.8. In Vivo Biosafety of Cu$_{2-x}$S@MnS CSNPs

The in vivo biosafety of nanomedicines is always a considerable concern for application in cancer theranostics. To test biosafety, nude mice were used as models and intravenously injected with the Cu$_{2-x}$S@MnS CSNPs suspension. Blood and tissue samples were collected and analyzed at different time points. The results showed that Cu$_{2-x}$S@MnS material treatment did not affect the normal range of blood biochemistry indicators (Figure S13, Supporting Information) including alanine aminotransferase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN), creatinine (CRE), and uric acid (UA), and the physiological morphology of tissues including heart, liver, spleen, lung, and kidney, observed from H&E-stained images in Figure S14 in the Supporting Information. Moreover, tissue samples were also digested with HNO$_3$/HCl to determine the Cu contents via ICP-AES measurements. As shown in Figure S15 in the Supporting Information, the Cu$_{2-x}$S@MnS material distribution reached a high level in liver and spleen at first, which could be possibly correlated with reticuloendothelial system (RES) uptake. This distribution decreased significantly at 72 h post injection, indicating elimination from the organisms within 3 days. These preliminary investigations confirmed the biosafety of Cu$_{2-x}$S@MnS CSNPs at the tested dose. However, additional systematic studies of pharmacokinetics, pharmacodynamics and pharmaco-immunology are still necessary for future clinical translation of such a material.
3. Conclusion

In conclusion, a novel nanosystem based on Cu$_{2-x}$S@MnS CSNPs was developed for cancer theranostics using a controllable hot-injection method. The external MnS shell mediates O$_2$ production to overcome hypoxia and regulates TME in a special tumor environment with excessive H$_2$O$_2$. The Cu$_{2-x}$S core is excited by a NIR laser, leading to an enhanced PD effect with an O$_2$ self-supplement condition and an improved PT effect via ROS and heat generation, resulting in successful eradication of subcutaneous tumors in CDX and PDX models. Moreover, the MR effect induced by the Mn-based nanomaterial can be used to noninvasively locate tumors and guide treatment. This multifunctional nanoplatform demonstrated effective tumor ablation and imaging, promoting clinical translation of nanomedicine.

4. Experimental Section

**Materials and Reagents:** Copper (II) acetate ($\text{Cu(CH}_3\text{COO)}_2$), manganese (II) acetate ($\text{Mn(CH}_3\text{COO})_2$), sodium carbonate ($\text{Na}_2\text{CO}_3$), cyclohexane ($\text{C}_6\text{H}_{12}$), and chloroform ($\text{CHCl}_3$) were obtained from Sinopharm Chemical Reagent Co. Ltd. 1-dodecanethiol ($\text{CH}_3(\text{CH}_2)_{11}\text{SH}$) and oleylamine (OM) were sourced from Aladdin Industrial Corporation. Tetrahydrofuran ($\text{C}_4\text{H}_8\text{O}$) was purchased from Shanghai Macklin Biochemical Co. Ltd. All chemicals were of at least analytical reagent grade and used without further purification.

**Synthesis of Cu$_{2-x}$S@MnS CSNPs:** The Cu$_{2-x}$S@MnS CSNPs were synthesized using a one-pot hot-injection method. In brief, 0.6 mmol Cu($\text{CH}_3\text{COO}$)$_2$ was dissolved in 20 mL of oleylamine under vigorous stirring, and 8 mL of 1-dodecanethiol was added. The mixture was heated to 220°C in an N$_2$ atmosphere and maintained for 15 min. Subsequently, 8 mL of oleylamine containing 1 mmol Mn($\text{CH}_3\text{COO}$)$_2$·4H$_2$O was injected into the hot solution, and the temperature of the solution was changed to 195°C. The reactions continued for another 20 min with different rates of temperature increase (i.e., 0.25, 0.55, and 1.00°C min$^{-1}$). Finally, solid black products were collected by centrifugation after cooling to room temperature.

Pure Cu$_{2-x}$S NPs were prepared using the procedures above but without the addition of Mn($\text{CH}_3\text{COO}$)$_2$·4H$_2$O and following another 20 min reaction.

**Surface Modification of Hydrophobic Cu$_{2-x}$S@MnS CSNPs:** The amphiphilic polymer composed of poly(maleic anhydride) and oleylamine was synthesized based on a protocol in the literature.$^{[50]}$ For surface modification of hydrophobic Cu$_{2-x}$S@MnS CSNPs, 150 µL of amphiphilic polymer stock solution (0.8 µm in CHCl$_3$) and 10 mL of hydrophobic Cu$_{2-x}$S@MnS CSNPs (500 ppm in CHCl$_3$) were combined and stirred for 4 h at room temperature. The solvent was removed by rotary evaporation, and 25 mL sodium carbonate solution (0.05 g mL$^{-1}$) was added to the flask and stirred for 15 min at room temperature to disperse the hydrophilic Cu$_{2-x}$S@MnS CSNPs. The solution was centrifuged and dispersed into PBS for further use.

To prepare the hydrophilic Cu$_{2-x}$S@MnS-FITC CSNPs, 5 mg of FITC was added into an amphiphilic polymer stock solution (20 mg) and stirred for 24 h in the dark until CHCl$_3$ was volatilized. The hydrophobic Cu$_{2-x}$S@MnS CSNPs were modified with this production using the above procedures.

**In Vitro PT Performances of Cu$_{2-x}$S@MnS CSNPs:** To measure the PT effect of Cu$_{2-x}$S@MnS CSNPs with different MnS shell thicknesses, 100 µL of aqueous dispersions containing pure Cu$_{2-x}$S NPs, thin-shell Cu$_{2-x}$S@MnS CSNPs, intermediate-shell Cu$_{2-x}$S@MnS CSNPs, and thick-shell Cu$_{2-x}$S@MnS CSNPs were each irradiated by an 808 nm laser (Shanghai Xilong Optoelectronics Technology Co., Ltd., China) at a power density of 0.72 W cm$^{-2}$. A thermal imaging camera (FLIR A300, USA) was used to record the temperature change.

To further investigate the PT effect of the selected thick-shell Cu$_{2-x}$S@MnS CSNPs, 100 µL of aqueous dispersions with different concentrations (0–500 ppm) were irradiated using the 808 nm laser for 5 min. In the photostability test, the sample was laser irradiated for 5 min and cooled naturally. The on–off process was repeated six times.

**O$_2$ Generation of Cu$_{2-x}$S@MnS CSNPs in Response to H$_2$O$_2$:** A portable dissolved oxygen meter was used to measure O$_2$ generation. First, the Cu$_{2-x}$S@MnS CSNPs were dispersed in 7 mL of aqueous solution with

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**Figure 8.** a) Representative H&E, TUNEL, and Ki-67 stained images of ex vivo tumor sections after various treatments in the HNSCC PDX model. Insert: Images with high magnification, scale bar: 20 µm. b) Percentage of positive proliferating cells calculated from Ki-67. c) Average tumor sizes collected from mice from different groups.
a concentration of 71 ppm, and different amounts of H2O2 were added. The dissolved oxygen concentration in the aqueous dispersions was recorded every 30 s after H2O2 was added. For investigation of the effect of the Cu2−xS@MnS CSNPs suspension concentration on O2 release, the amount of H2O2 was added and maintained at 71 × 10−3 m, and the Cu2−xS@MnS CSNPs concentration was changed from 0 to 143 ppm.

**ROS Production:** For ROS detection, the commercial chemical probe SOSG was used to detect its fluorescence intensity at 525 nm via fluorescence emission spectroscopy excited at 488 nm. SOSG (2.5 × 10−6 m) was added to 1 mL of Cu2−xS@MnS CSNPs suspension (0–16.67 ppm), and the mixture was kept in the dark and irradiated with an 808 nm laser (0.72 W cm−2) for 60 min. Fluorescence emission spectroscopy was recorded. To test ROS production in response to H2O2, laser irradiation was conducted after addition of H2O2 (71 × 10−3 m).

**Cell Uptake:** HeLa cells were seeded into a 96-well plate at 1 × 104 cells per well at 37 °C in the presence of 5% CO2 for 24 h. The Cu2−xS@MnS CSNPs suspensions were added to these wells at various concentrations (Cu concentrations were 10.709, 5.355, and 2.677 ppm, as measured by ICP-AES). After incubation for 6 and 12 h, the medium was removed, each well was washed three times with PBS, and HeLa cells were digested with HNO3/HCl to determine the Cu uptakes via ICP-AES measurements.

For visualization, HeLa cells were seeded into a 6-well plate and cocultured with Cu2−xS@MnS-FITC CSNPs. The medium was removed, and each well was washed three times with PBS. The cell nuclei were stained with DAPI for 20 min. Finally, the cells were observed by CLSM.

**Biocompatibility of Cu2−xS@MnS CSNPs:** The in vitro cytotoxicity was evaluated using a CCK assay. HeLa cells were seeded into a 96-well plate at 1 × 104 cells per well at 37 °C in the presence of 5% CO2. After incubation for 24 h, the Cu2−xS@MnS CSNPs suspensions were added to the cells at various concentrations and incubated for another 24 h. An amount of 200 µL complete medium with 10% (volume) CCK-8 dispersion was added to each well of the microtiter plate and incubated in the CO2 incubator for another 2 h. The optical density (OD) was measured at 490 nm using a microplate reader. All experiments were independently repeated three times.

**In Vitro Phototherapy Effect:** HeLa cells were seeded into a 96-well plate at 1 × 104 cells per well at 37 °C in the presence of 5% CO2 for 24 h. The Cu2−xS@MnS CSNPs suspensions were added to the cells at various concentrations. After 24 h, the cells were irradiated with an 808 nm laser at 0.72 W cm−2 for 7 min. After further incubation for another 2 h, the cell viability was detected using a CCK assay and observed on a CLSM after co-staining with calcein AM and PI for 20 min.

To study the effect of H2O2 on phototherapy, H2O2 (100 × 10−3 m) was added to the wells after Cu2−xS@MnS CSNPs suspension addition, and the cell viability was detected using a CCK assay after laser irradiation.

**Animals and Tumor Model:** All animal experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Shanghai Jiao Tong University School of Medicine. In the PDX model, tumor samples were obtained from Shanghai Ninth People’s Hospital with written informed consent from each patient and research ethics board approval in accordance with the Declaration of Helsinki. Herein, a tumor tissue sample from an HNSCC patient after surgical treatment was cut into ~5 mm pieces and implanted into the BALB/c nude mice (6–8 weeks old). For the PDX model, tumor samples were obtained from Shanghai Ninth People’s Hospital with written informed consent from each patient and research ethics board approval in accordance with the Declaration of Helsinki.

**MR Measurement:** For in vitro MR measurement, Cu2−xS@MnS CSNPs suspensions with different Cu concentrations were scanned under a 0.5 T small animal MR scanner (MesoMR23-060H-I) at room temperature. For MRI in vivo, HeLa tumor-bearing mice were scanned with the same MR scanner before and after intratumour injection with Cu2−xS@MnS CSNPs suspension (100 µL, 200 ppm). During the experiments, all mice were first anaesthetized using chloral hydrate (8%).

**Conflict of Interest**

The authors declare no conflict of interest.

**Keywords**

cancer treatment, Cu2−xS@MnS, patient-derived xenografts, photo/H2O2-responsive platforms, tumor cell line-derived xenografts

Received: June 13, 2019  
Revised: August 2, 2019  
Published online: August 27, 2019

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