Upconversion nanoparticles@AgBiS$_2$ core-shell nanoparticles with cancer-cell-specific cytotoxicity for combined photothermal and photodynamic therapy of cancers

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ABSTRACT

UCNPs@AgBiS$_2$ core-shell nanoparticles that AgBiS$_2$ coated on the surface of upconversion nanoparticles (UCNPs) were successfully prepared through an ion exchange reaction. The photothermal conversion efficiency of AgBiS$_2$ can be improved from 14.7% to 45% due to the cross relaxation between Nd ions and AgBiS$_2$. The doping concentration of Nd ions played a critical role in the production of reactive oxygen species (ROS) and enhanced the photothermal conversion efficiency. The NaYF$_4$:Yb/Er/Nd@NaYF$_4$:Nd nanoparticles endows strong upconversion emissions when the doped concentration of Nd ions is 1% in the inner core, which excites the AgBiS$_2$ shell to produce ROS for photodynamic therapy (PDT) of cancer cells. As a result, the as-prepared NaYF$_4$:Yb/Er/Nd@NaYF$_4$:Nd@AgBiS$_2$ core-shell nanoparticles showed combined photothermal/photodynamic therapy (PTT/PDT) against malignant tumors. This work provides an alternative near-infrared light-active multimodal nanostructures for applications such as fighting against cancers.

1. Introduction

During the past two decades, lanthanide ion-doped upconversion nanoparticles (UCNPs) have attracted tremendous attention owing to their unique capability to generate shorter wavelength emissions under the excitation of longer wavelengths [1–5]. Especially, UCNPs have been recognized as one kind of energy transducer for producing reactive oxygen species (ROS), enhancing energy migration for various applications, such as photodynamic therapy (PDT) [6,7], photothermal therapy (PTT) [8,9], and controlled drug delivery [10–12]. Among these, PDT has aroused great research interest in recent years due to its low systemic toxicity and minimal invasiveness. However, PDT for cancer treatment is hampered by tumor hypoxia, which involves sufficient oxygen, photosensitization and light excitation [13,14]. In addition, the traditional organic photosensitizer is inefficient to produce active oxygen and poor chemical stability, which leads to low efficacy for PDT in cancer treatment. After enormous efforts have been devoted, it was found that the combination of UCNPs with semiconductors can achieve the ideal efficacy of PDT to overcome oxygen dependence [15–17].

Additionally, a variety of semiconductors with excellent photothermal conversion abilities, including CuS, Bi$_2$S$_3$, gold (Au), carbon and metal chalcogenides, have been combined with UCNPs to achieve excellent photothermal and photodynamic efficacy [18–24]. In particular, bismuth (Bi)-based nanomaterials, such as Bi, Bi$_2$S$_3$, AgBiS$_2$ and Bi$_2$Se$_3$, have been proven to be promising candidates as superior photothermal conversion agents, owing to their light absorption coefficient,
heat dissipation rate and photothermal conversion efficiency [25–30]. Furthermore, Bi-based nanomaterials with a narrow band gap can also be used as catalytic materials, which have the ability to generate ROS under light irradiation. In the past few years, photothermal-enhanced photodynamic therapy has been recognized as efficient and non-invasive modalities for cancer treatment since thermal effects at an appropriate level can increase intratumoral blood flow and subsequently transport more oxygen into the tumor, resulting into yielding synergistic or combined therapeutic outcomes even in severely hypoxic solid tumors [31,32].

AgBiS₂ hollow nanospheres exhibited excellent chemical stability and good cancer-cell-specific cytotoxicity, which was synthesized by our previous reported protocol [33]. Herein, we proposed an efficient strategy to fabricate AgBiS₂-coated Nd³⁺-sensitized upconversion nanoparticles (denoted UCNPs) to construct unique UCNPs@AgBiS₂ core-shell nanoparticles (NPs) for enhanced photothermal conversion efficiency owing to the potential cross-relaxation pathways between the continuous energy band of AgBiS₂ and the ladder-like energy levels of Nd³⁺ ions (Scheme 1). The ROS production capability and photothermal conversion ability have been studied based on two different modes (up-/down-conversion luminescence). Antitumor experiments in vitro and in vivo were conducted upon 808 nm laser irradiation to demonstrate the privilege of the core-shell NPs. As expected, the as-prepared UCNPs@AgBiS₂ core-shell nanoparticles (NPs) with cancer-cell-specific cytotoxicity would show superior therapeutic efficacy.

2. Materials and methods

2.1. Materials

NaNdF₄@NaYF₄:Nd₀.2 (abbreviated as Nd@Nd), NaYF₄:Yb₀.3/Er₀.005/Ndₓ (X = 0, 0.005, 0.01, 0.03, 0.05)@NaYF₄:Nd₀.2 (abbreviated as UCNPs) core-shell NPs, UCNPs@AA-[Zn(OH)₄]²⁻ and UCNPs@ZnS nanoparticles showed good dispersability and were prepared according to our protocol reported previously [35]. Other chemicals were of analytic grade and used as received.

2.2. Synthesis of UCNPs@AgBiS₂ core-shell NPs

In a typical procedure, 0.045 mmol UCNPs@ZnS was added to 10 mL of ethylene glycol solution containing 0.09 mmol of thiourea to form milky dispersions. Subsequently, the solution was slowly heated to 130 °C under stirring. Then, AgNO₃ and Bi(NO₃)₃ (0.045 mmol) ethylene glycol solution were added to the above solution under constant stirring, which was maintained at 130 °C for 10 min. UCNPs@AgBiS₂ core-shell NPs were obtained by washing three times with ethanol and deionized water.

2.3. Cytotoxicity experiment

Typically, 4T1 (mouse breast cancer cells) cells were seeded in a 96-well plate at 1 × 10⁴ cells/well and then incubated with different concentrations of UCNPs@AgBiS₂ (0, 10, 20, 40, 80, and 160 μg mL⁻¹) for 12 h. Subsequently, laser irradiation (808 nm, 1 W cm⁻²) was performed for different times (0, 1, and 3 min), addition of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) solution and incubation for 4 h to form formazan. Finally, 100 μL dimethyl sulfoxide was added to dissolve and measure the absorbance at 570 nm with a microplate reader to determine the relative cell viability.
2.4. In vitro ROS assay

Typically, 4T1 cells were seeded in a 6-well plate at $1 \times 10^5$ cells/well and then incubated with UCNPs@AgBiS$_2$ (50 $\mu$g mL$^{-1}$) for 12 h. Subsequently, the cells were irradiated with an 808 nm laser (1 W cm$^{-2}$) for 3 min. Then, DCFH-DA (2',7'-dichlorodihydrofluorescein diacetate) was incubated for 0.5 h to form green fluorescent substance (DCF). Finally, the intracellular green fluorescence was monitored by confocal laser scanning microscopy (CLSM) and flow cytometry analysis.

2.5. Live/dead cell staining

Typically, 4T1 cells were seeded in a 24-well plate at $5 \times 10^4$ cells/well and then incubated with UCNPs@AgBiS$_2$ (50 $\mu$g mL$^{-1}$) for 12 h. Subsequently, the cells were irradiated with an 808 nm laser (1 W cm$^{-2}$) for 3 min. Then, calcein (AM) and propidium iodide (PI) were added for the staining of living and dead 4T1 cells and incubated to form different fluorescent substances. Digital fluorescence photographs of the cells were captured using a fluorescence microscope.

2.6. Apoptosis

Apoptosis quantitatively explored by flow cytometry. Usually, 4T1 cells were seeded into a 6-well plate at $1 \times 10^5$ cells/well and then incubated with UCNPs@AgBiS$_2$ (50 $\mu$g mL$^{-1}$) for 12 h. Subsequently, the cells were irradiated with an 808 nm laser (1 W cm$^{-2}$) for 3 min. After that, the cells were digested with trypsin and stained with Annexin V-FITC/PI, and the rate of apoptosis was quantitatively determined by flow cytometry analysis.

2.7. Characterization

The surface morphology, phase, fluorescence, optical properties, X-ray diffraction (XRD) and X-ray photoelectron spectra (XPS) of these products were investigated carefully according to our previous protocol or instruments [33]. Photothermal performance, ROS, hydroxyl radical (·OH) and singlet oxygen ($^1$O$_2$) detection were studied via our previously reported protocol. All animal experimental protocols were investigated carefully according to our previous protocol or instruments. All animal experiments were approved by the Ethical Committee of Anhui Medical University (approved number: LLSC20210077).

Fig. 1. (a) Schematic illustration of the synthesis of UCNPs@AgBiS$_2$ core-shell NPs. (b) FESEM, (c) TEM and (d) HRTEM images, (e) XRD pattern of UCNPs@AgBiS$_2$ core-shell NPs. (f) Representative STEM image and (g–m) elemental mapping images of Ag, Bi, S, F, Y, Yb and merged image of UCNPs@AgBiS$_2$ core-shell NPs.
3. Results and discussion

3.1. Synthesis and characterization of UCNPs@AgBiS\textsubscript{2} core-shell NPs

UCNPs@AgBiS\textsubscript{2} NPs were fabricated via an ion exchange reaction using UCNPs@ZnS core-shell NPs as sacrificial templates (Fig. 1a). UCNPs and UCNPs@AA-[Zn(OH)\textsubscript{4}]\textsuperscript{2−} and UCNPs@ZnS NPs with uniform morphology and excellent dispersion were synthesized according to our previous report (Fig. S1, supporting information) [35]. Fig. 1b and c showed that the as-prepared UCNPs@AgBiS\textsubscript{2} NPs consisted of uniform spherical structure with an average size in approximately 80 nm. In the high-resolution transmission electron microscopy (HRTEM) image taken from the marginal area of UCNPs@AgBiS\textsubscript{2} NPs, lattice spacings of 2.99 and 3.26 Å were assigned to the (110) and (111) planes of hexagonal NaYF\textsubscript{4} and cubic AgBiS\textsubscript{2}, respectively (Fig. 1d) [33]. Moreover, XRD patterns of the final samples were shown in Fig. 1e, which confirmed that the sample was composed of the cubic phase of AgBiS\textsubscript{2} (JCPDS No. 21–1178) and hexagonal NaYF\textsubscript{4} (JCPDS No. 28–1192). Therefore, based on the above analysis, AgBiS\textsubscript{2} was proven to be successfully coated on the surface of UCNPs.

Furthermore, as displayed in Fig. 1f–m and Fig. S2, the corresponding elements were confirmed by elemental mapping, and Ag, Bi, and S elemental signals were captured in the outer shell, while the other elements were detected inside, indicating that core-shell NPs were apparent with hexagonal UCNPs inside (~50 nm diameter) and the shell layer of AgBiS\textsubscript{2} (~30 nm shell thickness). The dynamic light scattering (DLS) size of UCNPs@AgBiS\textsubscript{2} was around 100 nm and the zeta potential was $-3.45 \pm 0.4$ mV (Fig. S3). The content of UCNPs@AgBiS\textsubscript{2}-related elements (e.g., F, Na, S, Y, Ag, Nd, Yb, Er, and Bi) was detected by Energy Dispersive X-Ray Spectroscopy (EDX) analysis (Table S1). The surface components and chemical states of the elements were further elucidated by XPS, in which the survey spectrum indicated that the as-prepared materials contained Ag, Bi, S, Y, Na, F, Yb, Nd and Er elements (Fig. S4). In addition, the binding energies of Ag located at 367.90 and 374.27 eV were deconvoluted to 3d\textsubscript{5/2} and 3d\textsubscript{3/2}, respectively, indicating the successful formation of pure AgBiS\textsubscript{2} NPs. Moreover, the binding energies for Y, Na, F, Yb, Nd and Er were weak, which was also clearly observed because of UCNPs embedded in the shell layer of AgBiS\textsubscript{2}. These results coherently verified that UCNPs@AgBiS\textsubscript{2} core-shell NPs were successfully fabricated.

3.2. Optical and photothermal properties of UCNPs@AgBiS\textsubscript{2} core-shell NPs

Desired NIR absorption and excellent photothermal conversion efficiency are the basis for the use of photothermal reagents in PTT. Fig. 2a showed digital photographs of UCNPs@AgBiS\textsubscript{2} aqueous solutions with different concentrations, and the color deepened as the concentration increased. According to the corresponding UV–vis–NIR absorption Fig. 2.

(a) Digital photograph of UCNPs@AgBiS\textsubscript{2} at various concentrations. (b) UV–Vis–NIR absorption spectra of UCNPs@AgBiS\textsubscript{2} dispersions at various concentrations. (c) Photothermal heating curve of UCNPs@AgBiS\textsubscript{2} dispersions at various concentrations. (d) Thermal images of UCNPs@AgBiS\textsubscript{2} solutions at various concentrations upon 808 nm laser irradiation (1 W cm\textsuperscript{2}). (e) Temperature change of different nanoparticles with different Nd doping concentrations under 808 nm laser irradiation. (f) Temperature changes of UCNPs@AgBiS\textsubscript{2} dispersions under 808 nm laser irradiation at various power densities. (g) Heating/cooling curve of UCNPs@AgBiS\textsubscript{2} nanoparticles after repeatedly turning on/off laser irradiation for five cycles. (h) Photothermal conversion efficiency (η) change of different nanoparticles with different Nd doping concentrations under laser on/off. (i) Schematic illustration of the generation of cross-relaxation pathways between Nd\textsuperscript{3+} ions and AgBiS\textsubscript{2}.
spectrum (Fig. 2b), UCNPs@AgBiS2 NPs with different concentrations showed wide and strong optical absorption in the NIR region, which proved the possibility of their high photothermal conversion performance. The distinguished photothermal effects with various concentrations of UCNPs@AgBiS2 NPs were investigated by an infrared camera with an 808 nm laser. Upon 808 nm laser irradiation, the temperature of the UCNPs@AgBiS2 NPs increased rapidly over time, indicating that UCNPs@AgBiS2 NPs had excellent photothermal effects (Fig. 2c–e, and Fig. S5). When the concentration of UCNPs@AgBiS2 suspension solution was kept at 100 μg mL⁻¹, the temperature increased from 25 to 56.5 °C after irradiation for 3 min. In contrast, only a slight increase of 2.3 °C took place for deionized water as a control group. Moreover, the power controllability and photothermal stability of UCNPs@AgBiS2 were explored, and the temperature of the UCNPs@AgBiS2 aqueous solution increased with laser power (Fig. 2f and g). Then, five laser ON/OFF cycles were employed to investigate the photostability of UCNPs@AgBiS2 core-shell NPs. In Fig. 2g and Fig. S6, after five laser cycles, the photothermal effect of UCNPs@AgBiS2 showed almost no obvious attenuation, highlighting its outstanding photostability. The time constant was 417.86 s, as shown in Fig. S7, the photothermal conversion efficiency (η) of the UCNPs@AgBiS2 (1% Nd) aqueous solution was calculated to be 27.5% on the basis of the heating-cooling profile, and the photothermal conversion efficiency increased with the Nd concentration (Fig. 2h). In contrast, the temperature of the Nd@Nd@AgBiS2 (100% Nd) suspension with a concentration of 100 μg mL⁻¹ was increased from 25 to 80.2 °C. As shown in Fig. 2h and Fig. S8, the photothermal conversion efficiency of Nd@Nd@AgBiS2 core-shell NPs were calculated to be 45.0%, which was higher than that of most widely studied PTT agents, such as Bi2S3 (26.8%, 28.1%), AgBiS2-TPP (23.5%), AgBiS2-PEI (21.3%), AgBiS2-PEI (35.2%) and AgBiS2 (36.51%) (Table S2) [26–29,36,37]. The doping of Nd ions facilitated the energy transfer and conversion of the as-prepared core-shell NPs, which could be attributed to the cross-relaxation (CR) process between Nd³⁺ ions and AgBiS2 (Fig. S9). The photons could be excited to the F⁵/₂ state and then descend to the F⁷/₂ state via non-radiation when the Nd³⁺ ions were exposed to 808 nm irradiation (Fig. 2i) [38,39]. In the F⁷/₂ state, the photon can be attenuated to a lower energy state by the irradiation process, resulting in 1008 nm (F⁷/₂ to 4I⁹/₂), 1058 nm (F⁷/₂ to 4I₁₁/₂), and 1332 nm (F⁷/₂ to 4I₁₃/₂) emission. CR between the F⁷/₂ to 4I₅/₂ and 4I₅/₂ to 6I₁₅/₂ states of different Nd³⁺ ions and other non-radiative transitioned to the ground state produces photothermal effects. When the photons in Nd@Nd dropped from 4F⁵/₂ to 4F⁷/₂, the photons in AgBiS2 could jump from the lower level to the higher level with the same energy difference as between 4F⁵/₂ and 4F⁷/₂, thus forming a shorter CR2 path, which can generate more heat energy in Nd@Nd. Moreover, the photothermal performance of UCNPs@AgBiS2 core-shell NPs could be adjusted by changing the concentration of Nd ions. Taken together, these results demonstrated that UCNPs@AgBiS2 NPs are excellent photothermal agents with outstanding photothermal performance under NIR laser irradiation.

Fig. 3. (a) The overlapping spectrum between the UV–Vis absorption spectra of UCNPs@AgBiS2 core-shell nanoparticles and the fluorescence spectra of UCNPs. (b) Fluorescence spectra of the UCNPs and UCNPs@AgBiS2 core-shell nanoparticles. The inset shows the corresponding enlarged images of UCNPs@AgBiS2. (c) The luminescence decays of the excited state levels of Er³⁺ at 521 and 540 nm for the UCNPs, UCNPs@AA-Zn(OH)₄, UCNPs@ZnS, and UCNPs@AgBiS2 core-shell nanoparticles, respectively. (d) Absorbance change at 650 nm of TMB in the presence of different nanoparticles with different Nd doping concentrations under 808 nm laser irradiation. (e) Intensity change at 425 nm of TAOH in the presence of different nanoparticles with different Nd doping concentrations under 808 nm laser irradiation, indicating the production of OH• species. (g) Absorbance change at 380 nm of ABDA in the presence of different nanoparticles with different Nd doping concentrations under 808 nm laser irradiation, indicating the production of ⋅OH species. (h) Schematic illustration of the enhanced generation of ROS between Er³⁺ ions and AgBiS2.
3.3. ROS production and detection for the UCNPs@AgBiS₂ core-shell NPs

The unique narrow band gap not only gives AgBiS₂ photothermal properties but also endows it the potential for ROS generation. As shown in Fig. 3a and Fig. S10, UCNPs@AgBiS₂ core-shell NPs possessed a wider and larger absorption peak at 550 nm, which indicated that the absorption peak of AgBiS₂ matched the upconversion luminescence (UCL) emission of the $^{4}S_{3/2} \rightarrow ^{4}I_{15/2}$ and $^{4}H_{11/2} \rightarrow ^{4}I_{15/2}$ transitions of Er$^{3+}$ [40]. The fluorescence emissions for the UCNPs@AgBiS₂ core-shell NPs were completely quenched (Fig. 3b), demonstrating the enhancement of fluorescence resonance energy transfer (FRET) efficiency between the UCNPs core and the AgBiS₂ shell, which agreed well with the aforementioned results. To further investigate the FRET efficiency of UCNPs@AgBiS₂ core-shell NPs upon 808 nm NIR laser excitation, the luminescence decays of the excited state levels of Er$^{3+}$ were detected for the UCNPs, UCNPs@AA-[Zn(OH)$_2$]$_2$, UCNPs@ZnS and UCNPs@AgBiS₂ NPs at 521 and 540 nm, respectively (Fig. 3c and d). Compared with UCNPs, UCNPs@AA-[Zn(OH)$_2$]$_2$ and UCNPs@ZnS, the FRET effect between UCNPs and AgBiS₂ was greatly enhanced, which demonstrated that high FRET efficiency was achieved. Previous studies revealed that composite nanostructure-incorporated UCNPs and semiconductors showed desired ROS production ability under NIR laser irradiation owing to their energy transfer between the core and shell components [41]. Then, 3,5,5-tetramethylbenzidine (TMB) was used to detect the generation of ROS, and the absorbance increased after the addition of UCNPs@AgBiS₂ NPs, indicative of ROS generation (Fig. 3e and Fig. S11). Furthermore, the ROS species were confirmed by terephthalic acid (TAOH), and 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA). -OH was confirmed by fluorescence spectra under the probe of TAOH, and $^{1}O_2$ was detected by UV–Vis spectra to verify the destruction of ABDA [33,42]. As shown in Fig. 3f-g and Fig. S12-13, the fluorescence intensity at 425 nm of TAOH increased and the absorption intensity at 380 nm of ABDA decreased with illumination time, indicating that UCNPs@AgBiS₂ could produce large amounts of ROS under 808 nm laser irradiation. Although AgBiS₂ theoretically had the ability to generate ROS under 808 nm laser irradiation, the ROS generated by pure AgBiS₂ was far less than that of UCNPs@AgBiS₂ under the same conditions [37]. In general, UCNPs@AgBiS₂ NPs excited by 808 nm laser displayed a favorable ability to produce ROS for PDT, which may be ascribed to the excellent energy transfer between AgBiS₂ and UCNPs. Based on the above results, the proposed ROS generation mechanism under the NIR response was proposed, including continuous Nd$^{3+}$ → Yb$^{3+}$ → activator energy transfer, which activated AgBiS₂ through energy transfer to generate ROS (Fig. 3h). First, Nd$^{3+}$ ions in the active core/shell UCNPs were excited to the $^{4}F_{5/2}$ state under 808 nm laser irradiation and then relaxed to the $^{4}F_{7/2}$ state under non-irradiation conditions. Energy could be transferred through the shell to nearby Yb$^{3+}$ ions and filled into their $^{4}F_{5/2}$ state, which eventually acted as an effective bridge to relay the energy to Er$^{3+}$ ions. Additionally, this energy transfer process initiated a typical upconversion process in the core, where Er$^{3+}$ ions were excited to high energy levels, such as $^{4}S_{9/2}$, $^{4}S_{3/2}$, and $^{4}H_{11/2}$. The excited electron of Er$^{3+}$ relaxed into the ground state and emitted green light [43–45]. AgBiS₂ could be activated by means of FRET and then react with O$_2$ and H$_2$O in the surrounding environment to produce -OH and $^{1}O_2$. As expected, the as-prepared UCNPs@AgBiS₂ core-shell NPs possessed excellent ROS generation performance for $^{1}O_2$ and -OH to kill tumor cells by combining PTT and PDT.

3.4. In vitro NIR activated PTT-PDT

The excellent photothermal/photodynamic effects of UCNPs@AgBiS₂ core-shell NPs prompted us to study their killing effect on cancer cells. Fig. S14 showed the confocal laser scanning microscopy (CLSM) images of the co-cultivation of Nile Red (NR) loaded UCNPs@AgBiS₂ with 4T1 cells, in which the concentration of UCNPs@AgBiS₂-NR were increased, the red fluorescence of NR continued to deepen in the cells, proving UCNPs@AgBiS₂ has cells ability to internalize. Then, human umbilical vein endothelial cells (HUVECs) and mouse mammary epithelium cells (HCC1) were employed as a normal cell model to evaluate the cytotoxic effect of UCNPs@AgBiS₂ and Nd@Nd@AgBiS₂ by MTT assay [46]. As shown in Fig. 4a and Fig. S15, cell viability was maintained above 95%, while the concentration of UCNPs@AgBiS₂ was up to 160 μg mL$^{-1}$. In addition, the spectrophotometric method with 3,3’-Biocyanin-PADAP was adopted to evaluate the leakage of Ag ions from UCNPs@AgBiS₂ within 24 h in aqueous solution [46, 47]. It was revealed that the UCNPs@AgBiS₂ showed superior chemical stability, and no leakage of Ag ions was observed (Fig. S16). In vitro cytotoxic effects of UCNPs@AgBiS₂ on 4T1 cells were investigated under NIR at different times (0, 1, and 3 min). The cell viability decreased slightly when treated with only UCNPs@AgBiS₂ or Nd@Nd@AgBiS₂, which could be attributed to the cell-specific cytotoxicity of AgBiS₂ (Fig. 4b-c and Fig. S17) [33]. Furthermore, almost no cells remained alive when the concentration was up to 160 μg mL$^{-1}$ under irradiation for 3 min. In contrast, the cells still maintained a high survival rate without NIR laser irradiation. In particular, the UCNPs@AgBiS₂ core-shell NPs showed the best therapeutic efficacy toward cancer cells owing to the production of ROS compared to Nd@Nd@AgBiS₂ core-shell NPs. Ascorbic acid (VC, 100 μg mL$^{-1}$) as a reducing agent, has been used to protect the cells against ROS and was added to 4T1 cells treated with UCNPs@AgBiS₂ upon irradiation with an NIR laser [48]. The Nd@Nd@AgBiS₂ core-shell NPs demonstrated better photothermal therapeutic efficacy, which could be attributed to their superior photothermal effect. Herein, UCNPs@AgBiS₂ core-shell NPs showed extraordinary therapeutic efficiency for combined PTT-PDT of cancer cells [49–51]. The CLSM images forthrightly demonstrated the production of ROS within the cell, which was due to the green fluorescence of DCF converted from the nonfluorescent ROS probe DCFH-DA (Fig. 4d) [52]. No green fluorescence representing ROS was observed when only treatment with PBS or NIR laser. When UCNPs@AgBiS₂ or Nd@Nd@AgBiS₂ was added, slight green fluorescence in 4T1 cells was observed, indicating that UCNPs@AgBiS₂ had the ability to catalyze H$_2$O$_2$ in the tumor microenvironment to generate ROS, which corresponded with the results of the cell experiments. The CLSM images illustrated the highest green fluorescence in the cytoplasm of the irradiated group. In addition, the UCNPs@AgBiS₂ group showed stronger green fluorescence than the Nd@Nd@AgBiS₂ group, suggesting that UCNPs could convert 808 nm light into higher energy visible light to excite AgBiS₂ with a low bandgap to produce more ROS. In addition, the flow cytometry techniques were also used to quantitatively analyze the production of intracellular ROS. In the presence of UCNPs@AgBiS₂ or Nd@Nd@AgBiS₂, the fluorescence intensity of DCF increased slightly, and the fluorescence intensity of the irradiated group was higher than that of the nonirradiated group, which was consistent with the CLSM images (Fig. 4e). A large amount of reinforced lethal intracellular ROS was generated by laser excitation of UCNPs@AgBiS₂, which was concluded by the results of CLSM observation and flow cytometry analysis [53]. Furthermore, the cancer cell killing effect of UCNPs@AgBiS₂ was investigated by living and dead cell staining with Calcein-AM and propidium iodide (PI) [54]. Fluorescence imaging of 4T1 cells stained with calcein-AM and propidium iodide (PI) revealed that UCNPs@AgBiS₂ core-shell NPs caused cancer cell death in a laser time-dependent manner (Fig. 4f). Finally, the flow cytometry with Annexin V-fluorescein isothiocyanate/propidium iodide (FITC/PI) staining was used to analyze apoptosis. As shown in Fig. 4g, the results showed that apoptosis was greatly increased in the presence of both Nd and UCNPs@AgBiS₂. The above data fully demonstrated that UCNPs@AgBiS₂ could efficiently kill cancer cells by combining PTT and PDT. Collectively, these results indicated that UCNPs@AgBiS₂ could efficiently convert 808 nm light into higher energy visible light to excite AgBiS₂, then increasing the production of ROS, thereby achieving a higher cell killing effect.
Fig. 4. (a) Cell toxicity of HUVECs after incubation with various concentrations of UCNPs@AgBiS$_2$ and Nd@Nd@AgBiS$_2$. (b–c) Apoptosis ablation of 4T1 cells incubated with various concentrations of UCNPs@AgBiS$_2$ and Nd@Nd@AgBiS$_2$ under irradiation (808 nm, 1.0 W cm$^{-2}$). ROS analysis of 4T1 cells stained by DCFH-DA. (d) CLSM images and (e) flow cytometry analysis. Scale bar = 50 μm. (f) Fluorescence microscopy images of 4T1 cells after different treatments as indicated. Scale bar = 500 μm. (g) Flow cytometric analysis of 4T1 cell apoptosis induced by different treatments with Annexin V-FITC/PI staining.
3.5. In vivo combined PTT-PDT

Encouraged by the excellent effect of UCNPs@AgBiS$_2$ NPs against 4T1 cells, 4T1 tumor-bearing BALB/c mice were employed to investigate the in vivo phototherapeutic effect of USP. The in vivo thermal behaviors of UCNPs@AgBiS$_2$ were accurately evaluated by tracking the heat signal under an infrared thermal camera on 4T1 tumor mice (Fig. 5a-c) [55]. The in-situ temperature of the tumors treated with UCNPs@AgBiS$_2$ rapidly increased under 808 nm laser irradiation (0.5 W cm$^{-2}$, 10 min). Because the temperature of the tumor regions reached 56.3$^\circ$C, the cells in tumor sites were ablated, and their malignant proliferation was inhibited effectively. In contrast, the temperature of tumors in the control group was not obviously changed. Inspired by the excellent photothermal effect of UCNPs@AgBiS$_2$ in vivo, the in vivo tumor growth inhibition effect was investigated after intratumoral injection. When the volume of the tumor reached around 100 mm$^3$, 4T1 cell-bearing mice were randomly divided into the following four groups (n = 5): 1) control group; 2) NIR laser-only group; 3) UCNPs@AgBiS$_2$ group; and 4) UCNPs@AgBiS$_2$ + NIR laser group. It was well known that the change in tumor volume and body weight within 14 days was the immediate performance of treatment effect and safety. During the treatment period, the body weight of each group maintained a steady increase (Fig. 5d), which indicated that the treatment of UCNPs@AgBiS$_2$ with PDT/PTT had no obvious systemic toxicity. Taken together, the tumor growth curves of all groups are shown in Fig. 5e. Group 4 treated with UCNPs@AgBiS$_2$ core-shell NPs under 808 nm laser irradiation caused the

![Fig. 5.](image_url)

*(Statistical analysis was performed using a t-test: ***P < 0.001)*.
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### Acknowledgment

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioactmat.2022.01.010.

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