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Actively Targeted Deep Tissue Imaging and Photothermal-Chemo Therapy of Breast Cancer by Antibody-Functionalized Drug-Loaded X-Ray-Responsive Bismuth Sulfide@Mesoporous Silica Core–Shell Nanoparticles

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Conflict of Interest

The authors declare no conflict of interest.

Supporting Information

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Abstract

A theranostic platform combining synergistic therapy and real-time imaging attracts enormous attention but still faces great challenges, such as tedious modifications and lack of efficient accumulation in tumor. Here, a novel type of theranostic agent, bismuth sulfide@mesoporous silica ($\text{Bi}_2\text{S}_3@ \text{mPS}$) core-shell nanoparticles (NPs), for targeted image-guided therapy of human epidermal growth factor receptor-2 (HER-2) positive breast cancer is developed. To generate such NPs, polyvinylpyrrolidone decorated rod-like Bi_2S_3 NPs are chemically encapsulated with a mesoporous silica (mPS) layer and loaded with an anticancer drug, doxorubicin. The resultant NPs are then chemically conjugated with trastuzumab (Tam, a monoclonal antibody targeting HER-2 overexpressed breast cancer cells) to form Tam- $\text{Bi}_2\text{S}_3@ \text{mPS}$ NPs. By in vitro and in vivo studies, it is demonstrated that the Tam- $\text{Bi}_2\text{S}_3@ \text{mPS}$ bear multiple desired features for cancer theranostics, including good biocompatibility and drug loading ability as well as precise and active tumor targeting and accumulation (with a bismuth content in tumor being ≈ 16 times that of nontargeted group). They can simultaneously serve both as an excellent contrast enhancement probe (due to the presence of strong X-ray-attenuating bismuth element) for computed tomography deep tissue tumor imaging and as a therapeutic agent to destruct tumors and prevent metastasis by synergistic photothermalchemo therapy.

Keywords

active tumor targeting; photothermal-chemo therapy; porous silica; theranostic nanoparticles; X-ray computed tomography

1. Introduction

Breast cancer is the most common cancer for women in the world. Especially, the human epidermal growth factor receptor 2 (HER-2) is overexpressed in 25% breast cancer patients and the HER-2 positive tumors are highly malignant, easy to metastasis, resistant to hormone and chemotherapy, and exhibit poor prognosis.^[1] To increase the efficiency of diagnosing and treating this type of breast cancer, it is urgent to develop a new method that can achieve both early accurate diagnosis and targeted therapy. Multifunctional theranostic nanoplateforms integrated with diagnostic and therapeutic functions have attracted considerable attention in cancer nanomedicine research.^[2] Currently, many studies have adopted passive targeting strategies, based on the enhanced permeability and retention (EPR) effect, to target nanoparticles (NPs) to tumors.^[3] However, the passive targeting strategies are still limited by tumor types.^[4] Modifying NPs with peptides^[5] or antibodies^[6,7] has been proposed to effectively solve the above problems because it further enhanced the accumulation in tumor sites.^[8] Moreover, integrating multiple types of therapeutic drugs and methods together on the same NPs will significantly enhance the efficacy of cancer therapy.

Many nanotheranostic platforms, which can be excited by magnetism, light, ultrasound, or X-ray, have been established in the past years, including nanomaterials of Fe_3O_4 ,^[9] Gd_2O_3 ,^[10] gold,^[11] carbon,^[12] and semiconductors.^[13] Among these nanomaterials, the NPs that can be applied in deep tissue imaging, such as X-ray computed tomography (CT) imaging, are more appealing for clinical use. X-ray-responsive NPs are particularly popular for their high spatial resolution in 3D reconstruction of interested tissues and areas by CT imaging. Bismuth (Bi)-based NPs hold great promise as novel X-ray CT imaging contrast agents due to their high atomic number, great ability in attenuating X-ray, and long residence time.^[14] Moreover, Bi is the earliest reported drug used in clinics and its biocompatibility has been carefully and comprehensively investigated.^[15] In addition, the narrow direct bandgap (≈ 1.3 eV) of bismuth sulfide makes it possible to absorb near infrared (NIR) light to initiate the photothermal cancer therapy. To date, various shapes of bismuth sulfide NPs have been exploited, including spheres^[16] and rods^[17] for photothermal therapy (PTT) or CT contrast imaging in addition to ternary nanomaterials Cu_3BiS_3 .^[18] Meanwhile, bismuth sulfide NPs with a wide range of size from 5 to 300 nm have also been investigated for in vivo CT imaging.^[19]

Previous reports suggested that Bi_2S_3 NPs can be an ideal nanotheranostic platform.^[20] However, so far, the surface functionalization on Bi_2S_3 NPs has been difficult, limiting the capability of loading drugs on the NPs, and the past studies on Bi_2S_3 NPs adopted only EPR-based passive tumor targeting, limiting the efficacy of targeted cancer therapy and imaging as well as the early cancer diagnosis. This study was designed to solve these problems. Specifically, we form a mesoporous silica (mPS) shell on the Bi_2S_3 NPs by a

modified Stöber method without using a surfactant such as cetyltrimethylammonium bromide and chloride, allowing us not only to conjugate functional molecules (such as an antibody that can target breast cancer along with a polyethylene glycol (PEG) linker to ensure the long circulation and active tumor targeting of the NPs) but also to load the chemotherapeutic drug (doxorubicin, DOX) in the porous shell (Scheme 1).

In our approach, water-soluble polyvinylpyrrolidone (PVP)-decorated Bi_2S_3 NPs (PVP- Bi_2S_3) were prepared by a facile hydrothermal method and then coated with a thin PS shell. The resultant NPs, termed $\text{Bi}_2\text{S}_3@\text{mPS}$, can function as an excellent photothermal agent as well as anticancer drug reservoir and also support further modification due to the abundant Si-OH groups on their surface. Trastuzumab (HER-2 monoclonal antibody, Tam), which specifically recognizes the HER-2 receptor on the HER-2 positive SKBR-3 breast cancer cells, was then conjugated to the as-synthesized $\text{Bi}_2\text{S}_3@\text{mPS}$ through a PEGbased linker (NHS-PEG-Mal, for improving in vivo biocompatibility and stability of the NPs and linking with the antibody). The resultant NPs, termed Tam- $\text{Bi}_2\text{S}_3@\text{mPS}$, were then loaded with an anticancer drug, DOX, to form Tam- $\text{Bi}_2\text{S}_3@\text{mPS}/\text{DOX}$ NPs due to the presence of a PS shell. The final Tam- $\text{Bi}_2\text{S}_3@\text{mPS}/\text{DOX}$ NPs were intravenously (i.v.) injected into tumorbearing mice model (generated by injection of SKBR-3 breast cancer cells) and then efficiently transported to the tumor site due to the active targeting of Tam. The NPs then function as CT contrast enhancement agents for the detection of the tumor sites for cancer diagnosis. In the meantime, the NPs will destruct the tumor by a combination of photothermal and chemotherapeutic therapy (i.e., photothermal-chemo therapy). The PTT arises from the photothermal effect of the Bi_2S_3 NPs in response to 808 nm NIR laser irradiation. The chemotherapy results from the DOX released from the NPs. The synergistic photothermal and chemotherapeutic effect of the Tam- $\text{Bi}_2\text{S}_3@\text{mPS}/\text{DOX}$ NPs effectively destructed the tumor cells without tumor recurrence. Thus, Tam- $\text{Bi}_2\text{S}_3@\text{mPS}/\text{DOX}$ NPs can serve as an effective tumor-targeting platform that can simultaneously achieve actively targeted cancer imaging/diagnosis (by CT) and photothermal-chemo therapy.

2. Results and Discussion

2.1. Design, Fabrication, and Characterization of Tam- $\text{Bi}_2\text{S}_3@\text{mPS}$

The hydrophilic PVP-decorated Bi_2S_3 (PVP- Bi_2S_3) NPs were first synthesized by a facile hydrothermal method. X-ray diffraction (XRD) was employed to investigate the composition and purity of the PVP- Bi_2S_3 NPs. All the peaks in the XRD pattern (Figure S1, Supporting Information) could be well indexed to the orthorhombic Bi_2S_3 crystal (JCPDS No. 65–2431). Silica was used to coat the NPs because it is biocompatible and can facilitate further antibody modification for clinical application.^[21] Moreover, when silica was porous, it can load drug due to the high specific surface area and large pore volume in addition to the well-established surface modification chemistry.^[22] Hence, we turned to a modified Stöber procedure^[23] to coat a porous layer on the PVP- Bi_2S_3 NPs (Figure 1a). Excitingly, during the silica-coating process, an mPS layer appeared on the NPs (Figure 1e,f). The mPS layer was formed because PVP molecules originally trapped in the NPs were dispersed into the silica shell during the silica-coating process. Hence, the mPS shell structure was obtained after the PVP molecules were washed away from the NPs finally.^[24]

Figure 1b,c shows the representative transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM) images of PVP-Bi₂S₃. The PVP-Bi₂S₃ NPs are rod-like with a diameter of about 26 nm and a length of up to 100 nm. The interplanar crystal spacing is ≈ 0.56 nm (Figure 1c), which is consistent with the lattice spacing of the (200) plane of Bi₂S₃. This result was further confirmed by selected area electron diffraction (SAED) (Figure 1d). After PS coating, Bi₂S₃@mPS NPs have a well-defined rod-like structure of around 130 nm in length and 60 nm in width (Figure 1e,f). The high-angle annular dark-field scanning TEM (HAADFSTEM) elemental mapping images confirmed the distribution of Bi, S, Si, and O elements, clearly confirming the presence of Bi₂S₃ cores and mPS shells. Nitrogen adsorption–desorption isotherm was employed to investigate the porous structure, the results (Figure 1g,h) confirmed that the Bi₂S₃@mPS possessed a higher specific surface area ($126.383 \text{ cm}^2 \text{ g}^{-1}$) and a larger pore volume ($0.348 \text{ cm}^3 \text{ g}^{-1}$). The average pore size was calculated to be 6.48 nm. After coated with mPS, the NPs zeta potential changed from neutral to -25 mV (Figure S2, Supporting Information).

To improve the in vivo behavior including reduced accumulation in the reticuloendothelial system and enhanced active targeting effect, the Bi₂S₃@mPS was further modified with HER-2 antibody Tam through a PEG linker. First, the Bi₂S₃@mPS NPs reacted with (3-aminopropyl) triethoxysilane (APTES) for 48 h in ethanol to form NPs terminated with an amino group. Then the resultant Bi₂S₃@mPS-NH₂ reacted with NHS-PEG-Mal via Michael addition reaction^[25] to form Bi₂S₃@mPS-PEG-Mal NPs. The activated Bi₂S₃@mPS-PEG-Mal NPs were then covalently coupled to thiolated Tam (SH-Tam) through thiol-maleimide linkages. To further prove the successful surface modification, the absorption spectrum and Fourier transform infrared spectroscopy (FTIR) spectrum were collected. As shown in Figure 2a, the PVP-Bi₂S₃ exhibited a broad absorption from 400 to 900 nm. After the NPs were coated with silica, an enhanced absorption band appeared in the whole spectrum region due to the abundant $-\text{OH}$ in silica (Figure 2a). When conjugated with Tam, the samples presented a new strong absorption band in 250–300 nm, which was in accordance with the protein peak in Tam (Figure 2a). Later, the FTIR was employed to investigate the successful conjugation of Tam (Figure 2b). The peak that appeared at 3428 cm^{-1} corresponds to the $-\text{OH}$ groups in Bi₂S₃@mPS and Tam-Bi₂S₃@mPS samples. The vibration that showed up in 1422 cm^{-1} could be assigned to COO^- in Tam-Bi₂S₃@mPS. The new peaks in 1102 and 539 cm^{-1} correspond to the vibration of $-\text{S}-\text{R}-$ group, which further confirmed the successful conjugation of Tam with Bi₂S₃@mPS-NH₂. Furthermore, the zeta potential of Bi₂S₃@mPS-NH₂ was 20 mV and the final Tam-Bi₂S₃@mPS was nearly neutral (Figure S2, Supporting Information), which was in agreement with the FTIR results.

2.2. Drug Loading, Releasing, and Photothermal Efficiency

It is worthwhile to note that the thin mPS shell ensures that the Bi₂S₃@mPS NPs work as a good drug carrier while maintaining the high photothermal conversion (PTC) efficiency of Bi₂S₃ NPs. To prove this hypothesis, drug-loading efficiency and photothermal effect were tested on the NPs. First, the common chemotherapeutic drug, doxorubicin (DOX), used in breast cancer therapy was selected as a model drug.^[26] It was loaded with Bi₂S₃@mPS by mixing the NPs and the drug under constant stirring for 24 h. The loading capacity was found to be as high as 60.85 mg/100 mg due to the large pore size and surface area of

Bi₂S₃@mPS (Figure 2c). The drug release rate was also tested in an acidic or neutral environment. The DOX showed a quick release at pH 5.0 but a very slow release at pH 7.4 (Figure 2d). Moreover, when the temperature rose from room temperature to 37 °C, a faster release of DOX from the Bi₂S₃@mPS occurred in neutral or acidic phosphate buffered solution (PBS) solution. This phenomenon suggests that our NPs hold great promise for selective drug release in tumor in vivo because the tumor microenvironment was acidic (pH 4–5 in lysosomes and pH 5–6 in endosomes).^[27]

Although the PVP-Bi₂S₃ and Bi₂S₃@mPS have broad absorption peaks in the NIR region (Figure 2a), the silica coating may hinder the photothermal effect of the NPs. Hence, we compared the photothermal properties between PVP-Bi₂S₃ and Bi₂S₃@mPS. The photothermal properties of two samples were tested under 808 nm NIR laser (1 W cm⁻²) with pure water as a control. Both the PVP-Bi₂S₃ and Bi₂S₃@mPS NPs showed a thermal response under the NIR irradiation. When 400 µg mL⁻¹ PVP-Bi₂S₃ was tested, the solution could reach up to 65 °C under 10 min continuous irradiation (808 nm, 1 W cm⁻²). However, the temperature of pure water could only reach 28 °C. Luckily, after coated with mPS, the resultant Bi₂S₃@mPS (with the same concentration of Bi as PVP-Bi₂S₃) solution also could reach as high as 60 °C (808 nm, 1 W cm⁻²). According to these data, the PTC efficiencies of PVP-Bi₂S₃ and Bi₂S₃@mPS were calculated to be ≈32.46% and 29.53%, respectively (Figure 3b,d; the detailed calculation method was presented in the Supporting Information). The excellent NIR to thermal conversion efficiency of Bi₂S₃@mPS NPs promises them to be an excellent photothermal nanoagent.

As supported by the high PTC of Bi₂S₃@mPS, the photothermal effect of Bi₂S₃@mPS and targeted Tam-Bi₂S₃@mPS was also investigated in vivo (Figure 3c). After i.v. injection of the solution of NaCl, Bi₂S₃@mPS, and Tam-Bi₂S₃@mPS NPs, the hyperthermia effect in the tumor site was carefully recorded by an IR camera when a NIR light was irradiating the tumor site. In Bi₂S₃@PS group, the temperature in tumor site was about 40 °C after 10 min irradiation (808 nm, 1 W cm⁻²). It was higher than the temperature in the control group due to the EPR effect, but still slightly lower than the reported minimum temperature (41 °C)^[28] needed to destruct tumors by hyperthermia. Impressively, the temperature in Tam-Bi₂S₃@mPS group (up to 46 °C) was above the minimum hyperthermia temperature due to their good targeting to the tumor sites.

2.3. Cell Viability

The in vitro biocompatibility of Bi₂S₃@mPS, Tam-Bi₂S₃@ mPS, and DOX was carried out on SKBR-3 cells by a standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. As shown in Figure 4a, there was a negligible effect on the cells even for the Bi₂S₃@mPS with a concentration of up to 400 µg mL⁻¹, which verified its good biocompatibility. While for Tam-Bi₂S₃@mPS (400 µg mL⁻¹), the survival rate was slightly lower than 80% because of the Tam toxicity toward SKBR-3 cells. With the decrease in the concentrations of Tam-Bi₂S₃@mPS NPs, the NPs showed low cytotoxicity on SKBR-3 cells. Considering the cell biocompatibility and photothermal effect, 200 µg mL⁻¹ was selected as the concentration of Tam-Bi₂S₃@mPS NPs used for the following experiments. Under 1 W cm⁻² NIR irradiation, cell viabilities decreased when Tam and/or DOX was on

the NPs (Figure 4b). In addition, due to the high toxicity of DOX in vitro, the DOX-loaded carriers showed severe toxicity to cancer cells in vitro. Especially in Tam-Bi₂S₃@mPS/DOX+NIR group, the cell viability was lower than 15% due to the synergistic effect of photothermal-chemo therapy (Figure 4b).

The hemolytic properties of the NPs (Figure 4c) were tested to understand the response of blood to the intravenous NPs administration in vivo. No visual red color occurred in the Bi₂S₃@mPS group with a gradient concentration ranging from 12.5 to 400 µg mL⁻¹ and PBS group, illustrating the good blood biocompatibility of the as-synthesized NPs. The red color in pure water group was attributed to the hemoglobin from erythrocyte.

Cell viability was further investigated by fluorescence live/dead staining. There were negligible dead cells (red cells) in the control, NIR, and Bi₂S₃@mPS NPs treatment groups. In contrast, all of the cells in Tam-Bi₂S₃@mPS/DOX+NIR group were dead with a round shape (red color), which was consistent with the MTT results. These results illustrated an enhanced combined photothermal-chemo effect for in vitro cancer therapy.

2.4. In Vitro Cancer Cell Targeting Effect

SKBR-3 breast cancer cells overexpress HER-2. HER-2 is associated with enhanced cell proliferation and survival, increased disease progression risk, and decreased overall survival rate. Tam is a famous monoclonal antibody directly against the HER-2 and has been clinically recommended for HER-2 positive cancers including breast cancer and gastric cancer.^[29] Therefore, it has been selected for in vitro and in vivo HER-2 positive breast cancer targeting in this study. The FITC-labeled NPs were investigated by fluorescence microscope and flow cytometry. After being cocultured with FITC-labeled NPs (Tam-Bi₂S₃@mPS NPs or Bi₂S₃@mPS NPs) or free FITC in dark for 24 h, a rigorous washing protocol was carried out to ensure that all noninternalized particles were removed from the cell surface prior to fluorescence imaging and flow cytometry analysis. The SKBR-3 cells cocultured with Tam-Bi₂S₃@mPS-FITC NPs presented strong and well-proportioned green fluorescence, whereas a very weak fluorescence was observed in Bi₂S₃@mPS-FITC under the same condition (Figure 5a). These results confirmed the cancer cell targeting capability of Tam-modified NP groups. The flow cytometry results further indicated the targeted effect (Figure 5b). Namely, after incubation with different groups for 24 h, the mean fluorescence intensity of Tam-Bi₂S₃@mPS group also showed the most distinguished enhancement (Figure 5c).

To further prove that Tam-Bi₂S₃@mPS NPs could selectively target HER-2 positive cancer cells, we examined the interaction of FITC-labeled Tam-Bi₂S₃@mPS NPs with HER-2 negative MCF-7 cells using the fluorescence microscopy. We found that MCF-7 cells incubated with Bi₂S₃@mPS-FITC or Tam-Bi₂S₃@mPS-FITC showed similar but very weak fluorescence from FITC (Figure S5, Supporting Information). This result implies that Tam-Bi₂S₃@mPS NPs could not target HER-2 negative cancer cells, further confirming the capability of Tam-Bi₂S₃@mPS in targeting HER-2 positive cancer cells.

2.5. In Vivo Targeted Imaging

The in vitro results encouraged us to further investigate Tam-Bi₂S₃@mPS in vivo. Bismuth-based nanomaterials such as Bi₂S₃ [30] and Bi₂Se₃ [31] are proved to be excellent candidates in CT contrast imaging agents due to their enhanced X-ray attenuation coefficient. We first investigated and compared the Hounsfield units (HU) values of Tam-Bi₂S₃@mPS and iobitridol (the third generation CT contrast agent as a gold standard) with different concentrations. The CT signal was found to be gradually enhanced with the increase of Tam-Bi₂S₃@mPS concentration (Figure 6a). Eventually, the Tam-Bi₂S₃@mPS NPs exhibited even a higher CT value per unit (42.58) with mass concentration than iobitridol (26.91) and iopromide (16.38) and previously reported Bi₂S₃ materials. [16,30,32] Moreover, we examined the noble metal gold nanorods under the same condition, and found the slope of the CT contrast value versus the concentration is ≈ 31.26 for the gold nanorods (Figure S6, Supporting Information), which is lower than that for the Tam-Bi₂S₃@mPS (42.58). Considering the strong enhancement of X-ray attenuation, we assessed the targeting and CT imaging effect of Tam-Bi₂S₃@mPS NPs at 2, 6, and 24 h after i.v. injection of NPs into tumor-bearing mice. As shown in Figure 6b, the tumor site exhibited an enhanced imaging contrast after i.v. injection of Tam-Bi₂S₃@mPS compared to the peritumoral tissue. Such a high contrast will be helpful for precise cancer diagnosis and resection in clinical application. Notably, the CT contrast image was still clear 24 h after i.v. injection, suggesting the excellent and stable accumulation of NPs in tumor site. These results indicate that the special rod-like structure of the Tam-Bi₂S₃@mPS NPs has advantages over the spherical NPs in interaction with target cells and prevention from extravasation, as illustrated by Mitragotri et al. [33] Meanwhile, with the increase in time, the spleen CT intensity was gradually decreased while there was only ignorable reduction in the CT intensity in the tumor site. It is worthwhile to note that the tumor profile was clearly revealed by CT imaging due to the rich blood vessels in this area although the NPs can hardly reach the inner part of the tumor site because of the heterogeneous and leaky distribution of vascular patterns. [34] We also compared the CT intensity of the main organs and tumors in the untargeting (i.e., non-Tam-modified) group with the targeting (i.e., Tam-modified) group (Figure 6). We found that the tumor site had almost no enhanced imaging in the untargeting group. Overall, as Figure 6b illustrates for in vivo imaging, 10 mg mL⁻¹ Tam-Bi₂S₃@mPS shows enough CT contrast for imaging because Tam-Bi₂S₃@mPS can target the tumor and thus accumulate within the tumor sites.

To further prove the targeting effect of Tam-modified group, the tissue distribution of bismuth was also carefully quantified by ICP-MS (Figure S3, Supporting Information) in tumor-bearing mice 24 h after intravenous injection. In Bi₂S₃@mPS group, average splenic uptake was determined to be 151.3 $\mu\text{g g}^{-1}$, while the uptake in liver, lung, and tumor site was 92.9, 117.5, and 12.44 $\mu\text{g g}^{-1}$, respectively. After modified with Tam, a significantly increased bismuth content (195.2 $\mu\text{g g}^{-1}$) was found in the tumor and this value was about 16 times that of the nontargeting Bi₂S₃@mPS group. In contrast, the bismuth in the peritumoral tissues was not so high in the Tam-modified group (Figure S2, Supporting Information), indicating the efficient targeting effect. It's worth noting that only little bismuth was found in the lung site in the Tam-Bi₂S₃@mPS group, probably because PEG and Tam modification on the NPs could avoid nonspecific aggregation and decrease the

accumulation in lung.^[35] Therefore, the Tam-modified NPs have a great potential in serving as a tumor-targeting CT contrast enhancement agent for cancer diagnosis.

2.6. In Vivo PTT and Chemotherapy Effect

The efficient Bi₂S₃ accumulation in the tumor site is beneficial to cancer diagnosis and therapy. The targeted photothermalchemo effect of Tam-Bi₂S₃@mPS/DOX was further investigated in vivo, which lays foundation for future anticancer application. As shown in Figure 7a,b, visual images of tumors in different groups on day 14 post treatment can be seen. In the Tam-Bi₂S₃@mPS+NIR group, though the tumor was inhibited by the hyperthermia effect during the therapy period, there were still some residual tumors after 14 d. Excitingly, the tumor volume in Tam-Bi₂S₃@mPS/DOX+NIR group became reduced significantly and the tumor even disappeared. A more detailed dynamic change in the tumor volume and body weight was recorded. As shown in Figure 7c, the tumors grew rapidly in the control, DOX, and Tam-Bi₂S₃@mPS/DOX groups. In the nontargeting Bi₂S₃@mPS/DOX+NIR or targeting Tam-Bi₂S₃@mPS+NIR groups, tumor size was not completely suppressed and still presented a growth trend due to the insufficient hyperthermia (for the lack of NP accumulation although with DOX) or lack of DOX toxicity at the tumor site. In contrast, the tumors in Tam-Bi₂S₃@mPS/DOX+NIR group became smaller and even disappeared over time, illustrating the excellent precise photothermal-chemo therapy. As Figure 7d shows, the body weight of the mice during the treatment always lies between 18 and 20 g for different treatment groups, suggesting that the treatment did not obviously influence the body weight. In addition, the histological changes of the tumors were also studied (Figure 7e). The histological staining of tumor sites in the control, DOX+NIR, and Tam-Bi₂S₃@mPS/DOX groups showed typical pathological characteristics of breast cancer and clear vascular structure, demonstrating negligible effect when only NIR light (i.e., without NPs) or Tam-Bi₂S₃@mPS/DOX (i.e., without NIR irradiation) was used to treat tumor. Tam-Bi₂S₃+NIR group showed that some tumor tissues were damaged but the recurrence of tumor was also found in this group. These results were consistent with the results on the tumor volume change (Figure 7c). For Tam-Bi₂S₃@mPS/DOX+NIR group, severe damage at the tumor site was identified, such as swelling cell nucleus, cell vacuoles, ambiguous intercellular gap as well as vague, and atrophy tumor structure. The hematoxylin and eosin (H&E) staining of tumor tissues demonstrated that NIR illuminated DOX-loaded nanocomposites caused more severe tumor cell damage and irreversible necrosis when compared with the other groups. Therefore, based on the excellent drug delivery ability and photothermal conversion effect, the NPs that include both the photothermal bismuth sulfide and DOX can also work as an efficient anticancer nanoagent in clinics.

2.7. H&E Staining

To further investigate the in vivo biocompatibility and therapy effect, the H&E stained images of the heart, liver, spleen, lung, kidney, intestine, brain, and peritumor muscle are collected (Figure S4, Supporting Information). There was no tissue damage or adverse effect in the Tam-Bi₂S₃@mPS/DOX+NIR treatment group. Namely, the glomerulus structure was clear and complete in the kidney section of the treatment group, hepatocytes were normal, and no pulmonary fibrosis or congestion was found, indicating the good biocompatibility of these NPs. However, in control groups, some SKBR-3 tumor cells appeared in peritumor

muscle and the tumor profile became vague, indicating the metabolism of the tumors in these groups. As expected, the histopathologic characteristics in the peritumoral muscle of Tam-Bi₂S₃@mPS+NIR and Tam-Bi₂S₃@mPS/DOX+NIR groups were completely normal due to targeting capability of the Tam on the NPs.

3. Conclusion

In conclusion, successful cancer-targeting Bi₂S₃ theranostic NPs were established. The multifunctional Tam-Bi₂S₃@mPS nanoplatfrom shows excellent biocompatibility, cancer-targeted bioimaging, as well as synergistic photothermal/chemotherapeutic therapy. Notably, thin PS coatings were formed on the PVP-Bi₂S₃ precursors, which guarantee the drug delivery effect but still maintaining good photothermal conversion efficiency. Meanwhile, after being modified with Tam, the nanocomposites show an effectively enhanced cancer-targeting effect. In addition, more than 20% Tam-Bi₂S₃@mPS nanocomposites were gathered in tumor site for in vivo imaging and therapy, suggesting that the NPs can work as a precise bomb for tumor tracing and synergistic photothermal-chemo therapy. More importantly, the simultaneous photothermal-chemo therapy based on Tam-Bi₂S₃@mPS/DOX under NIR laser could efficiently kill the tumor cells and prevent cancer metastasis. Hence, the Tam-Bi₂S₃@mPS/DOX NPs can provide significant contrast enhancement for CT imaging and cancer diagnosis as well as strengthen tumor ablation efficiency, enabling their bright future for clinical cancer diagnosis and therapy.

4. Experimental Section

Preparation of PVP-Bi₂S₃ NPs

The PVP-Bi₂S₃ NPs were prepared by a facile hydrothermal method. Briefly, 0.498 g bismuth nitrate pentahydrate was added to 70 mL ethylene glycol solution with constant stirring. After vigorous stirring for 30 min, 0.5 g PVP was added to the solution and stirred for 30 min. 2 mL deionized H₂O containing Na₂S·9H₂O (0.36 g) was added to the mixture dropwise and the mixture turned to black quickly. The solution was transferred into a 100 mL Teflon-lined autoclave, sealed, and heated up to 180 °C for 2 h in an electric furnace. Afterward, the autoclave was cooled naturally to room temperature, and the final products were collected by centrifugation and washed three times with ethanol and distilled water, respectively, to remove any possible remnants.

Preparation of Bi₂S₃@mPS and Bi₂S₃@mPS-NH₂

In a typical procedure, 20 mg PVP-Bi₂S₃ NPs were dissolved in 65 mL ethanol upon ultrasonication for 30 min. Subsequently, 2.8 mL concentrated ammonia aqueous solution (28%) was added dropwise and stirred for 30 min at room temperature. Then 65 µL tetraethoxysilane was dissolved in 6.5 mL ethanol and added dropwise to the solution at a rate of 0.5 mL min⁻¹ under vigorous stirring. After stirring for 6 h at room temperature, the products were separated by centrifugation and washed in sequence with ethanol and water. The final products were denoted as Bi₂S₃@mPS. For amino group modification, 20 mL (2 mg mL⁻¹) Bi₂S₃@mPS in absolute ethanol was allowed to react with APTES (1 mL) at room temperature for 48 h to form Bi₂S₃@mPS-NH₂. The final sample was again washed

with ethanol three times to get rid of free APTES. The concentration of NH_2 groups (nmol mL^{-1}) was measured with a Kaiser test kit.

Characterization

Optical absorption spectra of the samples were measured with a spectrophotometer (Perkin Elmer, USA) working in a spectral range from 200 to 1000 nm. The crystalline structure, size, and shape of the nanoparticles were observed by XRD and HRTEM. XRD was measured with a Siemens Kristalloflex 810 D-500 X-ray diffractometer (Karlsruhe, Germany) under an operating mode of 40 kV and 30 mA, with $\lambda = 1.5406 \text{ \AA}$ radiation. Bi_2S_3 NPs in solution were placed onto holey carbon-covered copper grids for HRTEM observation. The HRTEM images, HAADF-STEM image and element mapping were obtained from the particles with a Hitachi 2100 electron microscope with an accelerating voltage of 210 kV. FTIR spectra were measured on a PerkinElmer 580B IR spectrophotometer using the KBr pellet technique. N_2 adsorption/desorption isotherms were obtained on a Micromeritics ASAP Tristar II 3020 apparatus. Zeta potential measurements were determined with the Zetasizer Nano Z (Malvern, UK). UV-vis-NIR absorption spectra were measured by the multifunctional microplate reader (Thermo, USA). Pore size distribution was calculated by the Brunauer–Emmett–Teller method.

Cell Viability and In Vitro Photothermal-Chemo Therapy

The viability of PVP- Bi_2S_3 , Bi_2S_3 @mPS, and Tam- Bi_2S_3 @mPS NPs as well as DOX was carried out by breast cancer cell line SKBR-3 cells (purchased from the Type Culture Collection of the Chinese Academy of Sciences). Briefly, the cells were seeded onto the substrates at a density of 1×10^5 cells per cm^2 and cultured at 37°C for 12 h. Then Bi_2S_3 @mPS, Tam- Bi_2S_3 @mPS, and DOX with a gradient concentration of 0, 12.5, 25, 50, 100, 200, and $400 \mu\text{g mL}^{-1}$ were separately added to the plates. After the cells and the NPs or DOX were cocultured for 24 h, the MTT test was performed immediately after treatment.

Bi_2S_3 @mPS and Tam- Bi_2S_3 @mPS NPs loaded with $25 \mu\text{g mL}^{-1}$ DOX were cultured with SKBR-3 cells for another 6 h. Then the cells were washed with PBS and replaced with fresh medium and irradiated with or without 808 nm laser (1 W cm^{-2}) for 10 min. The standard MTT assay was carried out to determine the cell viabilities relative to the control untreated cells.

Live/Dead Staining

For viability staining studies, the cells were seeded on 96-well plates at a concentration of 5×10^4 cells cm^{-2} at 37°C with 5% CO_2 for 24 h. $100 \mu\text{L}$ of NPs (Bi_2S_3 @mPS and Tam- Bi_2S_3 @mPS) with or without $25 \mu\text{g mL}^{-1}$ DOX was added to the plate, then irradiated with or without 808 nm laser at a density of 1 W cm^{-2} for 10 min. The medium was removed and the adherent cells were subjected to Live/Dead staining following the manufacturer's protocol (Sigma, USA). The cells were then photographed using a fluorescence microscope (Olympus, BX51, Japan).

In Vitro Tam-Bi₂S₃@mPS Targeted

Fluorescence imaging and flow cytometry were carried out to evaluate the targeting capability of Tam-Bi₂S₃@mPS. The FITC labeling of the NPs was done following our previous report.^[36] Tam-Bi₂S₃@mPS-FITC, Bi₂S₃@mPS-FITC, or FITC were cocultured with HER-2 positive SKBR-3 cells or HER-2 negative MCF-7 cells for 24 h. The cells were washed three times with PBS, and then photographed using a fluorescence microscope (Olympus, BX51, Japan). For flow cytometry analysis, the same procedures were carried out, and then the cells were collected and analyzed with a BD FACSCalibur flow cytometer (BD, USA). The results were analyzed by FlowJo 7.6.1.

In Vivo Experiments—Animals

Female nude mice (6–8 weeks old female Balb/c) were purchased from Medical Experimental Animal Center of Guangdong Province. To establish the tumor model, 5×10^6 SKBR-3 cells were injected in the thymus gland of the mice. All animal studies were approved by Institutional Animal Care and Use Committee (IACUC) of Guangzhou General Hospital of Guangzhou Military Command.

In Vivo Experiments—In vitro and in vivo CT imaging

Different concentrations of Tam-Bi₂S₃@PS and iobitridol were put into 24-well plate under X-ray scanning for HU value measurement. Tumor-bearing nude mice were i.v. injected with 200 μ L of Bi₂S₃@mPS and Tam-Bi₂S₃@ mPS NPs (10 mg mL⁻¹) separately prior to imaging. After 2, 6, and 24 h, tumor-bearing mice were imaged by the small animal X-ray CT. In vivo CT imaging was performed on small mice X-ray CT (Aloka, Hitachi). Imaging parameters were as follows: scanning resolution 48 μ m, rotation angle 360°, and voltage 80 kv. The CT images were analyzed using amira VGStudio MAX 2.2. The whole mouse body experienced 360 scans, which lasted about 20 min for each mouse.

In Vivo Experiments—In vivo tumor therapy

When the tumor size reached 0.5×0.5 cm², the female SKBR-3 tumor-bearing mice were divided into six groups: 1) Tam-Bi₂S₃@mPS/DOX+NIR; 2) Tam-Bi₂S₃@ mPS+NIR; 3) Bi₂S₃@mPS+NIR; 4) Tam-Bi₂S₃@mPS/DOX; 5) DOX+NIR; and 6) Control (NaCl+NIR). After i.v. injection of the above materials, the 808 NIR laser (1 W cm⁻²) was applied to the tumor site for 10 min at 2 h postinjection. The photothermal effect was carefully recorded by an infrared (IR) camera. The changes of tumor volumes in all groups were carefully recorded every other day for a period of 14 d. The tumor sizes were measured by a caliper and calculated as follows

$$V = ab^2/2 \quad (1)$$

where V (mm³) is the volume of the tumor, and a (mm) and b (mm) are the tumor length and tumor width, respectively. After 14 d, the mice were anesthetized and killed, the tissues of main organs and tumor tissues were peeled from the mice and fixed in a 4% formaldehyde solution (pH = 7.0). The tissues were processed with paraffin embedding routinely. 3.5 μ m

thick sections were then cut and placed on glass slides, followed by staining with H&E. Finally, the pathological sections were observed at $\times 200$ magnifications using an inverted microscope (BX51, Olympus).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Synthesis and characterization of Bi_2S_3 nanoparticles. a) Synthetic procedure of $\text{Tam-Bi}_2\text{S}_3@m\text{PS}$ with a high targeting activity. First PVP-assisted Bi_2S_3 ($\text{PVP-Bi}_2\text{S}_3$) NPs were synthesized, followed by the deposition of mPS coating to form $\text{Bi}_2\text{S}_3@m\text{PS}$ NPs. Then a cancer-targeting antibody (Tam) was conjugated to $\text{Bi}_2\text{S}_3@m\text{PS}$ NPs through a PEG linker to form $\text{Tam-Bi}_2\text{S}_3@m\text{PS}$ NPs. Finally an anticancer drug DOX was loaded on the resultant NPs to form $\text{Tam-Bi}_2\text{S}_3@m\text{PS}/\text{DOX}$. b) TEM image of the hydrophilic $\text{PVP-Bi}_2\text{S}_3$ NPs. c) HRTEM image and d) SAED pattern of an individual $\text{PVP-Bi}_2\text{S}_3$ NP. e) TEM image of the $\text{Bi}_2\text{S}_3@m\text{PS}$ NPs. f) HAADF-STEM image of $\text{Bi}_2\text{S}_3@m\text{PS}$ NPs and the elemental mapping

of Si, O, S, and Bi from the highlighted area. g) Nitrogen adsorption/desorption isotherms of $\text{Bi}_2\text{S}_3@\text{mPS}$ NPs. h) Pore size distributions of $\text{Bi}_2\text{S}_3@\text{mPS}$ NPs.

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Figure 2.

Spectroscopic characterization as well as drug loading and release of different nanoparticles.

a) UV-vis spectra of PVP-Bi₂S₃, Bi₂S₃@mPS and Tam-Bi₂S₃@mPS. Inset: I) Image of Tam-Bi₂S₃@mPS aqueous solution (200 µg mL⁻¹); II) magnified region (600–900 nm) of the UV-vis spectra of PVP-Bi₂S₃, Bi₂S₃@mPS and Tam-Bi₂S₃@mPS. b) FTIR spectra of PVP-Bi₂S₃, Bi₂S₃@mPS, and Tam-Bi₂S₃@mPS. c) Drug-loading efficiency of DOX on Bi₂S₃@mPS. A DOX solution was added to Bi₂S₃@mPS solution. After drug loading, the mixture was centrifuged. The red and black curve represented the absorption spectrum of the original DOX solution and the supernatant, respectively, indicating the successful drug loading. d) Drug release from Bi₂S₃@mPS at pH 5 and 7.4 at room temperature and 37 °C.

Figure 3.

Photothermal properties of nanoparticles in vitro and in vivo. Quantitative temperature change of a) PVP-Bi₂S₃ c) and Bi₂S₃@mPS with different concentrations under 1 W cm⁻² 808 nm laser irradiation. Heating/cooling experiment of b) PVP-Bi₂S₃ and c) Bi₂S₃@mPS nanoparticles solution (400 µg mL⁻¹) under 1 W cm⁻² 808 nm laser irradiation. The laser was on from 0 to 600 s and then off from 600 to 1800 s. The PTC efficiencies were indicated as η . e) IR images of the animals captured every 2 min (from left to right) showing the temperature change at tumor sites under 1 W cm⁻² 808 nm laser irradiation. The light was only applied to the tumors during the measurements after i.v. injection of different samples. I, II, and III represent the Tam-Bi₂S₃@mPS, Bi₂S₃@mPS, and control (no particles) group, respectively. f) Quantitative temperature change at the tumor site in the Tam-Bi₂S₃@mPS,

Bi₂S₃@mPS, and control group derived from IR camera measurements shown in panel (e). These data confirm that Tam-Bi₂S₃@mPS NPs favor the accumulation of NPs at the tumor sites due to their tumor-targeting capability.

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Figure 4.

In vitro cancer therapy by nanoparticles. a) Cell viability of SKBR-3 cells after being cultured with different concentrations of $\text{Bi}_2\text{S}_3@\text{mPS}$, $\text{Tam-Bi}_2\text{S}_3@\text{mPS}$, and DOX for 24 h. b) Cancer cell killing effect of $\text{Bi}_2\text{S}_3@\text{mPS}$, $\text{Tam-Bi}_2\text{S}_3@\text{mPS}$, $\text{Bi}_2\text{S}_3@\text{mPS}/\text{DOX}$, and $\text{Tam-Bi}_2\text{S}_3@\text{mPS}/\text{DOX}$ under 808 nm laser irradiation for 10 min. c) The hemolytic ratio of $\text{Tam-Bi}_2\text{S}_3@\text{mPS}$ with different concentrations. NPs with different concentrations, purified water (+), and saline (–) are added to the centrifuge tubes to test the hemolytic properties of the NPs. The inset shows the picture of the centrifuge tubes after centrifugation. The slight red color at the bottom of the tubes in the NP group indicate the presence of few human red blood cells in the precipitates, suggesting the excellent blood compatibility of the NPs. d) Fluorescence micrographs of live/dead dye-stained SKBR-3 cells after cocultured with different nanoparticle groups with or without 808 nm laser irradiation (1 W cm^{-2}), showing

that Tam-Bi₂S₃@mPS/DOX+NIR group has the best photothermal killing effect on the cancer cells. Scale bar = 50 μ m.

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Figure 5.

In vitro assay verifying the internalization of nanoparticles by SKBR-3 cancer cells. a) Fluorescence images of SKBR-3 cells cocultured with free FITC as well as FITC-labeled $\text{Bi}_2\text{S}_3@m\text{PS}$ and Tam- $\text{Bi}_2\text{S}_3@m\text{PS}$. b) Flow cytometry analysis of SKBR-3 cells stained by the FITC-labeled $\text{Bi}_2\text{S}_3@m\text{PS}$ and Tam- $\text{Bi}_2\text{S}_3@m\text{PS}$. c) Quantitative analysis of fluorescence intensity of the SKBR-3 cells stained by free FITC as well as FITC-labeled $\text{Bi}_2\text{S}_3@m\text{PS}$ and Tam- $\text{Bi}_2\text{S}_3@m\text{PS}$ Scale bar=50 μm .

Figure 6.

In vitro and in vivo CT imaging enhanced by Tam-Bi₂S₃@mPS nanoparticles. a) CT value (HU) of Tam-Bi₂S₃@mPS and iobitridol with different concentrations under 80 kV. Inset: In vitro CT images of Tam-Bi₂S₃@mPS and iobitridol suspensions with different concentrations. b) In vivo CT images of SKBR-3 bearing nude mice recorded at 2, 6, and 24 h after intravenous injection of 200 μ L Tam-Bi₂S₃@mPS and Bi₂S₃@PS solution with a concentration of 10 mg mL⁻¹. The tumor site is highlighted by a red circle. This data suggests that tumor targeting of Tam-Bi₂S₃@mPS resulted in the accumulation of the NPs at the tumor sites, enabling the detection of tumors by CT.

Figure 7.

In vivo cancer therapy by different nanoparticles, showing that Tam-Bi₂S₃@mPS/DOX+NIR group has the best synergistic therapy performance. a) Representative photos of SKBR-3 bearing nude mice in different treatment groups 14 d after treatment. b) Representative photos tumors collected from different treatment groups of mice 14 d after treatment. c) Tumor volume growth curves of different treatment groups of mice during a period of 14 d. d) Dynamic body change of different treatment groups of mice during a period of 14 d. e) H&E staining of tumor tissues harvested from the different mice groups. Scale bar = 200 μ m.

Scheme 1.

Schematic illustration of the targeted Tam-Bi₂S₃@mPS theranostic nanoplatform.