Multifunctional $\text{Cu}_{1.94}\text{S}$ - Bi_2S_3 @polymer nanocomposites for computed tomography imaging guided photothermal ablation

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ABSTRACT The doping of radiocontrast agent such as bismuth (Bi) in copper chalcogenide nanocrystals for computed tomography (CT) imaging guided photothermal therapy (PTT) has drawn increasing attention. However, the doping of Bi often suffers from the weak CT signal due to the low Bi doping concentration and deteriorates the PTT efficacy of copper chalcogenides. Here we report a multifunctional nanoprobe by encapsulating both $\text{Cu}_{1.94}\text{S}$ and Bi_2S_3 nanocrystals into a biocompatible poly(amino acid) matrix with size of ~85 nm for CT imaging guided PTT. The amount of nanocrystals and the ratio of $\text{Cu}_{1.94}\text{S}$ -to- Bi_2S_3 in the multifunctional nanocomposites (NCs) are tunable toward both high photothermal conversion efficiency (~31%) and excellent CT imaging capability (27.8 HU g L⁻¹). These NCs demonstrate excellent effects for photothermal ablation of tumors after intratumoral injection on 4T1 tumor-bearing mice. Our study may provide a facile strategy for the fabrication of multifunctional theranostics towards simultaneous strong CT signal and excellent PTT.

Keywords: multifunctional nanoprobe, CT imaging, photothermal therapy, Bi_2S_3 , $\text{Cu}_{1.94}\text{S}$

INTRODUCTION

Nanomaterials have attracted tremendous interest over the past decades and been widely used in biomedical fields including fluorescence imaging, positron emission tomography (PET), magnetic resonance imaging (MRI), hyperthermia treatment, and so on [1–9]. Nanomaterials-based X-ray computed tomography (CT) has also drawn wide attention and been identified as a promising non-invasive diagnostic tool for clinical diagnosis due to its high resolution and the excellent penetration of X-ray [10,11]. Compared to the commonly used small molecule CT contrast agents such as iodinated molecules [12] that

often suffer from short imaging time and potential renal toxicity by their rapid kidney's clearance [13–15], the nanocrystal contrast agents such as Bi_2S_3 , TaO_x and Au nanoparticles (NPs) show several advantages including low toxicity, long imaging time, and high absorption coefficient [16–18]. In particular, bismuth-based NPs such as Bi_2Se_3 nanoplates and Bi_2S_3 nanorods have drawn significant attention [19,20], because bismuth element possesses high X-ray attenuation coefficient resulted from its high density (ρ) and atomic number (Z) [21]. Recently, Rabin *et al.* [22] synthesized Bi_2S_3 nanoplates which showed long vascular half-life and had considerable potential to achieve enhanced CT efficacy with a lower agent dose in the future clinical applications. Ai *et al.* [13] developed a facile strategy for the large-scale manufacture of Bi_2S_3 nanodots coated by oleic acid. After further surface modification by polyvinylpyrrolidone (PVP) through a versatile ligand exchange approach, these nanodots exhibited low cytotoxicity and high capabilities of angiography.

On the other hand, the integration of diagnosis and treatment, namely theranostics, is expected to improve therapeutic efficacy and reduce side effects through more personalized therapies for various diseases [23]. Fortunately, nanoplateforms which can integrate imaging moieties and therapeutic species in flexible ways have enjoyed broad attention in cancer diagnosis and treatment in the past decade [24]. Among diverse investigations, imaging-guided photothermal therapy (PTT) has been reported extensively and gained high recognition [25,26], because near-infrared (NIR) laser induced photothermal ablation causes minimal invasion and harm to normal tissues [27,28]. In the light of broad NIR absorption, high photothermal conversion efficiency (PCE), low cost, good photostability and excellent biocompatibility, copper

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Scheme 1 Schematic illustration for the fabrication and CT-guided PTT of the $\text{Cu}_{1.94}\text{S-Bi}_2\text{S}_3@\text{PSI}_{\text{OAm}}$ multifunctional nanocomposites.

chalcogenides (Cu_{2-x}S) have become a type of promising candidate to meet severe demands of photothermal ablation [29–32]. Most recently, our group has reported the synthesis of ultrahigh ^{19}F loaded $\text{Cu}_{1.75}\text{S}$ nanoprobe with small particle size (average size ~ 21 nm) for simultaneous ^{19}F magnetic resonance imaging (MRI) and PTT [33,34]. As for CT imaging-guided PTT [35,36], although much efforts have recently been concentrated on pursuing nanomaterials with not only the capacity of photothermal conversion but also CT imaging responses, some challenges remain to be addressed. For example, WO_{3-x} and gold nanorods possess both NIR and CT responses [12,23], but the NIR response resulted from oxygen vacancies on the surface of WO_{3-x} is easy to disappear with oxidization happened in the ambient environment; and due to the “melting effect”, the NIR absorbance peak and thus photothermal effects of gold nanorods will diminish after a long period of laser irradiation [37–39]. Additionally, Bi_2S_3 and Cu-Bi-S ternary nanocrystals such as Cu_3BiS_3 have been reported for dual-functional CT imaging and PTT [19,36,40]. However, the exploration and construction of ternary Cu-Bi-S nanomaterials with both strong NIR absorption and CT response is very limited because of their complicated stoichiometry and difficult preparation [36,40]. NIR response of these materials is

highly sensitive to crystalline phase, size, morphology, and bandgap engineering. When doping with high proportion of Bi, the crystallinity of Cu-Bi-S nanostructures will be affected, thus decreases their photothermal efficacy; while small doping amount of Bi will then lead to the loss of CT imaging response [41–43]. Therefore, it is still a challenge to fabricate the multifunctional nanocomposites (NCs) possessing not only high CT signal but also strong photothermal efficiency.

Here we present a facile and general strategy to fabricate multifunctional NCs by encapsulating both $\text{Cu}_{1.94}\text{S}$ and Bi_2S_3 nanocrystals into a biocompatible oleylamine functionalized poly-succinimide (PSI_{OAm}) matrix with size of ~ 85 nm (Scheme 1) [44]. The definite structure of PSI_{OAm} is shown in Fig. S1. The amount of $\text{Cu}_{1.94}\text{S}$ and Bi_2S_3 NPs in the nanocomposites can be flexibly tuned. As a result, these NCs demonstrate simultaneously strong CT signal and excellent photothermal efficiency. In addition, by means of the abundant carboxyl groups generated from alkaline hydrolyzation of the lactam rings in PSI_{OAm} , these NCs are highly water dispersible and bioconjugatable [45]. This makes it available to graft potential biotins onto the NCs, and thus endows the NCs with desirable and multiple functionalities toward CT imaging-guided photothermal ablation.

MATERIALS AND METHODS

Materials

Oleic acid (OA), oleylamine (OAm) and 1-octadecene (ODE) were purchased from Acros (Belgium). *N,N*-dibutylthiocarbamate ($\text{HS}_2\text{CNBut}_2$) was purchased from Pacific Ocean United Petro-Chemicals Co. Ltd. (Beijing, China). NaOH, $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, ethanol, methanol, cyclohexane and chloroform were supplied by Beijing Chemical Reagent Co. Ltd. (Beijing, China). Bismuth neodecanoate was obtained from Energy Chemical (Shanghai, China). Poly-succinimide (PSI) was obtained from Shijiazhuang Desai Chemical Company (Shijiazhuang, China). Sulfur powder was obtained from Alfa Aesar. *N,N*-dimethylformamide and 1-dodecanethiol were purchased from Sinopharm Chemical Reagent Co. Ltd. (Beijing, China). Dulbecco's modified eagle medium (DMEM), trypsin and phosphate buffered saline (PBS) were obtained from M&C Gene Technology (Beijing, China). Fetal bovine serum (FBS) was from Hangzhou Sijiqing Bioengineer Materials Ltd. (Hangzhou, China). Methyl thiazolyltetrazolium (MTT) was supplied by Amresco Inc. (USA).

Characterization

The morphology and size of nanomaterials were characterized by a JEM-1200EX (JEOL) transmission electron microscope (TEM) at 100 kV. Dynamic light scattering (DLS) size analysis of the nanoparticles was conducted using a Zetasizer Nano-ZS90 (Malvern) zeta and size analyzer. The powder X-ray diffraction (XRD) patterns were collected on a Bruker AXS D8-Advanced X-ray diffractometer with operation current and voltage of 40 mA and 40 kV, respectively. Fourier-transform infrared (FT-IR) spectra were acquired on a JASCO FT/IR-460 PLUS spectrometer (Tokyo). Photothermal performance was carried out with an FLIR-A600 infrared camera. The ratio of Cu-to-Bi in the fabricated $\text{Cu}_{1.94}\text{S-Bi}_2\text{S}_3@\text{PSI}_{\text{OAm}}$ nanocomposites was measured by inductively coupled plasma optical emission spectrometry (ICP-OES) on a Thermo Scientific icap 600 series system. UV-vis-NIR absorbance spectra of the as-prepared $\text{Cu}_{1.94}\text{S}$ NPs and $\text{Cu}_{1.94}\text{S-Bi}_2\text{S}_3@\text{PSI}_{\text{OAm}}$ NCs were measured with a UV-3600 spectrophotometer (Shimadzu). Fluorescence measurements were performed on an F-4600 spectrophotometer (Hitachi). *In vitro* CT imaging was carried out on a multispectral photoacoustic tomography scanner (CRI Maestro 2). *In vivo* CT scanning was performed using a Siemens Inveon device (Germany).

Synthesis of hydrophobic $\text{Cu}_{1.94}\text{S}$ NPs

$\text{HS}_2\text{CNBut}_2$ (86.2 mg) and $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ (96.8 mg) were dissolved in ethanol (1.0 mL) to form $\text{Cu}(\text{S}_2\text{CNBut}_2)_2$ precursors. Then, the mixture of oleic acid (8.0 mL) and 1-dodecanethiol (4.0 mL) was heated to 190°C under nitrogen purging. Thereafter, the $\text{Cu}(\text{S}_2\text{CNBut}_2)_2$ precursor was injected into the above hot solution which immediately turned black. After keeping at 180°C for 15 min, the resultant solution was cooled to room temperature naturally and the final product was collected by precipitating and washed with ethanol for twice. The as-synthesized $\text{Cu}_{1.94}\text{S}$ NPs were then dispersed in chloroform for later use.

Synthesis of hydrophobic Bi_2S_3 nanorods

Bismuth neodecanoate (130 mg) was dissolved in a mixture containing OA (4 mL) and 1-octadecene (8 mL). The mixture was then heated to 120°C under continuous magnetic stirring and a gentle nitrogen flow. Afterwards, 6 mg of sulfur powder dissolved in OAm (0.8 mL) was injected into the above solution. The resultant mixture solution was kept at 105°C for 15 min. After cooling to room temperature, the final product of Bi_2S_3 NRs was obtained by precipitating and washed with ethanol for twice and then redispersed into chloroform for further use.

Preparation of PSI_{OAm}

The coating polymer PSI_{OAm} was prepared according to our previous report [44]. Briefly, 1.6 g of PSI was dissolved in 32 mL of dimethyl sulfoxide under magnetic stirring. Then the mixture solution was heated to 90°C followed by adding 2.17 mL of OAm. The resultant solution was maintained at 100°C for 5 h, and then cooled to room temperature naturally. Finally, the product (PSI_{OAm}) was harvested after purification and then stored in chloroform for next experiments.

Synthesis of $\text{Cu}_{1.94}\text{S-Bi}_2\text{S}_3@\text{PSI}_{\text{OAm}}$ NCs

In typical, PSI_{OAm} (30 mg), $\text{Cu}_{1.94}\text{S}$ (3.7 mg) and Bi_2S_3 (0.7 mg) were dissolved in 1.0 mL of chloroform and the resultant mixture was then added into 10 mL of NaOH (5.0 mmol L^{-1}) aqueous solution. After ultrasonication treatment for 6 min, the chloroform was removed by evaporation at 55°C. Finally, the $\text{Cu}_{1.94}\text{S-Bi}_2\text{S}_3@\text{PSI}_{\text{OAm}}$ NCs were collected by centrifugation and washed with water and then redispersed into 1.0 mL of deionized water for later applications.

Evaluation of photothermal performance

To evaluate the PCE [27,46] of the as-synthesized NCs, the

temperature change of the NCs colloidal solution was recorded under continuous irradiation of 808-nm laser with a power density of 1.0 W cm^{-2} until the solution reached a steady-state temperature and then the laser was shut off. The heating and cooling temperature trends of samples were recorded by a FLIR thermal camera. The PCE was determined by using Equation (1):

$$\eta_{\tau} = \frac{hA(T_{\max} - T_{\text{amb}}) - Q_0}{I(1 - 10^{-A_{\lambda}})}, \quad (1)$$

where h and A are the heat transfer coefficient and the surface area of the container, respectively. $T_{\max}$ and T_{amb} are the maximum temperature of system and ambient temperature, respectively. Q_0 (in unit of mW) refers to the rate of heat input due to light absorption by the solvent and the sample container. I is the laser power (in unit of mW) and A_{λ} is defined as the absorbance of the NCs suspension at 808 nm. The value of hA is calculated by Equation (2):

$$\tau_s = \frac{m_D C_D}{hA}, \quad (2)$$

where τ_s is time constant of the sample system, m_D and C_D are the mass and heat capacity of deionized water, respectively. And C_D is approximated to be $4.2 \text{ J mg}^{-1} \text{ K}^{-1}$. The heat energy (Q_0) of the sample container and solvent without NCs was measured independently, using the following Equation (3):

$$Q_0 = hA(T_{\max} - T_{\text{amb}}). \quad (3)$$

Cytotoxicity tests

The cytotoxicity of the as-prepared NCs was measured by MTT assay. In brief, HeLa cells were firstly transferred to 96-well plates (2.0×10^4 cells per well) and incubated for 24 h. Then nanocomposite colloids were added to the corresponding wells at different concentrations. For the groups to be treated with NIR light irradiation, after incubating the cells with various concentrations of the NCs for 30 min, the wells were placed under the 808-nm laser at the output power density of 1.0 W cm^{-2} for 5 min. Afterwards, all groups of cells were incubated for another 24 h prior to further measurements by MTT assay.

In vivo CT and PTT experiments

Female mice (BALB/c) between 4 and 5 weeks of age (weighing 20–24 g) were used for imaging-guided therapy. All animal experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee from Beijing University of Chemical

Technology (BUCT). The Institutional Animal Care and Use Committee from BUCT has approved the animal experiments reported in this paper. Tumor-bearing mice were prepared by subcutaneous inoculation of approximate 1×10^6 4T1 cells (in PBS, pH 7.4) in the right legs of mice. Tumors were allowed to grow for 1 week, during which time solid tumors typically formed.

For *in vivo* CT imaging, mice bearing tumors of around 100 mm^3 were subjected to CT scanning before and after intra-tumorally injected with $100 \mu\text{L}$ of NCs solution (24 mg mL^{-1} in PBS). CT data were acquired using X-ray voltage biased to 50 kVp with a $670 \mu\text{A}$ anode current with projection angles of 720° .

PTT experiments were carried out through an STL808CF-10W instrument. For *in vivo* measurements, tumor-bearing mice were exposed to 808-nm laser irradiation with a power density of 1.0 W cm^{-2} for 10 min after administration of $100 \mu\text{L}$ of NCs solution (24 mg mL^{-1} in PBS).

RESULTS AND DISCUSSION

Fabrication and characterization of NCs

Both $\text{Cu}_{1.94}\text{S}$ and Bi_2S_3 nanocrystals were first synthesized by hot-injection method, and then simultaneously encapsulated into PSI_{OAm} matrix to form a multifunctional $\text{Cu}_{1.94}\text{S}-\text{Bi}_2\text{S}_3@ \text{PSI}_{\text{OAm}}$ composite nanoprobe in which $\text{Cu}_{1.94}\text{S}$ and Bi_2S_3 served as photothermal and CT contrast agents, respectively (Scheme 1). Here, the lactam ring in PSI chain is easily opened *via* aminolysis by OAm to form PSI_{OAm} with a long hydrophobic side-chain [47], and the lactam part also can be readily hydrolyzed to carboxyl group under alkaline condition. Together with its amphiphilic and biodegradable properties, the PSI_{OAm} that has been extensively used for surface functionalization of inorganic nanomaterials and construction of drug delivery nanocapsules [48,49], was employed as a coating agent to fabricate the nanocomposites. The morphology and size of the hydrophobic $\text{Cu}_{1.94}\text{S}$ and Bi_2S_3 precursors and hydrophilic $\text{Cu}_{1.94}\text{S}-\text{Bi}_2\text{S}_3@ \text{PSI}_{\text{OAm}}$ nanocomposites were characterized by TEM (Fig. 1a–d) and DLS analysis (Fig. 1e). The $\text{Cu}_{1.94}\text{S}$ nanocrystals are uniform nanoparticles (NPs) with an average diameter of $\sim 4.1 \text{ nm}$ (Fig. 1a), while Bi_2S_3 nanocrystals are shown as nanorods with average length and diameter of $\sim 15.7 \text{ nm}$ and $\sim 2.4 \text{ nm}$, respectively (Fig. 1b). Accordingly, the as-fabricated water dispersible $\text{Cu}_{1.94}\text{S}-\text{Bi}_2\text{S}_3@ \text{PSI}_{\text{OAm}}$ NCs are well distributed nanospheres (Fig. 1c), and both of the $\text{Cu}_{1.94}\text{S}$ nanoparticles and Bi_2S_3 nanorods are clearly observed in the highly magnified TEM image of single $\text{Cu}_{1.94}\text{S}-\text{Bi}_2\text{S}_3@ \text{PSI}_{\text{OAm}}$ nanosphere

Figure 1 TEM images of $\text{Cu}_{1.94}\text{S}$ NPs (a), Bi_2S_3 nanorods (b) as well as $\text{Cu}_{1.94}\text{S-Bi}_2\text{S}_3@\text{PSI}_{\text{OAm}}$ NCs (c and d), and DLS size distribution of NCs (e).

(Fig. 1d). The average DLS diameter of the as-prepared NCs was measured to be 84.7 ± 9.2 nm (Fig. 1e). This size (in the range of 10–200 nm) is desirable for passive accumulation of the NCs in tumor sites by escaping kidney filtration (less than 5.5 nm) and elimination by the macrophages in reticuloendothelial system (RES) [25,50–52]. While if only Bi_2S_3 nanocrystals were used to fabricate the nanocomposites, only the Bi_2S_3 nanorods can be seen in the as-fabricated $\text{Bi}_2\text{S}_3@\text{PSI}_{\text{OAm}}$ nanospheres (Fig. S2).

The crystal structure of $\text{Cu}_{1.94}\text{S}$ and Bi_2S_3 nanocrystals was further identified by XRD (Figs S3 and S4). Correspondingly, the XRD pattern of NCs (Fig. 2a) also revealed the co-existence of $\text{Cu}_{1.94}\text{S}$ and Bi_2S_3 nanocrystals. The PSI_{OAm} coating was characterized by the FITR spectroscopy (Fig. 2b). The characteristic absorption bands of the lactam ring and its aminolyzed product around 1720 and 1654 cm^{-1} for the stretching vibrations of carboxyl group ($\nu_{\text{C=O}}$) were observed in $\text{Cu}_{1.94}\text{S-Bi}_2\text{S}_3@\text{PSI}_{\text{OAm}}$ NCs, which were in accordance with those in PSI_{OAm} [45], indicating

that PSI_{OAm} has been successfully coated onto the NCs. Additionally, as shown in Fig. S5, the as-prepared NCs can be well dispersed and stable in both PBS (pH 7.4) and FBS media, which is highly desired for their potential *in vivo* applications.

The PCE was also evaluated. As shown in Fig. 2c, the NCs with only Bi_2S_3 (0.7 mg) demonstrated a very weak PCE of 10.8% (Sample 1). By fixing the dosage of Bi_2S_3 (0.7 mg) and increasing the amount of $\text{Cu}_{1.94}\text{S}$, the PCE of the $\text{Cu}_{1.94}\text{S-Bi}_2\text{S}_3@\text{PSI}_{\text{OAm}}$ NCs was enhanced to 21.1% (Sample 2), 30.9% (Sample 3) and 33.2% (Sample 4), respectively. The molar ratio of Cu-to-Bi in the NCs was measured by ICP-OES and calculated to be 9.07:1, 17.2:1 and 29.0:1 (Table S1). It is clear that further increment of $\text{Cu}_{1.94}\text{S}$ dosage causes a negligible enhancement of PCE. Therefore, to obtain simultaneously desired CT imaging and photothermal ablation efficacy, the ratio of Cu-to-Bi in the NCs was confirmed and set at 17.2:1. It is noteworthy that the PCE (30.9%) of the as-fabricated $\text{Cu}_{1.94}\text{S-}$

Figure 2 (a) XRD pattern of $\text{Cu}_{1.94}\text{S}$ - Bi_2S_3 @ PSI_{OAm} NCs. (b) FTIR spectra of the $\text{Cu}_{1.94}\text{S}$ NPs, Bi_2S_3 nanorods, PSI_{OAm} and the NCs. (c) The monitored temperature profiles of the NCs synthesized with various Cu-to-Bi ratios, under the irradiation of 808-nm laser (1.0 W cm^{-2}) for 6 min, followed by cooling naturally with laser light turned off. The dosage of Bi_2S_3 is fixed at 0.7 mg and the ratio of Cu-to-Bi in NCs was 9.07:1 (S2), 17.2:1 (S3) and 29.0:1 (S4), respectively. The nanocomposite sample prepared with only Bi_2S_3 (0.7 mg) was denoted as S1. The concentration of the NCs was 0.3 mg mL^{-1} .

Bi_2S_3 @ PSI_{OAm} NCs is better or comparable to that of different nanoplatforms for PTT in previous reports [19,53–55].

Evaluation of *in vitro* photothermal efficacy of NCs

In order to investigate the applicability of the as-prepared NCs in PTT, *in vitro* evaluation of their photothermal efficacy was first performed. As for the choice of the irradiation light wavelength, although the absorption of the $\text{Cu}_{1.94}\text{S}$ NPs at 980 nm or 1500 nm was stronger than that at 808 nm (Fig. S6), yet under 980-nm or 1500-nm laser irradiation, the temperature increase of pure water was much higher than that under 808-nm laser [56]. In view of biomedical applications, inducing minimal harm to normal tissue is the prerequisite. As a result, the 808-nm laser was utilized for the further investigation. Different factors that may affect the photothermal responses such as the power densities of NIR light (Fig. 3) and the concentrations of NCs (Fig. S7) were evaluated. As shown in Fig. 3a and Fig. S7a, during the irradiation of 808-nm light, the

photothermal images were getting brighter (stronger) along with ascending of the power densities and the concentrations of NCs, respectively. Moreover, under continuous light irradiation with different power densities, the temperature of $\text{Cu}_{1.94}\text{S}$ - Bi_2S_3 @ PSI_{OAm} NCs colloidal solution (0.4 mg mL^{-1}) increased step by step along with increasing of the power density of the laser, and finally reached 43.5°C at the power density of 1.2 W cm^{-2} (Fig. 3b). Alternatively, by fixing the power density at 1.0 W cm^{-2} , the temperature went up along with the increase of the concentration of NCs. Notably, the temperature of pure water increased less than 1.0°C from room temperature under 808-nm light irradiation for 7 min (Fig. S7b).

Cytotoxicity and photothermal therapy investigation on HeLa cells

In addition, the cytotoxicity of the as-prepared NCs was investigated by MTT assay (Fig. 4a). As a control, while the cells were cultured with PBS solution, the cell viability was

Figure 3 (a) Photothermal images of $\text{Cu}_{1.94}\text{S-Bi}_2\text{S}_3@\text{PSI}_{0\text{Am}}$ NCs colloids under the irradiation of 808-nm light at different power densities and time intervals. (b) The temperature elevation profiles of the NCs colloids under continuous irradiation of 808-nm light at various power densities. The NCs concentration was 0.4 mg mL^{-1} .

Figure 4 Cell viability tests of NCs in the absence (a) and presence (b) of 808-nm light irradiation (1.0 W cm^{-2} , 5 min) after incubation with HeLa cells for 24 h.

recorded as 100% in the absence of NIR irradiation. As shown in Fig. 4a, the cell viability of the NCs was still over 90% within 24 h even at high dosage up to $400 \mu\text{g mL}^{-1}$, indicating that the as-prepared $\text{Cu}_{1.94}\text{S-Bi}_2\text{S}_3@\text{PSI}_{0\text{Am}}$ NCs possess excellent biocompatibility.

Next, we investigated the PTT performance of the NCs on HeLa cells under 808-nm laser irradiation (1.0 W cm^{-2} , 5 min) (Fig. 4b). As compared to the control (the cells incubated with PBS and without NIR light irradiation), over 96% cells were still alive after incubation with PBS for 24 h under the laser irradiation (1.0 W cm^{-2} , 5 min), suggesting that the short-time irradiation of NIR light caused only negligible damage to cells. However, once HeLa cells incubated with NCs were exposed to the light irradiation (808 nm, 1.0 W cm^{-2}) for 5 min, a low dosage

of NCs would give rise to a significant cell ablation due to the superior photothermal capability of the as-prepared NCs. To be specific, at $120 \mu\text{g mL}^{-1}$ of NCs, the cell viability was dramatically decreased to 38% owing to the excellent effect of PTT. Such specific PTT performance would make the as-prepared NCs to be great promising drug candidates for cancer treatment at a lower dosage.

In vitro CT imaging assessment of $\text{Cu}_{1.94}\text{S-Bi}_2\text{S}_3@\text{PSI}_{0\text{Am}}$ NCs

In addition to the photothermal ablation functionality, CT imaging property of the as-prepared NCs was further assessed *in vitro*. As shown in Fig. 5, the Hounsfield unit (HU) values increased linearly with the concentrations of NCs (in PBS solution), and the slope of the HU value for

Figure 5 *In vitro* CT images (a) and plot (b) of CT values (HU) versus the concentrations of Cu_{1.94}S-Bi₂S₃@PSI_{OAm} NCs colloidal solution. The concentration of Bi in each colloidal solution is 0.0, 0.09, 0.18, 0.36, 0.78, and 1.14 mg mL⁻¹.

Figure 6 (a) *In vivo* CT images of 4T1 tumor-bearing mice before and after intra-tumor injection of NCs nanoprobe solution (100 μL, 24 mg mL⁻¹); *In vivo* photothermal images of the tumor-bearing mice after intra-tumor injection of NCs under irradiation (808 nm, 1.0 W cm⁻²) for different time intervals (b), and temperature evolution profile (c) of the tumor under 808-nm laser irradiation (1.0 W cm⁻²) for 0–10 min.

NCs is about 27.8 HU L g⁻¹, which is higher than that of WS₂ nanosheets (22.01 HU L g⁻¹) [36] and commercially used iobitridol (16.4 HU L g⁻¹) [19] because of the large attenuation coefficient of Bi element. It should be noted that, according to the ICP-OES results, the content of Bi in NCs is only 3 wt.%. Therefore, the as-fabricated multifunctional Cu_{1.94}S-Bi₂S₃@PSI_{OAm} NCs could provide an equivalent contrast CT imaging sensitivity at a lower dose compared to the clinical iodinate agent, which is highly

beneficial for contrast agents as it reduces potential adverse effects to patients.

CT imaging guided photothermal therapy

To further validate the efficacy and potential applicability of the as-fabricated NCs, *in vivo* studies were assessed with tumor-bearing mice (Fig. 6). As shown in Fig. 6a, a distinct difference can be observed in CT images of tumor area before and after administration of the NCs. In addition,

Figure 7 Evolution plots of the tumor size (a) and the mouse body weight (b) after photothermal treatment under 808-nm laser irradiation with a power density of 1.0 W cm^{-2} for 10 min. $100 \mu\text{L}$, 24 mg mL^{-1} of the nanoprobe solution was administered by intra-tumor injection; $100 \mu\text{L}$ of PBS solution (pH 7.4) was injected for the control group.

the photothermal property of NCs was evaluated by irradiation of tumor-bearing mice with 808-nm laser at different time intervals. The tumor site shows much brighter photothermal images compared with the surrounding matrix (Fig. 6b). The temperature at the tumor sites quickly increased from 33 to 48°C within 4 min (Fig. 6c), which is highly desirable for PTT. However, at the current stage, it is aimed to achieve and demonstrate the multifunctional performance of the fabricated NCs in both superior *in vivo* CT imaging and PTT. In the future, we further need to develop desired targeting ability for these NCs to improve their performance in active recognition to the cancerous lesions.

After photothermal treatment, the changes of tumors size with initial volume of $100\sim 130 \text{ mm}^3$ were recorded. As shown in Fig. 7a, the size of tumors was decreasing after treatment with NCs, and the tumors have completely disappeared after 2 weeks, indicating the superior effect of PTT. As for the control group, the tumor size increased from ~ 100 to $\sim 330 \text{ mm}^3$ during the two weeks of post-treatment. Meanwhile, the body weight of the mice slightly increased for the treated group, while decreased for the

control group (Fig. 7b). These observations demonstrate the $\text{Cu}_{1.94}\text{S}-\text{Bi}_2\text{S}_3@\text{PSI}_{\text{OAm}}$ NCs have great potentials as multifunctional platforms for CT imaging guided PTT of tumors.

CONCLUSIONS

In summary, we present a multifunctional nanoplatform for CT imaging-guided PTT of tumors, by means of encapsulating $\text{Cu}_{1.94}\text{S}$ and Bi_2S_3 nanocrystals into PSI_{OAm} matrixes. The excellent photothermal performance of $\text{Cu}_{1.94}\text{S}$ NPs and high levels of X-ray attenuation of Bi_2S_3 nanorods were well combined together in nanocomposites with tunable $\text{Cu}_{1.94}\text{S}$ -to- Bi_2S_3 ratio. When the molar ratio of Cu-to-Bi of 17.2:1 (measured by ICP-OES) was applied, the as-synthesized NCs demonstrated high PCE ($\sim 31\%$) and superior CT imaging efficacy (27.8 HU g L^{-1}), indicating the great potentials in simultaneous CT imaging and photothermal ablation of tumor. In view of the generality and flexible adjustment for fabrication of nanocomposites, this study may open up a new avenue for the development of remote and noninvasive therapies under a desirable imaging guidance in biomedical fields.

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Author contributions Wang L proposed the research direction and guided the project. Lu X, Li Y and Bai X designed and performed the experiments. Lu X, Hu G and Wang L analyzed and discussed the experimental results, and drafted the manuscript. All the authors checked and approved the manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

Supplementary information Experimental details are available in the online version of the paper.

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多功能 $\text{Cu}_{1.94}\text{S}$ - Bi_2S_3 @polymer纳米探针用于CT成像指导的光热治疗

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摘要 铋掺杂硫化铜纳米晶用于CT成像指导的光热治疗已经引起了人们的广泛关注. 然而, 低剂量的铋掺杂CT信号弱, 高剂量铋掺杂又会减弱硫化铜的光热效果. 本文报道了一种多功能纳米探针, 用两亲性高分子将油溶性 $\text{Cu}_{1.94}\text{S}$ 和 Bi_2S_3 纳米晶同时封装于约85 nm大小的纳米微球中, 用于CT成像指导光热治疗. 多功能纳米探针中 $\text{Cu}_{1.94}\text{S}$ 和 Bi_2S_3 纳米晶的量和比例能够灵活调节, 从而得到较高的光热转换效率(~31%)和良好的CT成像能力(27.8 HU g L^{-1}). 这些纳米探针在4T1荷瘤小鼠进行瘤内注射后, 显示了良好的CT成像与肿瘤光热治疗效果. 该复合纳米探针的制备方法还可用于其他多功能纳米探针的制备.