Photothermo-responsive Cu₇S₄@polymer nanocarriers with small sizes and high efficiency for controlled chemo/photothermo therapy

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ABSTRACT Drug delivery systems (DDSs) have been getting more and more attention in the field of cancer therapy with the development of nanotechnology. But remote and noninvasive controlled drug release for improving treatment efficacy and reducing side effects faces great challenge. We report a kind of “smart” nanocomposites (NCs) that is sensitive to the surrounding temperature by grafting a layer of thermosensitive polymer, poly(*N*-isopropylacrylamide) (pNIPAm), on the surface of single Cu₇S₄ nanoparticle (NP) via atom-transfer radical polymerization (ATRP). These NCs demonstrate a photothermal conversion efficiency of 25.4% under 808-nm near infrared (NIR) light irradiation and a drug loading content of 19.4% (drug/total NCs, w/w) with a lower critical solution temperature (LCST) of ~38°C. At normal physiological temperature (37°C), only 10.8% of the loaded doxorubicin (DOX) was released at physiological pH value (pH 7.4) within 10 h. In the presence of 808-nm irradiation, due to the temperature increment as a result of photothermal effects, DOX was rapidly released.

Keywords: drug delivery, copper chalcogenide, atom transfer radical polymerization, near infrared light, chemo/photothermo-therapy

INTRODUCTION

Drug delivery systems (DDSs) in a controlled fashion have attracted extensive attention in recent decades [1,2]. Up to now, smart drug delivery platforms triggered by stimuli such as pH [3–6], temperature [7], magnetic field [8–10] and glutathione existing in cytoplasmic matrix [11,12] have been designed, which overcome the current shortcoming of DDSs that release drugs following a predetermined rate after administration, irrespective of the patients' needs [13]. These smart DDSs offer the possibility to remotely and noninvasively control the drug

release, which would substantially improve treatment efficacy and reduce side effects [14]. Particularly, DDSs that encapsulate components displaying extensive near infrared (NIR) light absorption within a polymer matrix for photothermal-responsive drug release have been the focus due to the deep penetration ability and biological safe features of NIR irradiation [15–17]. So far, one of the most frequently studied polymers is poly(*N*-isopropylacrylamide) (pNIPAm), of which the conformation is sensitive to the surrounding temperature [18]. To be specific, when the surrounding temperature is below the lower critical solution temperature (LCST), the polymer matrix is in its swelling state, whereas at temperature higher than LCST, the polymer matrix will shrink, leading to the release of pre-loaded drugs. In addition, incorporating functional co-monomers would help to adjust the LCST [19]. With regarding to drug delivery, the LCST should not be lower than the normal human body temperature, otherwise, the drugs could be leaked out before being delivered to the desired sites. Besides, the pNIPAm polymer matrix should have a quick response toward temperature changes, that is, once the temperature is higher than LCST, a conformation change would immediately occur and result in quick drug release. Therefore, to fabricate a DDS with proper LCST as well as quick responses toward temperature changes is highly desirable [20].

In another aspect, some of the light-triggered photothermal DDSs also own the potential for photothermal therapy, hence, affording a combination of thermotherapy and chemotherapy with regarding to cancer treatment [21,22]. To date, various photothermal agents have been employed to generate heat from light, which results in ablation and

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succeeding death of cancer cells [23]. Gold nanomaterials such as nanorods [24], nanoshells [25], nanostars [26] and nanocages [27,28] are one of the mostly investigated agents due to their easy accessibility. Yet, recent results indicated that these kinds of photothermal agents suffer from poor photostability under continuous irradiation of NIR light [29,30]. Such issue has driven researchers to explore novel types of photothermal agents. For example, tungsten disulfide [31], palladium nanosheets [32], graphene [33,34], carbon nanotubes [35], bismuth sulfide [36], molybdenum disulfide [37] and even black phosphorus quantum dots [38] are utilized to kill cancer cells by hyperthermic effects. In addition to the aforementioned nanomaterials, copper chalcogenides (Cu_{2-x}S) [39–42] are another important hotspot nanomaterials and become a promising candidate to meet severe demands of photothermal ablation due to their high photothermal conversion efficiency, broad NIR absorption, low cost, high photostability and excellent biocompatibility [43–46]. Qu *et al.* utilized hollow CuS nanospheres to load hydrophobic anticancer drug, which demonstrates the feasibility of copper chalcogenide in DDS and underscores their potential in biological and medical applications [47]. Therefore, nanoplateforms based on Cu_{2-x}S nanocrystals possess great performance in photothermal therapy and DDSs, and hold potential application for clinical therapy of tumors [48].

Herein, we developed a universal and facile strategy to fabricate a layer of thermo-sensitive polymer matrix on the surface of single Cu_7S_4 nanoparticle (NP) by atom-transfer radical-polymerization (ATRP) method [49,50]. The afforded polymer matrix exhibits sensitive responses toward temperature changes with LCST at around 38°C . The as-synthesized $\text{Cu}_7\text{S}_4@\text{p}(\text{NIPAm-AAm-MBA})$ nanocomposites ($\text{Cu}_7\text{S}_4\text{NCs}$) possess high photothermal efficiency, adequate drug loading content and excellent biocompatibility as well as suitable dynamic light scattering (DLS) sizes. *In vitro* experiments indicated their excellent performance for photo-responsive controlled drug release as well as photothermal therapy. The type of synergistic photothermal ablation and chemotherapy could greatly promote the treatment of cancers.

MATERIALS AND METHODS

Materials

The oleylamine (OAm), 1-octadecene (ODE), 3-bromopropylamine hydrobromide and *N,N*-dimethylformamide were purchased from Aldrich. Chloroform (CHCl_3), dimethyl formamide (DMF), ethanol, $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, paraformaldehyde, sodium dodecyl sulfate (SDS), acry-

lamide (AAm) and NaOH were purchased from Beijing Chemical Reagent co. Ltd. The poly-succinimide (PSI) ($M_w = 5570$ Da, $M_w/M_n = 1.03$) was obtained from Shiji-azhuang Desai Chemical Company. *N,N*-dibutyldithiocarbamate (S_2CNBut_2) was obtained from the Pacific Ocean United (Beijing) Petro-Chemicals Co. Ltd. *N*-isopropyl acrylamide (NIPAm) and *N,N'*-methylene bisacrylamide (MBA) were purchased from Tokyo Chemical Industry and purified by *n*-hexane and ethanol for twice. CuBr and 1,1,4,7,7-pentamethyldiethylenetriamine (PMDETA) were obtained from Tianjin Fuchen Chemical Reagent Factory. And CuBr was purified by acetic acid and ethanol for three times. Doxorubicin hydrochloride (DOX-HCl) was bought from Shanghai Rising Chemical Co. Ltd. For cell culture, Dulbecco's modified eagle medium (DMEM), pancreatin and phosphate buffer solution (PBS) were obtained from M&C Gene Technology. Fetal calf serum was purchased from Hangzhou Sijiqing Bioengineer Materials Ltd. Methyl thiazolyltetrazolium (MTT) was obtained from Amresco Inc. and used for cell culture and cytotoxicity tests.

Characterization

Transmission electron microscopy (TEM) images were recorded on a Hitachi H-800 transmission electron microscope operating at 100 kV. Dynamic light scattering (DLS) particle size analysis of the samples were measured using a Zetasizer Nano-ZS90 (Malvern) zeta and size analyzer. ^1H NMR spectra were recorded with a Bruker AV600 NMR spectrometer (^1H frequency is 600.13 MHz). Bruker AXS D8-Advanced X-ray diffractometer with Cu K α radiation ($\lambda = 1.5418 \text{ \AA}$) was employed to test powder X-ray diffraction (XRD) patterns with operation current and voltage at 40 mA and 40 kV, respectively. The photoluminescence measurements were carried out on an F-4600 spectrophotometer (Hitachi). UV-3600 UV-vis-NIR spectrophotometer (Shimadzu) equipped with a plotter unit was employed to measure the absorption spectra. The photothermal performance was carried out with an FLIR camera. Confocal laser scanning microscopy (CLSM) picture was observed using TCS SP5 microscopes (Leica).

Synthesis of hydrophobic Cu_7S_4 NPs

The Cu_7S_4 NPs were prepared according to the previous report of our group [42]. Briefly, $\text{HS}_2\text{CNBut}_2$ (43.1 mg, 0.1 mmol) and $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ (48.3 mg, 0.2 mmol) were first dissolved in ethanol (1.0 mL) to form $[\text{Cu}(\text{S}_2\text{CNBut}_2)_2]$ precursors. Then, the mixture of oleylamine (4.0 mL) and octadecene (6.0 mL) was heated to 210°C under nitrogen atmosphere for 15 min to remove oxygen with magnetic stirring. Thereafter, the $\text{Cu}(\text{S}_2\text{CNBut}_2)_2$ precursor was injected

into the above hot solution, and the solution became black immediately. After keeping at 190°C for 15 min, the resulted solution was cooled to 80°C naturally and the products were washed with ethanol for twice. The as-synthesized Cu₇S₄ NPs were dispersed in CHCl₃ finally.

Preparation of PSI_{OAm-Br}

1.60 g of PSI was dissolved in DMF (32 mL). Then 3-bromopropylamine hydrobromide (265.5 mg) and OAm (1.63 mL) were added at 100°C with continuous magnetic stirring. After 10 h, 120 mL of methanol was added to precipitate the product. After centrifugation, the polymer was dissolved in CHCl₃ for later use.

Preparation of Cu₇S₄@PSI_{OAm-Br}

1.0 mL of CHCl₃ containing both Cu₇S₄ (4.0 mg) and PSI_{OAm-Br} (30 mg) was added to 9 mL of deionized water. Then 1.0 mL of NaOH (0.1 mol L⁻¹) aqueous solution was added to the above mixture. After ultrasonication (350 W, 6 min) treatment, an oil-in-water emulsion was obtained. Subsequently, the chloroform was removed by rotary evaporation. The final product was dispersed in deionized water after centrifugation for twice.

Preparation of Cu₇S₄@p(NIPAm-AAm-MBA) NCs

3.0 mg of the as-prepared Cu₇S₄@PSI_{OAm-Br} was added to a three-neck flask containing NIPAm (100 mg), AAm (28 mg), MBA (5.6 mg), SDS (15 mg), PMDETA (5 µL) and deionized water (12 mL) under continuous magnetic stirring in nitrogen atmosphere. Then CuBr (2.5 mg) as catalysts was added to the system at 30°C. After 9 h, the reaction system was quenched by being exposed to air for 30 min. The catalysts were removed through at least three times of centrifugation. Finally, the as-prepared NCs were dispersed in deionized water after purification.

DOX loading into NCs

2.0 mL of DOX-HCl aqueous solution (4.0 mg mL⁻¹) was added to PBS buffer (pH 7.4) containing the NCs (1.2 mg mL⁻¹, 2.0 mL) with continuous stirring at ambient temperature (37°C) for 48 h. After that, the obtained DOX-loaded nanocomposites (NCs-DOX) were washed by PBS (4.0 mL, pH 7.4) for three times. All supernatants should be collected for DOX loading content calculation.

Controlled drug release from NCs-DOX

For photothermo-triggered DOX release, 4.0 mL of PBS buffer (pH 7.4) containing NCs-DOX (2.0 mg) was stirred at 25, 37, 41 and 50°C, respectively. The mixture was centrifuged at a certain time interval. The supernatant

was collected and the precipitate was redispersed into the same volume PBS buffer. The amount of released DOX was measured by detecting the fluorescence of DOX in the supernatant. For photothermal controlled DOX release, the NCs-DOX PBS suspension (1.0 mg mL⁻¹) was placed in an incubator at 37°C. The sample was then exposed to the irradiation of 808-nm laser with output power density of 1.0 W cm⁻² for a certain period. The measurement of the amount of DOX was carried out via the same process mentioned above.

Cytotoxicity tests

The cytotoxicity and photothermal therapy performance of NCs and NCs-DOX under 808-nm laser were measured by MTT assay. Specifically, HeLa cells were firstly transferred to 96-well plates. Each well contained approximately 2.0 × 10⁴ cells. And these cells were incubated for 24 h. Then NCs and NCs suspension with different concentrations were added to corresponding wells. For the groups treated with NIR light irradiation, after incubating the cells with different concentration of the materials for 30 min, the well was placed under the 808-nm laser with the output power density of 1.0 W cm⁻² for 5 min. Afterwards, all groups of cells were incubated for 24 h and 48 h, respectively, prior to further MTT assay measurement.

Cell imaging

HeLa cells were cultured in a 12-well cell culture plate for 24 h under standard incubation condition. Then the PBS suspension of NCs-DOX was added into the cell culture well and the final concentration of NCs-DOX was 50 µg mL⁻¹. In contrast, the NCs-DOX stock suspension was exposed to 808-nm light with the output power density of 1.0 W cm⁻² for 10 min, and the suspension was then added into the cell culture well with the final concentration of 50 µg mL⁻¹ NCs-DOX. After incubation for 6 h, the cells were washed with PBS for three times and fixed with 10% of formaldehyde solution for 15 min at 37°C for CLSM observation.

RESULTS AND DISCUSSION

Synthesis and characterization of nanocomposites

The NCs were synthesized by *in situ* polymerization of pNIPAm on the surface of Cu₇S₄ NPs via ATRP (Scheme 1). The hydrophobic Cu₇S₄ nanocrystals were prepared by following previously reported methods [42] and characterized by powder XRD (Fig. S1, Supplementary information), of which the two distinct sets of diffraction peaks could be assigned to the anilite Cu₇S₄ (JCPDS no. 23-0958). The amphiphilic PSI derivatives (denoted as PSI_{OAm-Br}) were

Scheme 1 Scheme for the synthesis of photothermo sensitive nanocomposites and NIR light triggered release of DOX.

modified with oleylamine and 3-bromopropylamine (Fig. S2) via aminolysis reaction, which was confirmed by ^1H NMR spectrum (Fig. S3). Polyaspartic acid (PASP), due to its high water-soluble and biodegradable property, has become a kind of excellent functional materials for drug delivery and encapsulation of NPs [51,52]. The lactam ring of PSI is easy to be opened by amino group, which forms PASP derivatives with a side-chain functional group [53–56]. Here, $\text{PSI}_{\text{OAm-Br}}$ was soluble in chloroform because of oleylamine groups. To transfer hydrophobic Cu_7S_4 NPs into water, NaOH aqueous solution was mixed with the mixture chloroform solution containing $\text{PSI}_{\text{OAm-Br}}$ and hydrophobic Cu_7S_4 NPs [57]. Then the van der Waals interaction between the long alkyl chain on the amphiphilic $\text{PSI}_{\text{OAm-Br}}$ and the hydrophobic ligands on the Cu_7S_4 NPs facilitated the encapsulation of hydrophobic Cu_7S_4 NPs by means of ultrasonication. The average size of Cu_7S_4 nanocrystals was about 13.5 nm from TEM image (Fig. 1a) with DLS size of 14.9 nm in chloroform (Fig. 1c). After being functionalized with $\text{PSI}_{\text{OAm-Br}}$ and transferred into water, there were no obvious changes on their morphology (Fig. S4a) as compared with that of hydrophobic Cu_7S_4 nanocrystals. However, the DLS size of $\text{Cu}_7\text{S}_4@ \text{PSI}_{\text{OAm-Br}}$ was increased to around 23.1 nm (Fig. S4b).

To graft thermosensitive polymer on $\text{Cu}_7\text{S}_4@ \text{PSI}_{\text{OAm-Br}}$, the alkyl bromide was used as an initiator to initiate the *in situ* polymerization with ATRP strategy. ATRP as a kind of controlled radical polymerization strategy displays distinct advantages such as active polymer chain ends and narrow distribution of molecular weight in fabri-

cating core-shell composite nanomaterials, especially in modifying nanoparticles with a layer of polymer based on a “graft-from” method [58–60]. Monomers including NIPAm and AAm as well as the cross-linker MBA are involved in the chain propagation when catalyzing by CuBr with the ligand PMDETA. Finally, a core-shell structure of $\text{Cu}_7\text{S}_4@ \text{p}(\text{NIPAm-AAm})$ NCs was obtained, of which the TEM results imply a well-defined interface between the polymer layer and the Cu_7S_4 core (Fig. 1b) with a DLS size of ~ 70 nm (Fig. 1d). To verify the thermo-responsive properties, DLS analysis of these NCs under different temperatures from 25 to 55°C was performed. As shown in Fig. 1e, the size of the NCs shrunk dramatically from ~ 70 to ~ 31 nm when the temperature was increased above 38°C . However, when the temperature was kept higher than 40°C , the size remained the same, indicating an LCST of $\sim 38^\circ\text{C}$. The average thickness of the polymer shell on the surface of Cu_7S_4 NPs calculated by formula $(D_{25} - D_{41})/2$ is 19.5 nm. Here, D_{25} and D_{41} represent the DLS size of NCs at 25 and 41°C , respectively. Additionally, the robustness of thermo-responses toward temperature changes was demonstrated by switching the temperature between 37 and 41°C (Fig. 1f).

Photothermal performance

Prior to applications in photothermal therapy as well as controlled photothermo-responsive drug release, the photothermal conversion efficiency was evaluated. The as-prepared copper chalcogenides (Cu_7S_4) display a broad absorption (Fig. S5) in the NIR region, yet, choices of the

Figure 1 TEM images (a, b) and DLS size distribution (c, d) of Cu_7S_4 NPs (a, c) and the NCs (b, d), respectively. (e) DLS size evolution plot of the NCs versus different temperatures. (f) DLS size analysis of the NCs by switching the temperature between 37 and 41 $^{\circ}\text{C}$.

irradiation light wavelength should also consider their potential effects on biological tissues. Although the absorption of the NCs at 980 nm was stronger than that at 808 nm, yet under 980-nm laser irradiation, the temperature increase of pure water was much higher than that under 808-nm laser (Fig. S6). In view of biomedical applications, inducing minimal harm to normal tissue is the prerequisites; therefore, 808-nm diode laser was utilized for the further investigation. Initially, different factors that may affect the photothermal responses such as power densities (Fig. 2a) and the concentration of NCs (Fig. 2b) were evaluated. As shown in Fig. 2a, under continuous illumination of 808-nm light, by using irradiation with different power

densities, it was found higher power density would induce higher temperature elevation at the same irradiation time interval. Alternatively, by fixing the power density at 1.0 W cm^{-2} , the temperature increased along with the increase of NCs concentration (Fig. 2b). Notably, when irradiated by NIR light, the temperature of the aqueous suspension underwent a rapid increase and reached a plateau within 4 min, which was a superb merit for photothermal therapy and controlled light-responsive drug release. By following previous reports [42,61], the photothermal conversion efficiency of the Cu_7S_4 NCs under 808-nm laser (0.5 W cm^{-2}) was also measured. Briefly, under continuous irradiation, the temperature of the NCs suspension reached a plateau.

Figure 2 The temperature elevation profiles of the NCs suspension with continuous irradiation under 808-nm light: (a) 0.4 mg mL⁻¹ of NCs suspension at different power densities; (b) different concentrations of NCs suspension at the same power density (1.0 W cm⁻²). The corresponding photothermal images after irradiation for 6 min were placed at the right side. (c) The temperature change profile of the NCs suspension under NIR irradiation (808 nm, 0.5 W cm⁻²) for 6 min and then the light source was shut off. (d) The linear fitting of the time from the cooling period of (c) vs. negative natural logarithm of driving force temperature.

The irradiation source was then switched off while keeping monitoring the temperature changes to determine the rate of heat transfer from the system (Fig. 2c). The photothermal conversion efficiency was determined by using Equation (1).

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

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

where h is the heat transfer coefficient, A is the surface area of the container. $T_{\max}$ and T_{amb} are the maximum temperature of system and ambient temperature, respectively. In this case, the value of $(T_{\max} - T_{\text{amb}})$ is 8.37°C according to Fig. 2c. 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; here

$I = 500$ mW) and A_λ is defined as the absorbance of the NCs suspension at 808 nm (here $A_\lambda = 0.703$). The value of hA is obtained according to Equation (2), where τ_s is a time constant of the sample system. By using the linear time data from the cooling period vs. negative natural logarithm of driving force temperature, the time constant for heat transfer was determined to be 217.9 s (Fig. 2d). m_D and C_D are the mass (1.0047 g) and heat capacity of deionized water, respectively. And C_D was approximated to 4.2 J mg⁻¹ K⁻¹. The heat energy (Q_0) of the sample container and solvent was measured independently and found to be 60.5 mW. As a result, the heat conversion efficiency (η_T) of the Cu₇S₄ NCs under 808-nm laser was finally calculated to be 25.4%, which was higher than that of reported gold nanoshells (13%, 800 nm), gold nanorods (21%, 800 nm) [62] and Cu_{2-x}Se (22%, 980 nm) [63]. It should be mentioned that the polymer shell of the NCs demonstrates a negative impact on the overall photothermal conversion ef-

efficiency compared with the photothermal conversion efficiency of pure Cu_7S_4 nanocrystals [42].

DOX controlled release from nanocomposites

DOX as model drugs was loaded onto the polymer matrixes of nanocarriers through physical adsorption and the DOX-loaded NCs were termed as NCs-DOX. The thermo-sensitive polymer was grafted onto a single Cu_7S_4 nanoparticle, affording a large specific surface area and thus a high drug loading capacity. Here the DOX loading content was up to 19.4 % in weight based on the fluorescence measurement [64] (Fig. S7). The drug release profile of the NCs-DOX in PBS buffer under different temperatures (Fig. 3a) revealed that at physiological temperature (37°C), to a large extent, the NCs remained intact, with merely 10.8% of DOX leakage within 10 h. Nevertheless, at an elevated temperature (41°C), a significant drug release (45.9%) was observed within 10 h. If the temperature was further increased to 50°C , nearly 64.8% of DOX was released within 10 h. Meanwhile, the step wise drug release was successfully achieved by switching on/off NIR laser alternately (Fig. 3b). In the absence of NIR light irradiation, only a slight drug leakage was observed. However, once exposed to 808-nm NIR light (1.0 W cm^{-2}) for 10 min, an additional 9.5% of DOX was released from the nanocarriers rapidly (from 4.8% to 14.3%). Once shutting off the light source, DOX release was negligible. When radiated by NIR light again, quick drug release was observed immediately, indicating that the drug release from the NCs-DOX could be manipulated simply by controlling the NIR light irradiation [65]. In another aspect, the repeated drug release also implies that the photothermal response of Cu_7S_4 NPs is very stable [66], allowing for drug release

for multicycles. The photo-activated on-demand drug delivery in nanocapsules exhibits promising potential for enhanced anti-cancer efficacy.

Cytotoxicity tests and photothermo-chemo therapy evaluation

Prior to biomedical applications, the cytotoxicity of the as-prepared NCs was investigated by MTT assay (Fig. 4). As a control, when the cells were cultured with PBS, the cell viability was set as 100% in the absence of 808-nm irradiation. When the laser irradiation (1.0 W cm^{-2} , 5 min) was introduced, as shown in Fig. 4, over 95% cells were still alive after 48 h, suggesting that the short-time irradiation (1.0 W cm^{-2}) gave rise to negligible damage to the cells. In the absence of light irradiation, the cell viability was still over 94.5% (91.7%) within 24 h when the dosage of NCs (NCs-DOX) was up to $300\text{ }\mu\text{g mL}^{-1}$. After incubation for 48 h, the cell viability was over 85.7% in the presence of NCs-DOX ($300\text{ }\mu\text{g mL}^{-1}$) but no laser irradiation (1.0 W cm^{-2} , 5 min). All the results indicate that the as-prepared Cu_7S_4 NCs are highly biocompatible. As for the NCs-DOX, the high cell viability implies that the NCs-DOX remains intact with negligible drug leakage in the absence of light irradiation [67].

To verify the applicability for photothermally controlled photothermo-therapy, HeLa cells incubated with the NCs were exposed to NIR light irradiation (808 nm, 1.0 W cm^{-2}) for 5 min, followed by incubation for 24 and 48 h, respectively. It was found that even short-time irradiation enabled a significant cell ablation due to the good photothermal performance of Cu_7S_4 cores in the nanocarriers. For a concentration of $100\text{ }\mu\text{g mL}^{-1}$, merely 60% of cell viability was observed after being incubated for 48 h. Meanwhile, a

Figure 3 (a) DOX release profile of the NCs-DOX in PBS buffer (pH 7.4) at different temperatures; (b) DOX release profile of the NCs-DOX in PBS buffer (pH 7.4) in the absence and presence of 808-nm light (10 min, 1.0 W cm^{-2}).

Figure 4 Cell viability tests of the NCs and NCs-DOX on HeLa cell lines with and without 808-nm irradiation (1.0 W cm^{-2} , 5 min) for a period of 24 (a) and 48 h (b), respectively.

synergistic photothermo-chemo therapy has been demonstrated by employing Cu_7S_4 NCs-DOX. In one aspect, the NIR light induced the photothermo-responsive drug release and in another aspect, it also caused hyperpyrexia, leading to cell ablation. With $100 \mu\text{g mL}^{-1}$ of Cu_7S_4 NCs-DOX, about 40% of cell viability was observed. Such synergistic photothermo-chemo therapy performance makes the as-prepared Cu_7S_4 NCs-DOX greatly promising for cancer treatment [68].

Fluorescence cell imaging

As shown in the MTT assay, the NCs-DOX demonstrates very good biocompatibility in the absence of laser irradiation. However, in the presence of laser irradiation (1.0 W cm^{-2} , 5 min), due to the synergetic effects of released

DOX and photothermal ablation, after incubation with the NCs-DOX for 48 h, nearly 85% cells were dead. The photothermal controlled drug release was further investigated via the fluorescence imaging. As shown in Figs 5a–c, when the HeLa cells were incubated with NCs-DOX for 6 h in the absence of laser irradiation, the red fluorescence of DOX was observed only outside of the nuclei and no red fluorescence could be detected in the nuclei. Fluorescence spectrum (Fig. 5g) via line-scanning along the line in Fig. 5b also indicates that the fluorescence is mainly located at the two sides. All the results suggest that the NCs-DOX was immobilized in cytoplasmic matrix and could not enter the nucleus (Figs 5a–c), which indicated that DOX could not be released from the NCs even in endocellular microenvironment in the absence of light irradiation [69]. However,

Figure 5 Fluorescence imaging of HeLa cell lines incubated with DOX-loaded NCs that were irradiated with (a–c) and without (d–f) 808-nm (1.0 W cm^{-2}) light for 10 min. (g and h) The line-scanning fluorescence spectra along the line in the circle of (b) and (e), respectively.

when the NCs-DOX suspension was exposed to the NIR irradiation (808-nm, 1.0 W cm^{-2}) for 10 min before culturing with cells, DOX was rapidly released from the nanocapsules and entered the nuclei (Figs 5d–f). As shown in the line-scanning fluorescence spectrum (Fig. 5h) along the line in Fig. 5e, the fluorescence is distributed evenly along the line, suggesting that the DOX has entered the nuclei. All the results further demonstrate that these NCs may be a kind of excellent drug carriers and capable of responding to the photothermal effects in an NIR light-responsive manner for controlled drug release.

CONCLUSION

In summary, we for the first time fabricated a novel nanoplatform with small particle sizes for remote and non-invasive NIR-light controlled drug delivery by grafting a thin layer of thermo-sensitive polymer on the hydrophobic Cu_7S_4 nanocrystals via ATRP. Due to the good photothermal performance of the encapsulated Cu_7S_4 core, these drug nanocarriers can serve for not only photo-responsive chemotherapy but also photothermo therapy. In addition, these nanocarriers are highly sensitive to the variation of the surrounding temperature around the LCST ($\sim 38^\circ\text{C}$), which facilitates well controlled drug release from the nanocarriers and tremendously reduces side effects to normal tissues, because only a mild (or short-time) NIR irradiation is needed to cause the slight temperature increment (1.0°C) from normal body temperature ($\sim 37^\circ\text{C}$) to the LCST ($\sim 38^\circ\text{C}$). This study may open up a new avenue for the development of other metal chalcogenide-based nanocarriers for remote and noninvasive NIR-light controlled synergetic photothermo/chemo therapy.

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Author contributions Wang L proposed the research direction and guided the project. Li Y and Xu M designed and performed the synthesis of the nanocarriers. Bai X performed the *in vitro* experiments. Hu G, Xu S and Wang L analyzed and discussed the experimental results, and drafted the manuscript. All the authors checked the manuscript.

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

Supplementary information Supporting data are available in the online version of the paper.

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光热响应的 Cu_7S_4 @pNIPAm纳米载体应用于可控的药物/光热治疗

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摘要 抗癌药物的靶向安全输送及远程无损控制释放是提高肿瘤治疗效率并降低毒副作用的前提, 但挑战巨大. 本文通过原子转移自由基聚合技术在单颗粒 Cu_7S_4 光热纳米粒子表面嫁接一层温敏高分子, 从而设计了一种近红外光热控制智能纳米药物胶囊. 该载药纳米胶囊具有较高的抗癌药物(阿霉素)负载能力(19.4%)和良好的光热转换效率(25.4%), 并具有合适的药物释放温度($\sim 38^\circ\text{C}$). 在正常生理条件下(37°C , pH 7.4), 10 h后仅有10.8%的阿霉素释放, 但在808-nm近红外光的照射下, Cu_7S_4 将光能转换成热, 诱导温敏高分子快速收缩, 从而导致所负载的阿霉素被挤出而释放, 达到远程光控药物释放的目的. 与此同时, Cu_7S_4 的光热效应还可用于肿瘤的热疗, 该复合纳米胶囊对癌症的化疗与热疗协同疗法将显著提高癌症治疗效果.