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ARTICLE

Aqueous Synthesis of PEGylated Copper Sulfide Nanoparticles for Photoacoustic Imaging of Tumors

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By integrating high imaging sensitivity and high resolution in a single modality, photoacoustic (PA) imaging emerges as a promising diagnostic tool for clinical applications. Benefiting from the absorption in near-infrared region (NIR), copper sulfide nanoparticles (NPs) as contrast agent are potentially useful for increasing the sensitivity of PA imaging. However, the aqueous synthesis of size-tunable, biocompatible and colloidal stable copper sulfide NPs remains challenging due to the intrinsic dipole-dipole interactions among particles. In this work, aqueous synthesis of PEGylated copper sulfide NPs with controllable size between 3–7 nm was developed. The particle size-dependent contrast enhancement effect of the copper sulfide NPs for PA imaging was carefully studied both *in vitro* and *in vivo*. Although the contrast enhancement effect of the copper sulfide NPs is proportional to particle size, the *in vivo* studies revealed that copper sulfide NPs smaller than 5 nm presented higher tumor imaging performance especially at tumor boundary site, which was further discussed in combination with the pharmacokinetic behaviors of differently sized particles.

Introduction

Photoacoustic (PA) imaging is a newly emerging biomedical modality that provides both high spatial resolution and strong biochemical contrast, benefiting from the combined merits from ultrasound imaging and optical imaging.^{1–4} Based on the ultrasonic signal generated from the PA effect, it is possible to obtain high spatial resolution images with optical contrast in a region up to 5–6 cm deep in biological tissues, which breaks through the optical diffusion limit.^{5,6} Meanwhile, PA imaging provides inherently background-free detection attributed to the fact that nonabsorbing tissue components present no background, and it overcomes the speckle artifact of ultrasound imaging.^{3,7} Relying on the endogenous optical absorbers in different tissues, especially the rich optical absorption from oxyhemoglobin and deoxyhemoglobin in red blood cell, numerous applications by using PA imaging have been exploited for visualizing biological structures at an anatomical or even functional level.^{8–10}

To further enhance the contrast in PA images, exogenous absorbers in the NIR region known as PA contrast agents are intensely investigated over the past decade.¹ Owing to the

intrinsic absorptive property, plasmonic nanomaterials, such as gold nanocrystals are widely applied as potential PA contrast agents.^{5,11–13} However, relatively larger size (>35 nm TEM size) are commonly needed to obtain absorption in NIR region for the above-mentioned nano-absorbers, resulting in rapid clearance by the reticuloendothelial (RES) system from intravenous delivery.⁵ Thus, the imaging performance is largely limited by the poor bioavailability of the contrast agents (herein, the bioavailability means an effective accumulation of the nanoparticles delivered through intravenous injection at the tumor site for imaging). Benefiting from the strong localized surface plasmon resonance (LSPR) effect originated from free holes, copper sulfide nanoparticle provide an alternative to Au particle-based photoacoustic contrast agent due to its inherent low hole density and high hole effective mass.^{14–17} In addition, its size-dependent NIR absorption behavior in small size regime (<10 nm) favors the *in vivo* bioapplications for tumor imaging.^{15,18–21}

Apart from the significant PA contrast enhancement effect of small copper sulfide NPs, water solubility and colloidal stability are basic requirements for their applications in biological system. Up to now, the synthesis of water-soluble copper sulfide NPs is mainly achieved through two synthetic routes: indirect organic-phase synthesis followed by a ligand-exchange process, and direct aqueous-phase synthesis. Albeit yielding high-quality NPs with narrow size distribution and high crystallinity, organic-phase synthetic route is strongly limited by the high-cost, unfriendly experimental conditions and complicated ligand-exchange process.^{22–26} Alternatively, direct aqueous synthetic route provides a greener approach for

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^c Electronic Supplementary Information (ESI) available: XRD results, theoretical hydrodynamic size, temporal evolution of TGA-capped copper sulfide NPs, concentration-dependent growth-inhibition of HeLa cells. See DOI: 10.1039/x0xx00000x

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acquiring water-soluble copper sulfide NPs.^{27–29} Nevertheless, due to the inherent dipole-dipole interaction, strong interactions among the NPs often lead to particle aggregation.^{25,30,31} Consequently, it remains challenging to develop efficient synthetic strategy for achieving aqueous copper sulfide NPs with excellent colloidal stability and size tunability.

Following on from our previous studies on the synthesis of copper chalcogenide and their *in vivo* tumor imaging applications,^{31–33} herein we report our recent results on the aqueous synthesis of water-soluble copper sulfide NPs with controllable size between 3–7 nm. The thiol-poly(ethylene glycol) (HS-PEG) was introduced as surface ligand for providing water solubility, colloidal stability as well as biocompatibility. The effects of particle size on the optical property, cytotoxicity and *in vivo* PA imaging of tumor were investigated.

Experimental Section

Chemicals

Copper (I) chloride (CuCl, 97%), Thioacetamide (TAA, 99%), Sodium hydroxide (NaOH, 96%) were purchased from Alfa Aesar. Thiol-PEG (HS-PEG, molecular weight 2000) was purchased from Tianjin Goldenbio Co., Ltd. Ether, and ethanol were analytical reagent grade and purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. Millipore quality deionized water (Milli-Q water) (resistivity is 18.2 MΩ·cm) was utilized in the experiments. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, 98%), dimethyl sulfoxide (DMSO), Dulbecco's Modified Eagle Medium (DMEM, high glucose), and fetal bovine serum (FBS) were bought from Biodee Biotechnology Co., Ltd., Beijing, China. Other solvents and chemicals were used without further purification.

Synthesis of the PEGylated copper sulfide NPs

In brief, 0.6 g (0.3 mmol) of HS-PEG was dissolved in 15 mL of water, and then 0.015 g (0.15 mmol) of CuCl was introduced while stirring. Nitrogen gas was introduced to purge the reaction solution for 1 h. The pH value of the reaction mixture was adjusted to 9.00 by dropwise addition of 0.5 M NaOH aqueous solution. After that, 0.0056 g (0.075 mmol) of TAA was then introduced while stirring. The resultant mixture was then heated to 90°C, and the reaction was allowed for 17 h under nitrogen protection. During this process, the color of the reaction solution progressively changed from light yellow to yellow, orange, and finally dark brown. A series of aliquots were extracted at 90°C for monitoring the particle growth. Then the solution was cooled to room temperature. Upon addition of ethanol into the resultant solution (vol:vol = 2:1), the resultant NPs were precipitated in ether and isolated by centrifugation. By being redispersed in ethanol and subsequently precipitated with ether for three cycles, the nanocrystals finally obtained were redispersed in water for further experiments. The particle solution was subsequently purified by ultrafiltration at 5000 g using a 10 kD ultrafiltration

device (Millipore). The condensed solution was then ready for further experiments.

Cytotoxicity assay of the PEGylated copper sulfide NPs

MTT assays were performed to assess the cytotoxicity of the copper sulfide NPs. Specifically, HeLa cells were first seeded in 96-well plates at a density of 4000 cells per well and cultured for about 24 h in DMEM supplemented with 10% FBS. Then, the cells were washed with PBS and incubated with copper sulfide NPs at different concentrations at 37°C for 24 h. Subsequently, the cells were washed twice with PBS followed by further proliferating in the culture medium for 48 h; subsequently, 20 µL of MTT with a concentration of 5 mg/mL was added and allowed to react with the cells for 4 h before the addition of 150 µL of DMSO for dissolving of the precipitation. Finally, the absorption of each solution was measured at 490 nm on a microplate reader (Thermo, Varioskan Flash).

Animal model

The tumor models used were established upon subcutaneous injection of HeLa cells ($\sim 5 \times 10^5$) into male BALB/c nude mice (4–6 weeks old) at the flank region of the right hind leg. The tumor imaging studies were carried out 10 days after the inoculations of tumor cells.

All animal experiments reported herein were carried out according to a protocol approved by Peking University Institutional Animal Care and Use Committee.

Photoacoustic (PA) imaging

In vitro PA imaging of PEGylated copper sulfide NPs in different concentrations were acquired by a multispectral optoacoustic tomography system (iThera Medical, inVision 256) at wavelength of 850 nm. For *in vivo* PA imaging studies, a nude mouse bearing subcutaneous tumor was anesthetized by 1% isoflurane delivered via a nose cone, and then the PEGylated copper sulfide NPs (3 mg Cu/kg body weight) were injected via the tail vein. *In vivo* PA imaging was acquired at different time points post injection by the multispectral optoacoustic tomography system at wavelength of 850 nm. The averaged PA signals of tumor regions were extracted with the multispectral optoacoustic tomography software.

Pharmacokinetics of the PEGylated copper sulfide NPs

Male, 6-week-old Sprague Dawley rats were adopted for the pharmacokinetic studies of differently sized PEGylated copper sulfide NPs, after they were intravenously injected into groups of rats ($n = 4$). The dose level was the same as that for imaging experiments. Blood samples were obtained via retro-orbital blood collection at 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 24 h, and 48 h postinjection. The red blood cells were removed from the collected blood by centrifugation at 1000 rpm. The blood were weighed, and eroded with concentrated nitric acid and H₂O₂ for measuring the Cu content in the plasma with ICP-OES.

Characterization

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Absorption spectra were recorded at room temperature on a Cary 50 UV-Vis spectrophotometer. Transmission electron microscopy (TEM) images were taken on FEI Tecnai20 and JEM-2100F microscopes working at an accelerating voltage of 200 kV. The metal composition was determined by the Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) method using a Thermo Fisher IRIS Intrepid II XSP. Powder X-ray diffraction (XRD) patterns were obtained with a Regaku D/Max-2500 diffractometer equipped with a Cu K α 1 radiation ($\lambda = 1.54056 \text{ \AA}$). Dynamic light scattering (DLS) measurements were carried out at 298.0 K with a Nano ZS (Malvern) equipped with a solid-state He-Ne laser ($\lambda = 633 \text{ nm}$) for measuring the hydrodynamic size of the resultant NPs. PA imaging was acquired by a multispectral optoacoustic tomography system (iThera Medical, inVision 256).

Results and discussion

Synthesis and characterization of the PEGylated copper sulfide NPs

In brief, the PEGylated copper sulfide NPs were prepared by a

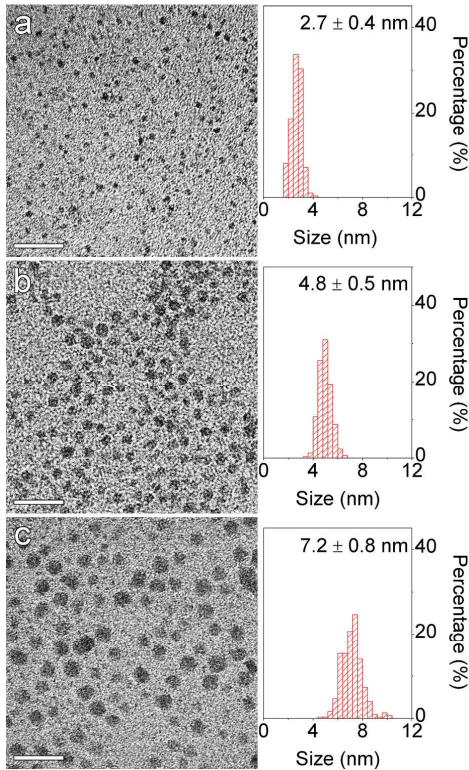


Figure 1. TEM images of PEGylated copper sulfide NPs acquired at reaction time of 1 h (a), 5 h (b), and 17 h (c) respectively, together with the corresponding size histograms. All scale bars correspond to 20 nm.

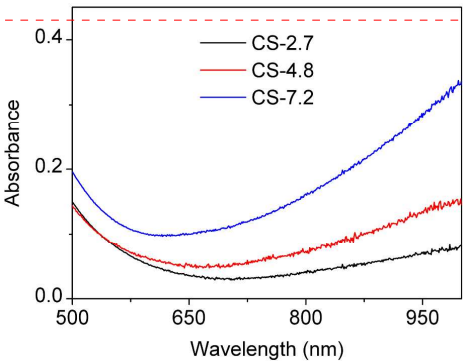


Figure 2. Absorption spectra of the PEGylated copper sulfide nanoparticles of 2.7 nm (black), 4.8 nm (red), and 7.2 nm (blue).

reaction heating the mixture of CuCl and TAA at 90°C in the presence of HS-PEG in water of pH = 9.00. HS-PEG was serving as a capping agent to ensure the water solubility of the NPs. Through a slow hydrolysis of TAA, differently sized NPs were obtained by different reaction times. Figure 1 presents the TEM images of the resultant NPs obtained at the reaction time of 1 h, 5 h, and 17 h. The NPs are uniform with average particle size of 2.7 nm, 4.8 nm, and 7.2 nm, respectively. The resultant NPs presented a crystalline structure close to chalcocite by X-ray diffractometry (XRD) as displayed in Figure S1 in Electronic Supplementary Information (ESI). Owing to the long chain of HS-PEG ligands,³⁴ the overall size of the NPs from the current reaction is much smaller than those prepared by using small molecular surface capping agents such as sodium citrate.¹⁸ Further particle size analysis reveal that the relative standard deviation firstly decreased for particles and then slightly increased, i.e. 14.8% (2.7 nm), 10.4% (4.8 nm) and 11.1% (7.2 nm). With further prolonged reaction time, the particle size distribution greatly increased, which suggest that Ostwald ripening process started to play a determined role governing the particle size.³⁵ Therefore, the above particle samples, denoted as CS-2.7, CS-4.8 and CS-7.2, were chosen for the following studies on particle size-dependent optical property, cytotoxicity and tumor imaging performance together with pharmacokinetic behaviors.

Optical property of the PEGylated copper sulfide NPs

For PA imaging application, the contrast enhancement performance of copper sulfide NPs strongly depends on their optical property.^{14,36} As displayed in Figure 2, copper sulfide NPs present a broad NIR absorption band that can be attributed to the LSPR effect arising from free holes originating from the copper vacancy.^{14,16} Theoretically, the LSPR energy is proportional to the copper vacancy density,¹⁴ which explains the strong particle size-dependent LSPR intensity shown in Figure 2 as well as the strongest LSPR intensity of CS-7.2.

Colloidal stability of the PEGylated copper sulfide NPs

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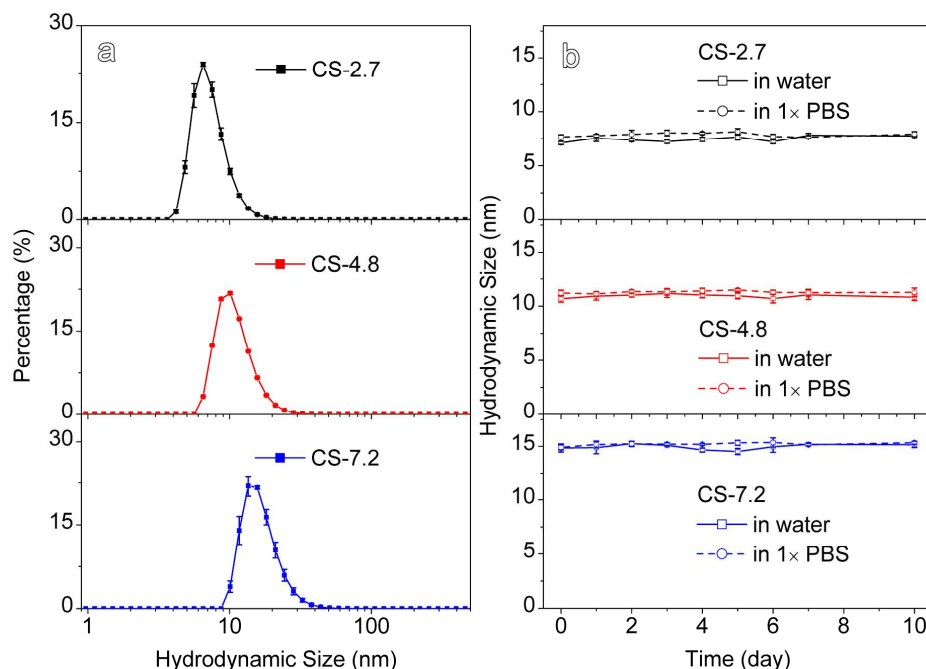


Figure 3. The hydrodynamic size profiles of as-prepared 2.7 nm (black), 4.8 nm (red), and 7.2 nm (blue) copper sulfide nanoparticles, respectively (a), and temporal evolutions of the hydrodynamic sizes of the corresponding samples in water (square) and 1x PBS (circle), respectively (b).

To evaluate the colloidal stability of the as-prepared NPs, the hydrodynamic size of PEGylated copper sulfide NPs dispersed in water and PBS buffer was characterized by DLS. As shown in Figure 3a, all particle samples exhibit single scattering peaks in water locating at 7.1 nm, 10.7 nm, and 14.9 nm for CS-2.7, CS-4.8, and CS-7.2, respectively. Moreover, the experimental hydrodynamic sizes are in good agreement with the theoretical predictions as list in Table S1, indicating that the PEGylated copper sulfide NPs form no aggregates in water.³⁷

Long-term (ca. 10 days) observations further demonstrate that the resultant particles possess excellent colloidal stability in water as well as PBS buffer that is commonly used for mimicking the biological conditions. All these data support that HS-PEG can effectively render copper sulfide NPs satisfying water solubility and colloidal stability by suppressing the dipole-dipole interactions among copper sulfide NPs, probably due to the steric hindrance effect of HS-PEG. To verify this hypothesis, thioglycolic acid (TGA) was used instead of HS-PEG in the synthesis of copper sulfide NPs. The following DLS studies revealed that the TGA-capped NPs gradually formed large agglomerates within days (Figure S2).

Cytotoxicity of the PEGylated copper sulfide NPs

The cytotoxicity of the PEGylated copper sulfide NPs was evaluated through MTT assays on the proliferation of HeLa cells. As is shown in Figure 4, all the PEGylated copper sulfide NPs present nearly no toxicity to HeLa cells at a concentration below 3 mg/L. The cell viability starts to decrease when the particle concentration is higher than 5 mg/L, but remains above 40% even when the particle concentration reaches 25 mg/L. The positive correlation of the cell viability with the particle size may be caused by the enhanced ion leakage of smaller NPs.^{38–40} However, according to the literature reports,^{18,21} if a dose of the copper sulfide NPs around 3 mg/kg is suitable for PA imaging, the current copper sulfide NPs are nearly nontoxic. Further theoretical fittings revealed that the

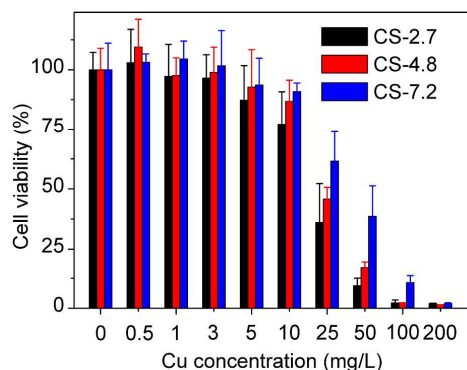


Figure 4. Viability of HeLa cells recorded after being incubated with differently sized PEGylated copper sulfide nanoparticles with different Cu concentrations.

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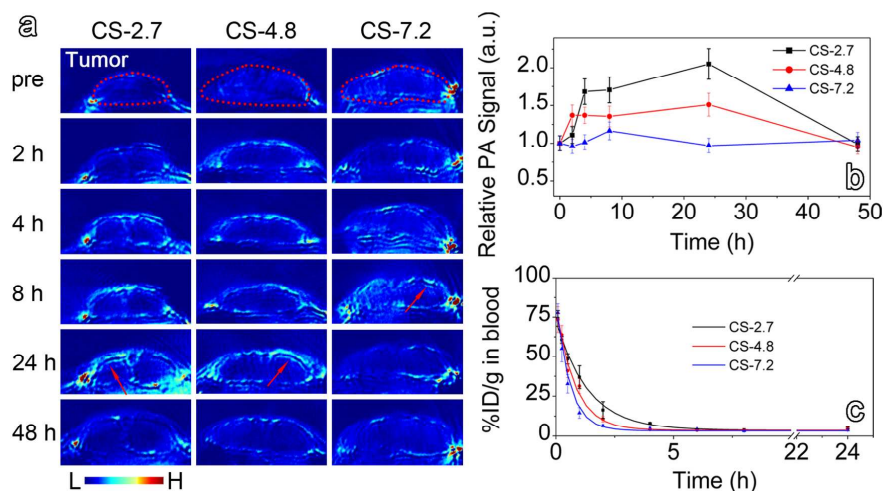


Figure 6. *In vivo* photoacoustic images acquired pre- and at different time points after intravenous injection of the differently sized copper sulfide nanoparticles into the mouse bearing subcutaneous tumors (a), temporal evolutions of the integrated photoacoustic signals recorded from the tumor region (b), blood clearance profiles of differently sized copper sulfide nanoparticles in rats ($n = 4$) (c). The solid lines in frame c are theoretical fitting curves obtained through single-compartment model for depicting the clearance behaviors of the nanoparticles.

IC_{50} (50% inhibitory concentration) values of CS-2.7, CS-4.8, CS-7.2 are 5.7 $\mu\text{mol/L}$, 1.5 $\mu\text{mol/L}$, 0.6 $\mu\text{mol/L}$, respectively, if the IC_{50} values are expressed against particle concentration rather than copper concentration (Figure S3).

In vitro PA imaging of the PEGylated copper sulfide NPs

The PA imaging performance of PEGylated copper sulfide NPs was evaluated on a multispectral optoacoustic tomography system at room temperature. The upper frame of Figure 5

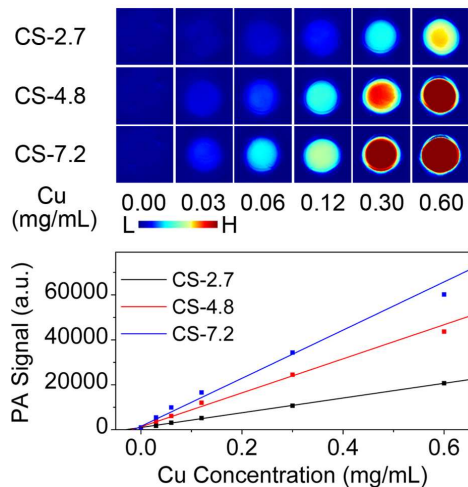


Figure 5. *In vitro* photoacoustic imaging (Upper frame) and the correspondingly quantified PA signal (Lower frame) of aqueous solutions containing differently sized PEGylated copper sulfide nanoparticles.

shows three sets of the PA images of aqueous solutions containing NPs of CS-2.7, CS-4.8, and CS-7.2. It can be seen that all the three NPs are presenting dramatic PA effect. The quantified PA signals against Cu concentration reveal that the contrast enhancement effect is positively correlated to the particle-size due to the size-dependent absorbance of copper sulfide NPs.^{3,5,41}

In vivo PA imaging of tumors with the PEGylated copper sulfide NPs

To further show the PA contrast enhancement effect of the as-prepared copper sulfide NPs in tumor imaging, a subcutaneously transplanted tumor model bearing with HeLa xenografts was adopted. 10 days after the inoculation of tumor cells, three groups of BALB/c nude mice ($n = 4$ per group) with comparable tumor sizes of 5–7 mm were selected to evaluate the PA imaging performance of copper sulfide NPs that were intravenously injected through tail vein. A set of PA images of tumor region acquired before and at different time points post-injection are presented in Figure 6a. The overall contrast of the tumor sites was gradually enhanced and the enhancement effects at tumor boundary (indicated by red arrows) became more significant, which is greatly meaningful for imaging guided surgery. Such boundary enhancement effect was previously explained by the unique feature of absorption contrast in PA imaging.^{42,43}

To further quantitatively evaluate the performance of the copper sulfide NPs, the PA signals of the region of interest (ROI) in each set of images are normalized and presented in Figure 6b. The quantified results reveal that smaller particles produce dramatically increased contrasts for the tumor site than larger particles in the first 4 h of post-injection.

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Thereafter, the PA signals of tumors enhanced by both CS-2.7 and CS-4.8 NPs keep increasing and reach maximum values at around 24 h, while the PA signals of tumors enhanced by CS-7.2 NPs stop increasing after 8 h of post-injection. According to the results shown in the lower frame of Figure 5, the PA signal generated with CS-7.2 NPs is approximately 62 times of that with CS-2.7 NPs if normalizing the concentration by the molar concentration of NPs rather than Cu ions, i.e., larger copper sulfide NPs produce stronger PA contrast than smaller copper sulfide NPs *in vitro*. While the quantified results shown in Figure 6b reveals that the maximum contrast produced by CS-2.7 NPs (24 h) is 1.76 times of that achieved by CS-7.2 NPs (8 h) although the dose of CS-2.7 is only 18 times higher than that of CS-7.2 NPs according to the molar concentration of nanoparticles. These quantified results suggest that the overall contrast enhancement of smaller particles is more significant than that of larger ones for *in vivo* tumor imaging, which may be attributed to more effective tumor uptake of smaller NPs than bigger ones. To confirm this hypothesis, further pharmacokinetic studies of differently sized PEGylated copper sulfide NPs were carried out on the Sprague Dawley rats and the results shown in Figure 6c demonstrate that the blood half-time of the copper sulfide NPs is inversely proportional to the particle size, e.g., 33 min for CS-2.7, 25 min for CS-4.8, and 20 min for CS-7.2. Since long blood half-time is in favour of the extravasation of copper sulfide NPs from blood to the tumor through enhanced permeability of tumor blood vessels, the smaller particles presented better PA contrast enhancement effect *in vivo*.^{44,45}

Conclusions

In summary, we have successfully synthesized monodispersed PEGylated copper sulfide NPs through an aqueous synthetic route. The surface PEGylation rendered copper sulfide NPs excellent colloidal stabilities in both water and PBS buffer apart from significant LSPR effect. The low cytotoxicity of the copper sulfide NPs enabled them potentially useful as contrast agent for PA imaging. Although larger particles exhibit stronger contrast enhancement effect, the following tumor imaging studies suggest that the small particles can more effectively enhance the contrast of tumor boundary due to prolonged blood half-time, which highlights the importance of strict particle size control for sensitive tumor imaging. It is deserve to mention that the tumor boundary can more significantly be enhanced by copper sulfide particles smaller than 5 nm, which is clinically meaningful for developing PA contrast agents for imaging guided tumor therapy.

Acknowledgments

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References

1. L. M. Nie, X. Y. Chen, *Chem. Soc. Rev.*, 2014, **43**, 7132-7170.
2. X. Yang, S. E. Skrabalak, Z.-Y. Li, Y. Xia, L. V. Wang, *Nano Lett.*, 2007, **7**, 3798-3802.
3. L. V. Wang, S. Hu, *Science*, 2012, **335**, 1458-1462.
4. T. Harrison, J. C. Ranasinghesagara, H. Lu, K. Mathewson, A. Walsh, R. J. Zemp, *Opt. Express*, 2009, **17**, 22041-22046.
5. W. Li, X. Chen, *Nanomedicine (Lond.)*, 2015, **10**, 299-320.
6. L. H. V. Wang, *Nat. Photonics*, 2009, **3**, 503-509.
7. L. H. V. Wang, *Dis. Markers*, 2003, **19**, 123-138.
8. R. Schulze, G. Zangerl, M. Holotta, D. Meyer, F. Handle, R. Nuster, G. Paltauf, O. Scherzer, *J. Biomed. Opt.*, 2011, **16**, 086002.
9. J. A. Viator, J. Komadina, L. O. Svaasand, G. Aguilar, B. Choi, J. S. Nelson, *J. Invest. Dermatol.*, 2004, **122**, 1432-1439.
10. Y. Wang, S. Hu, K. Maslov, Y. Zhang, Y. Xia, L. V. Wang, *Opt. Lett.*, 2011, **36**, 1029-1031.
11. J. V. Jokerst, A. J. Cole, D. Van de Sompel, S. S. Gambhir, *ACS Nano*, 2012, **6**, 10366-10377.
12. C. Kim, E. C. Cho, J. Chen, K. H. Song, L. Au, C. Favazza, Q. Zhang, C. M. Cobley, F. Gao, Y. Xia, L. V. Wang, *ACS Nano*, 2010, **4**, 4559-4564.
13. L. Nie, S. Wang, X. Wang, P. Rong, A. Bhirde, Y. Ma, G. Liu, P. Huang, G. Lu, X. Chen, *Small*, 2014, **10**, 1585-1593.
14. J. M. Luther, P. K. Jain, T. Ewers, A. P. Alivisatos, *Nat. Mater.*, 2011, **10**, 361-366.
15. X. Liu, X. Wang, B. Zhou, W.-C. Law, A. N. Cartwright, M. T. Swihart, *Adv. Funct. Mater.*, 2013, **23**, 1256-1264.
16. X. Liu, M. T. Swihart, *Chem. Soc. Rev.*, 2014, **43**, 3908-3920.
17. Y. Zhao, H. Pan, Y. Lou, X. Qiu, J. Zhu, C. Burda, *J. Am. Chem. Soc.*, 2009, **131**, 4253-4261.
18. G. Ku, M. Zhou, S. L. Song, Q. Huang, J. Hazle, C. Li, *ACS Nano*, 2012, **6**, 7489-7496.
19. S. Goel, F. Chen, W. B. Cai, *Small*, 2014, **10**, 631-645.
20. M. Zhou, G. Ku, L. Paeon, C. Li, *Nanoscale*, 2014, **6**, 15228-15235.
21. K. Yang, L. Zhu, L. M. Nie, X. L. Sun, L. Cheng, C. X. Wu, G. Niu, X. Y. Chen, Z. Liu, *Theranostics*, 2014, **4**, 134-141.
22. Y. Wu, C. Wadia, W. Ma, B. Sadler, A. P. Alivisatos, *Nano Lett.*, 2008, **8**, 2551-2555.
23. Q. Y. Lu, F. Gao, D. Y. Zhao, *Nano Lett.*, 2002, **2**, 725-728.
24. C.-H. Kuo, Y.-T. Chu, Y.-F. Song, M. H. Huang, *Adv. Funct. Mater.*, 2011, **21**, 792-797.
25. Z. Zhuang, Q. Peng, B. Zhang, Y. Li, *J. Am. Chem. Soc.*, 2008, **130**, 10482-10483.
26. C. G. Tian, Z. H. Kang, E. B. Wang, L. Gao, C. L. Wang, L. Xu, C. W. Hu, *Mater. Lett.*, 2005, **59**, 1156-1160.
27. G. X. Ma, Y. L. Zhou, X. Y. Li, K. Sun, S. Q. Liu, J. Q. Hu, N. A. Kotov, *ACS Nano*, 2013, **7**, 9010-9018.
28. H. L. Cao, X. F. Qian, C. Wang, X. D. Ma, J. Yin, Z. K. Zhu, *J. Am. Chem. Soc.*, 2005, **127**, 16024-16025.
29. L. D. Partain, P. S. McLeod, J. A. Duisman, T. M. Peterson, D. E. Sawyer, C. S. Dean, *J. Appl. Phys.*, 1983, **54**, 6708-6720.
30. S.-C. Liufu, L.-D. Chen, Q. Yao, F.-Q. Huang, *J. Phys. Chem. C*, 2008, **112**, 12085-12088.
31. L. X. Yi, M. Y. Gao, *Cryst. Growth Des.*, 2011, **11**, 1109-1116.
32. W. Han, L. X. Yi, N. Zhao, A. W. Tang, M. Y. Gao, Z. Y. Tang, *J. Am. Chem. Soc.*, 2008, **130**, 13152-13161.
33. K. Ding, L. H. Jing, C. Y. Liu, Y. Hou, M. Y. Gao, *Biomaterials*, 2014, **35**, 1608-1617.
34. K. De Nolf, R. K. Capek, S. Abe, M. Sluydts, Y. Jang, J. C. Martins, S. Cottenier, E. Lifshitz, Z. Hens, *J. Am. Chem. Soc.*, 2015, **137**, 2495-2505.
35. Z. A. Peng, X. G. Peng, *J. Am. Chem. Soc.*, 2001, **123**, 1389-1395.
36. J. Grodzanov, M. Najdoski, *J. Solid State Chem.*, 1995, **114**, 469-475.

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37 R. A. Sperling, T. Liedl, S. Duhr, S. Kudera, M. Zanella, C. A. J. Lin, W. H. Chang, D. Braun, W. J. Parak, *J. Phys. Chem. C*, 2007, **111**, 11552-11559.

38 Y. Pan, S. Neuss, A. Leifert, M. Fischler, F. Wen, U. Simon, G. Schmid, W. Brandau, W. Jahnke-Dechent, *Small*, 2007, **3**, 1941-1949.

39 M. V. D. Z. Park, A. M. Neigh, J. P. Vermeulen, L. J. J. de la Fonteyne, H. W. Verharen, J. J. Briedé, H. van Loveren, W. H. de Jong, *Biomaterials*, 2011, **32**, 9810-9817.

40 M. Baek, M. K. Kim, H. J. Cho, J. A. Lee, J. Yu, H. E. Chung, S. J. Choi, *Journal of Physics: Conference Series*, 2011, **304**, 012044.

41 X. D. Wang, Y. J. Pang, G. Ku, X. Y. Xie, G. Stoica, L. H. V. Wang, *Nat. Biotechnol.*, 2003, **21**, 803-806.

42 Z. Guo, L. Li, L. V. Wang, *Med. Phys.*, 2009, **36**, 4084-4088.

43 L. Nie, M. Chen, X. Sun, P. Rong, N. Zheng, X. Chen, *Nanoscale*, 2014, **6**, 1271-1276.

44 H. Maeda, H. Nakamura, J. Fang, *Adv. Drug Deliv. Rev.*, 2013, **65**, 71-79.

45 N. Bertrand, J. Wu, X. Xu, N. Kamaly, O. C. Farokhzad, *Adv. Drug Deliv. Rev.*, 2014, **66**, 2-25.

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