Supporting Information

A high quantum yield Ag_2S quantum dot@polypeptide-engineered hybrid nanogels for targeted second near-infrared fluorescence/photoacoustic imaging and photothermal therapy

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Materials and Methods

1 Materials

Restriction endonuclease BamHI, NheI, SpeI, and T4 DNA ligase were obtained from New England Biolabs Inc. (Beijing, China). Diethyldithiocarbamic acid silver salt (AgDDTC, 98%), dodecanethiol (DT, 98%), ampicillin, kanamycin were purchased from Sinopharm Group Chemical Reagent Co., Ltd (Shanghai, China). Isopropyl-*β*-D-thiogalactoside (IPTG), calcein AM, octadecene (ODE, 90%) were obtained from Sigma-Aldrich, Inc. (St. Louis, MO). Nickelnitrilotriacetic acid (Ni-NTA) separation column was purchased from Qiagen China (Shanghai) Co., Ltd. Ultrapure water (≥18.2 MΩ) purified by the Milli-Q system (Millipore, USA) was used for preparation of all solutions.

2 Synthesis and purification of the coil-coiled polypeptides

PQE9PC_{10}A plasmid was a gift from Prof. David Tirrell at the California Institute of Technology, Pasadena, CA. PC_{10}A and PC_{10}ARGD polypeptides were prepared according to our previously reported method.[1] The synthesized polypeptides were analyzed on a Bruker Reflex III reflectron MALDI-TOF mass spectrometer. PC_{10}A (MS: 20932.6 Da, the theoretical calculation of molecular weight: 20858.5 Da), PC_{10}ARGD (MS: 22371.4 Da, the theoretical calculation of molecular weight: 22295.9 Da).

3 Synthesis of DT coated Ag_2S QD

DT coated Ag_2S QDs were prepared according to previous reports with some modifications.[2] Briefly, 76.8 mg AgDDTC was added into a solution containing 6 g DT and 30 g ODE under argon protection. The solution was heated to 100 °C to remove water for 10 min. The solution was stirred and heated to 140 °C for 10 min, and the solution turned dark brown immediately after being heated to 140 °C. Hexane was added to terminate the reaction.

After cooled down to room temperature, the solution was centrifuged and washed twice with acetone. Hydrophobic Ag_2S QD were re-dispersed in chloroform for further using.

4 Preparation of polypeptide nanogels and Ag_2S QD@polypeptide nanogels

Various concentrations (0.5%, 0.1%, 0.05%, 0.01% w/v) $PC_{10}A$ or $PC_{10}ARGD$ were prepared in ultrapure water, and all the samples were heated in 100 ^oC boiling water for 5 minutes. After cooling and renaturation, $PC_{10}A$ or $PC_{10}ARGD$ nanogels were successfully prepared.

Ag_2S QD@polypeptide hybrid nanogels were prepared by ultrasonic treatment. $PC_{10}A(RGD)$ (0.025% w/w) were prepared in ultrapure water, and the solution was heated in 100 $^\circ$ C boiling water for 5 minutes. After cooling to room temperature, different concentration (0.03, 0.06, 0.125, 0.25, 0.5, 1 mg mL^{-1}) hydrophobic Ag_2S QD were added, and the mixtures were treated ultrasonically for 5 min. Clarified water-soluble Ag_2S QD@$PC_{10}A(RGD)$ hybrid nanogels were obtained. The morphology of $PC_{10}A(RGD)$ nanogels, Ag_2S QD, and Ag_2S QD@$PC_{10}A(RGD)$ hybrid nanogels were measured on a G2 20 U-Twin transmission electron microscope (TEM). The UV-visible absorbance spectra of Ag_2S QD and Ag_2S QD@$PC_{10}A(RGD)$ hybrid nanogels were determined by a UV-2550 UV-vis spectrophotometer (Shimadzu, Japan) at room temperature. The fluorescence spectra of Ag_2S QD, and Ag_2S QD@$PC_{10}A(RGD)$ hybrid nanogels were collected by near infrared spectroscopy (Ocean Optics, USA), a NIR laser (λ = 810 nm) as light source (Changchun New Industries Optoelectronics Technology, Changchun, China). The fluorescence images of Ag_2S QD@$PC_{10}A(RGD)$ hybrid nanogels solution with different concentration were collected on a homemade built fluorescence imaging system.

5 Fluorescence quantum yields (QY)

The QY of oil-soluble Ag_2S QD and Ag_2S QD@PC_{10}ARGD were measured using indocyanine green (ICG) in DMSO as reference (QY = 13%) under the excitation of an 810 nm laser. A series of solutions of ICG in DMSO, Ag_2S QD in chloroform, and Ag_2S QD@PC_{10}ARGD aqueous solution with the same absorbance values at 810 nm were prepared, respectively. The fluorescence spectra of the solutions were measured. The fluorescence intensities were plotted against the absorbance values measured at 810 nm and the slopes were obtained by linear fitting. The QY were calculated by the previously reported mehod.[3]

6 Stabilities of PC_{10}ARGD nanogels and Ag_2S QD@PC_{10}ARGD hybrid nanogels

The hydrodynamic size and zeta potential of PC_{10}ARGD nanogel (0.1% w/w) and Ag_2S QD@PC_{10}ARGD hybrid nanogels (0.1% w/w of nanogels and 0.5 mg mL^{-1} Ag ion) at pH 7.4 under the condition of 4 ºC, 25 ºC, and 37 ºC were determined daily on a ZS90 Nanosizer within six weeks.

7 Photothermal effect of Ag_2S QD@PC_{10}ARGD hybrid nanogels

In order to evaluate the photothermal effect, 0.3 mL of Ag_2S QD@PC_{10}ARGD (0.03, 0.06, 0.125, 0.25, 0.5, 1 mg mL^{-1} Ag ion) was directly exposed to a NIR laser (Changchun New Industries Optoelectronics Technology, Changchun, China) at 2.5 W cm^{-2} for 8 minutes. The NIR laser was placed 5 cm over the test tube. The temperature increase was recorded using a EasIR-9 Thermal Imager (Wuhan Guide Infrared Co., Ltd, China). At the same time, the photothermal effect of Ag_2S@PC_{10}ARGD (0.03 mg mL^{-1} Ag ion) under different power density of laser irradiation was measured.

8 Photothermal stability and photothermal conversion efficiency of Ag_2S QD@PC_{10}ARGD hybrid nanogels

Ag_2S QD@PC_{10}ARGD (0.25 mg mL^{-1} Ag ion) was irradiated with a NIR laser (810 nm, 2.5 W cm^{-2}) for 5 min, and the laser was shut off to make the solution cool down to room

temperature without laser for 10 min. The temperature was recorded using a EasIR-9 Thermal Imager. This laser ON/OFF process was repeated for another four cycles to monitor photothermal stability.

To calculate the photothermal conversion efficiency, 0.3 mL of Ag_2S QD@PC_{10}ARGD aqueous solution (1 mg mL^{-1} Ag ion) was irradiated at 2.5 W cm^{-2} in an EP tube. The laser was removed when the temperature of solution rose no longer. The solution was cooled down to room temperature. The temperature was recorded every 10 seconds during the experiment with a EasIR-9 Thermal Imager.

9 *In vitro* cytotoxicity

HeLa cells were seeded in 96-well plates (10^4 cells per well) and cultured in a cell incubator (5% CO_2, 37 ºC). After incubation for 24 h, the medium was discarded, and cells were washed twice with PBS. Following incubation with PC_{10}ARGD or Ag_2S QD@PC_{10}ARGD with various concentrations in serum-free DMEM for another 48 h, The incubated cells were assayed for cell viability with 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT). The cells were washed twice with PBS, and MTT (20 μL, 5 μg mL^{-1}) solution was added to each well. After incubation for 4 h, the medium was discarded, and the insoluble purple formazan crystals were dissolved with DMSO (150 μL). The absorbance at 490 nm was recorded with a microplate reader (BioTek ELX808IU, USA). The absorbance was directly correlated with cell quantity, and cell viability was calculated by assuming 100% viability in the control.

10 *In vitro* NIR fluorescence imaging

HeLa and MCF-7 cells were seeded in 6-well plates in their respective meida at a density of 1×10^6 cells per well and cultured in a cell incubator (5% CO_2, 37 ºC). After incubation for 22 h, the cells were washed twice with PBS. The cells were incubated with

$Ag_2S@PC_{10}ARGD$ or $Ag_2S@PC_{10}A$ (0.25 mg mL^{-1} Ag ion) for 4 h. The cells were washed twice with cold PBS and trypsinized. The cells were centrifuged at 1000 g for 8 min in a 1.5 mL EP tube. The collected cells were imaged on a homemade built fluorescence imaging system. The system consists of a fiber-coupled 748-nm diode laser (B&W Tek, U.S.), a two-dimensional scanner (WN202WA100X100, Winner Optical Instruments Group Company, China), an InGaAs CCD camera (XEVA-353, XenIC,Belgium), and an EMCCD camera (DU897, Andor, U.K.).[4]

11 *In vitro* photoacoustic imaging

HeLa and MCF-7 cells were seeded in 6-well plates in their respective meida at a density of 1×10^6 cells per well and cultured in a cell incubator (5% CO_2, 37 ºC). After incubation for 22 h, the cells were washed twice with PBS. The cells were incubated with $Ag_2S@PC_{10}ARGD$ or $Ag_2S@PC_{10}A$ (0.25 mg mL^{-1} Ag) for 4 h. The cells were washed twice with PBS and trypsinized. The cells were centrifuged at 1000 g for 8 min in a 1.5 mL EP tube. The collected cells were imaged on a homemade built photoacoustic imaging system. A dye laser (Credo, Sirah Laser und Plasmatechnik,Germany) pumped by a Nd:YLF laser (IS8II-E, EdgeWave GmbH, Germany) was employed as the wavelength-tunable irradiation source.[5]

12 *In vitro* photothermal therapy

HeLa cells were seeded in 24-well plates at a density of 1×10^4 cells per well and cultured in a cell incubator (5% CO_2, 37 ºC). After incubation for 24 h, the cells were washed twice with PBS. The cells were incubated with $Ag_2S@PC_{10}ARGD$ or $Ag_2S@PC_{10}A$ (0.25 mg mL^{-1} Ag ion) for 2 h. The cells were washed twice with PBS. PBS (200 μL) was added in each well. Subsequently, multiple regions of cells were exposed to an 810 nm laser at a power density of 2.5 W cm^{-2} for 10 min, and cells were stained with Calcein AM for 10 min. The

cells were washed twice with PBS and imaged immediately with a 20 × objective on an inverted microscope (Olympus IX71, Japan).

13 *In vivo* toxicity of Ag_2S QD@PC_{10}ARGD hybrid nanogels

To study the potential toxicity of Ag_2S QD@PC_{10}ARGD hybrid nanogels, female BALB/C mice were randomly divided into two groups (n = 20 per group). Ag_2S QD@PC_{10}ARGD (1 mg mL^{-1} Ag ion, 100 μL) was intratumorally injected into female BALB/c mice. In control, the other group was injected with PBS. Blood samples were collected at different time points (0, 6 h, 1, 3, 7, and 15 d) after injection and used for the biochemical analysis of liver enzymes and blood analysis. Main organs (heart, lungs, spleen, kidneys, liver, and small intestine) were also collected at different time points, fixed with 4% paraformaldehyde, and embedded in paraffin for hematoxylin and eosin (H&E) staining analysis. All animal experiments were approved by the Animal Experimental Ethics Committee of Huazhong University of Science and Technology.

14 *In vivo* fluorescent imaging and photoacoustic imaging

HeLa cells (10^6 cells per 100 μL of PBS) were injected into the back of female BALB/c mice (6 weeks old). When the tumor volume reached 60-100 mm^3, Ag_2S QD@PC_{10}ARGD hybrid nanogels (0.025% w/w PC_{10}ARGD, 1 mg mL^{-1} Ag ion, 100 μL) was intratumorally injected into the tumor-bearing mice. At different times (0, 6, 12, 24, 48, 60, and 72 h), the mice were scanned on a homemade built PA imaging system or a homemade built fluorescence system to collect the PA or NIR II fluorescence signals. Image reconstruction was analyzed using MATLAB. All animal experiments were approved by the Animal Experimental Ethics Committee of Huazhong University of Science and Technology.

15 *In vivo* photothermal therapy

HeLa cells (10^6 cells per 100 μL of PBS) were injected into the back of female BALB/c mice (6 weeks old). The HeLa tumor-bearing mice were divided into four groups (five mice per group): (a) Ag_2S QD@PC_{10}ARGD (0.025% w/w PC_{10}ARGD, 1 mg mL^{-1} Ag ion) with

laser, (b) PBS with laser, (c) PBS without laser, (d) Ag_2S QD@PC_{10}ARGD (0.025% w/w PC_{10}ARGD, 1 mg mL^{-1} Ag ion) without laser. When the tumor volume reached 60-100 mm^3, 100 μL of Ag_2S QD@PC_{10}ARGD (0.025% w/w PC_{10}ARGD, 1 mg mL^{-1} Ag ion) or PBS was injected intratumorally, separately. The tumors in the group of a and b were irradiated by the 810 nm laser for 10 min at 2.5 W cm^{-2}. The temperature changes of the tumor sites were recorded during irradiation using an infrared thermal images system (Wuhan Guide Infrared Co., Ltd, China). After treatment, the mice of weight and tumor size were monitored every day within 18 days, and the volume was calculated using the following formula: tumor volume = (tumor length) × (tumor width)^2/2.^{6} H&E staining of tumor sites were applied at 18 day after treatment. Experimental results were presented as mean ± SD. All animal experiments were approved by the Animal Experimental Ethics Committee of Huazhong University of Science and Technology.

Results and Discussion

Prior to the use of Ag_2S QD@PC_{10}ARGD hybrid nanogels for tumor targeting imaging and PTT, an MTT assay was used to investigate the in vitro cytotoxicity of PC_{10}ARGD nanogels and Ag_2S QD@PC_{10}ARGD hybrid nanogels. HeLa cells were incubated with different concentrations (0.05%, 0.1%, 0.5% and 1% w/w) of PC_{10}ARGD nanogels for 48 h to investigate the cytotoxicity of PC_{10}ARGD nanogels. As shown in Fig. S8 (ESI†), the viabilities of the cells incubated with PC_{10}ARGD nanogels were above 90%, indicating that these nanogels are nontoxic. Therefore, PC_{10}ARGD nanogels can be used as a good coating material for preparation of hybrid nanomaterials. In addition, the cytotoxicity of Ag_2S QD@PC_{10}ARGD hybrid nanogels was also measured by the MTT assay (Fig. S9, ESI†). Within 48 h, the viabilities of cells incubated with Ag_2S QD@PC_{10}ARGD hybrid nanogels containing Ag ions under 0.5 mg mL^{-1} were above 90%. These results indicated that such

Ag2S QD@PC10ARGD hybrid nanogels showed good biocompatibility and were suitable for application in biomedical fields.

References

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Amino acid sequences of $PC_{10}A$ and $PC_{10}ARGD$

$PC_{10}A$: MRGS**[6H]**GSGDL**[P]**GTSYRDPMG**[C_{10}]**ARMPT**[A]**IG DHVAPRDTSW

$PC_{10}ARGD$: MRGS**[6H]**GSGDL**[P]**GTSYRDPMG**[C_{10}]**ARMPT**[A]**IG DHVAPRDTSYAVTG**RGD**SPASSTSW

Abbreviations for peptide domains:

[6H]: HHHHHH

[P]: APQMLRELQETNAALQDVRELLRQQVKEITFLKNTVMESDAS

[A]: SGDLENEVAQLEREVRSLEDEAAELEQKVSRLKNEIEDLKAE

[C_{10}]: $[AGAGAGPEG]_{10}$ (hydrophilic random coil)

Fig. S1 The sequences of the polypeptides used in the study.

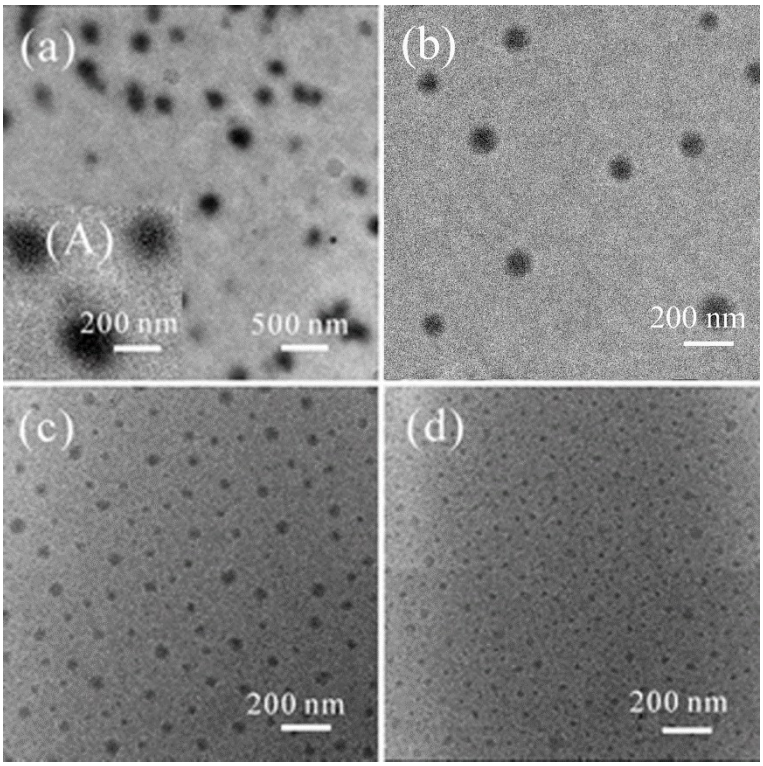

Fig. S2 TEM images of polypeptide $PC_{10}ARGD$ nanogels with different size at different concentrations. (a) 0.5% w/w, 200-300 nm; (b) 0.1% w/w, 100-150 nm; (c) 0.05% w/w, 50-100 nm; (d) 0.01% w/w, 20-50 nm. Inset (A) is the amplification of (a).

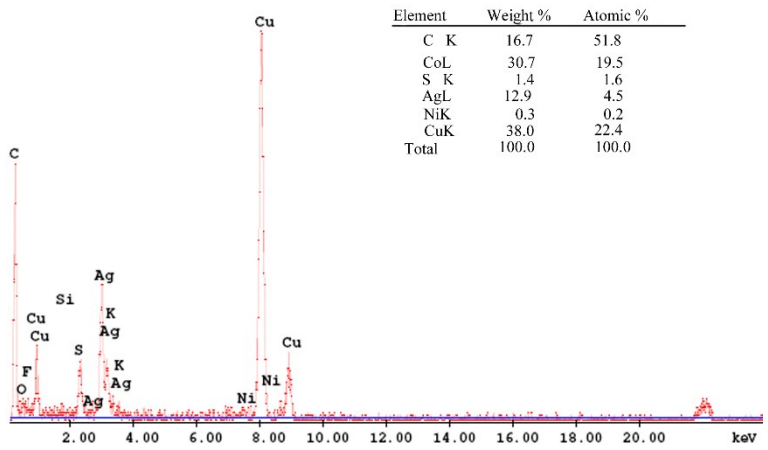

Fig. S3 The energy dispersive X-ray spectrum of Ag_2S QD@PC_{10}ARGD hybrid nanogels.

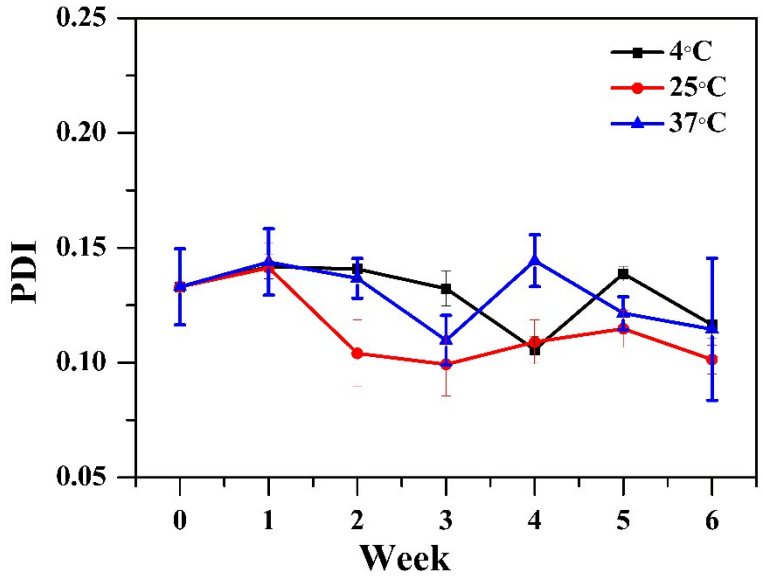

Fig. S4 The polydispersity index of Ag_2S QD@PC_{10}ARGD hybrid nanogels.

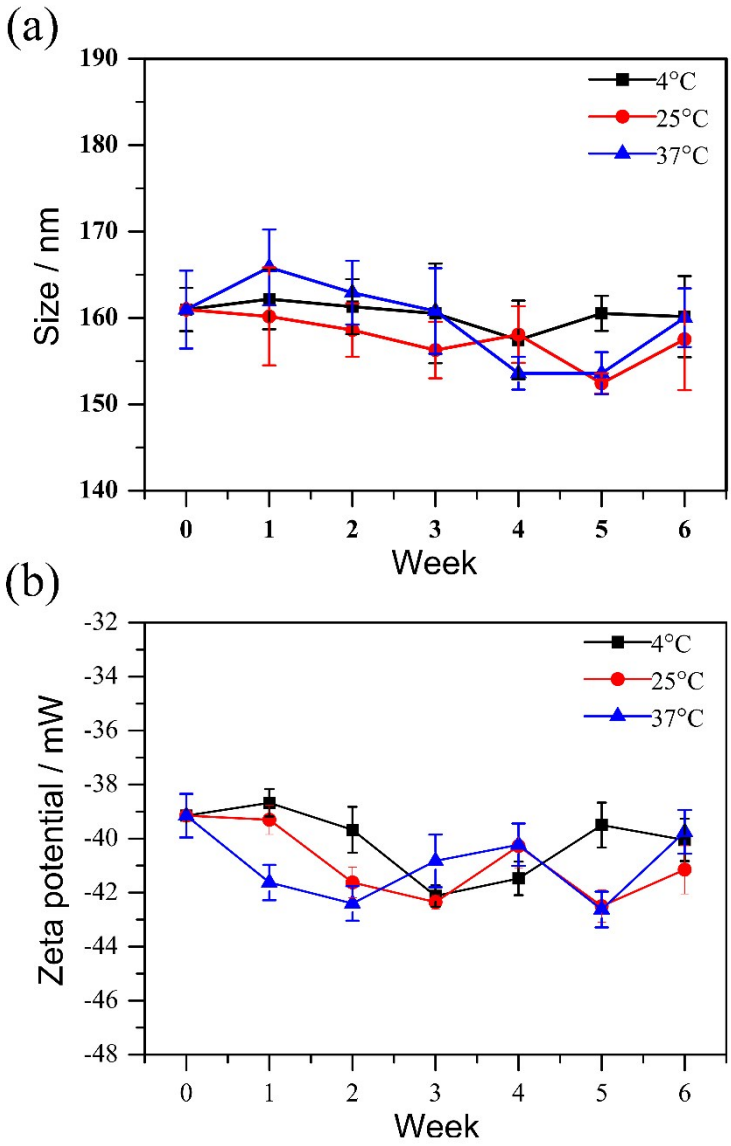

Fig. S5 The sizes (a) and zeta potentials (b) of Ag_2S QD@PC_{10}ARGD hybrid nanogels (0.1% w/w PC_{10}ARGD, 0.5 mg mL^{-1} Ag ion) were monitored at 4 ºC, 25 ºC, and 37 ºC within six weeks.

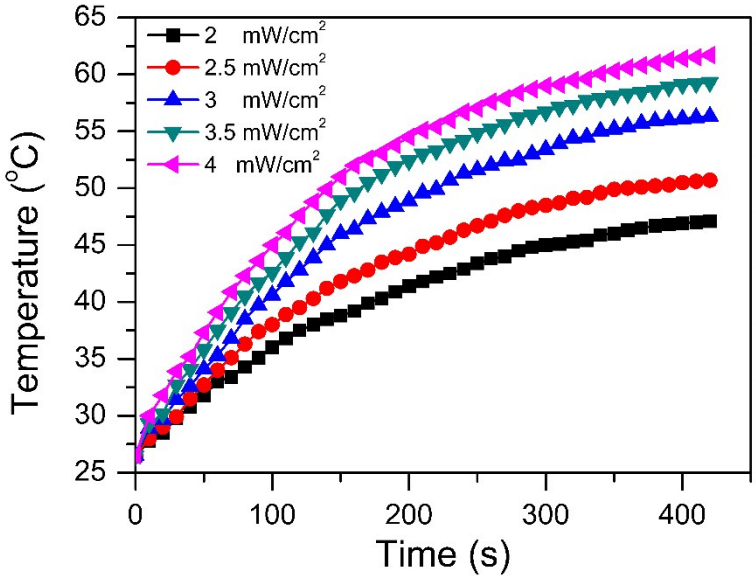

Fig. S6 Temperature changes of Ag_2S QD@PC_{10}ARGD hybrid nanogels upon irradiation using a NIR laser (λ = 810 nm) at different laser power densities. The concentration of Ag ion is 0.03 mg mL^{-1}.

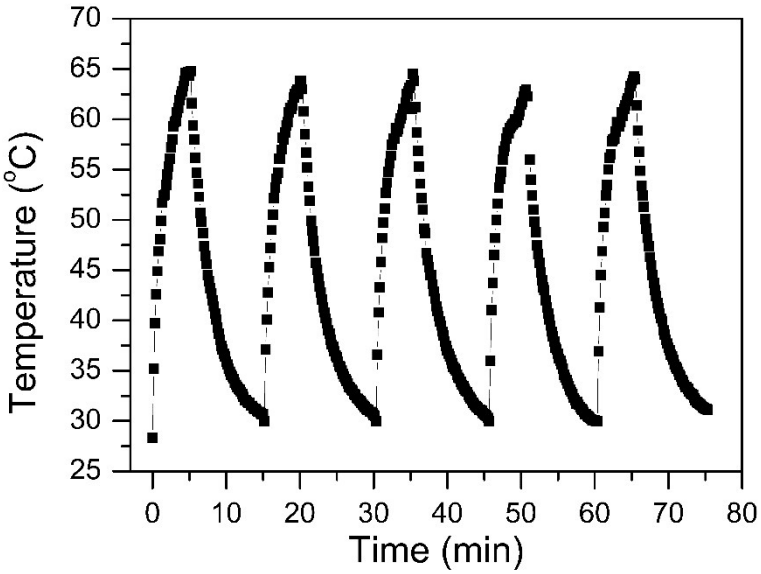

Fig. S7 Temperature elevation of Ag_2S QD@PC_{10}ARGD nanogels under five irradiation/cooling cycles using a NIR laser (2.5 W cm^{-2}, λ = 810 nm). The concentration of Ag ion is 0.25 mg mL^{-1}.

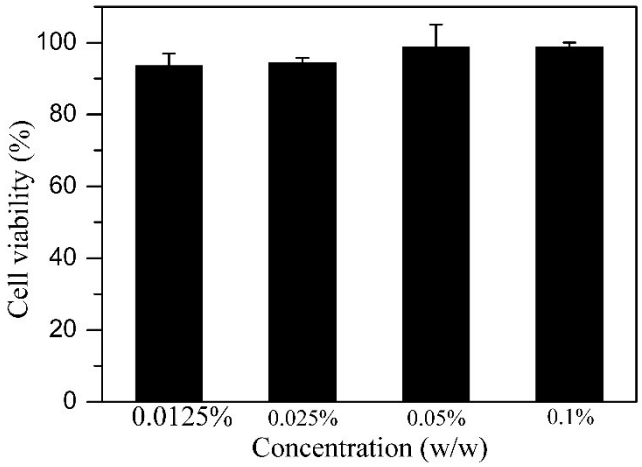

Fig. S8 The cell viability of HeLa cells incubation at different concentration of PC_{10}ARGD polypeptide.

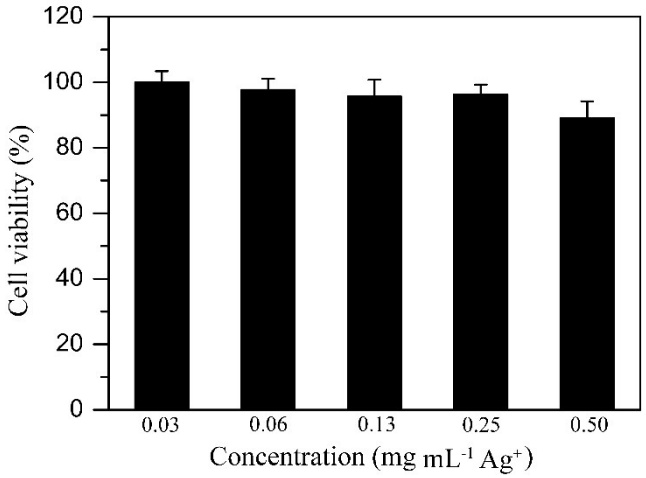

Fig. S9 The cell viability of HeLa cells incubation at different concentration of Ag_2S QD@PC_{10}ARGD hybrid nanogels.

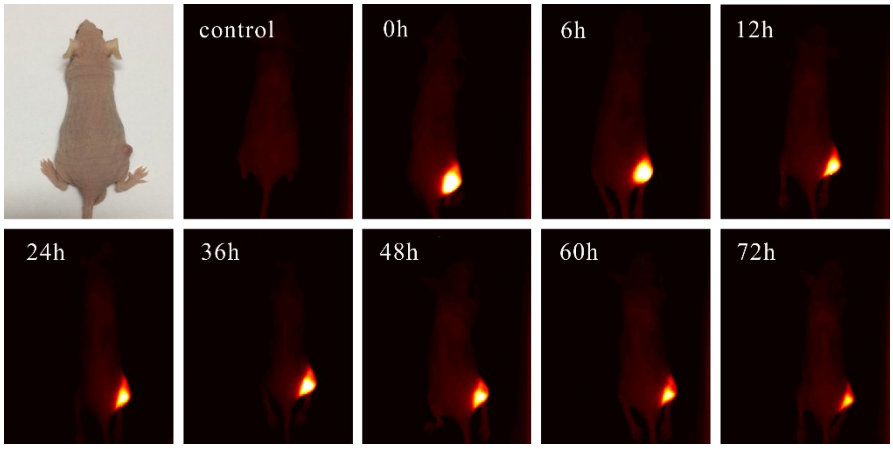

Fig. S10 *In vivo* NIR II fluorescence imaging of HeLa-tumor-bearing mice treated with 100 μL Ag_2S QD@PC_{10}ARGD (0.025% w/w PC_{10}ARGD, 1 mg mL^{-1} Ag ion). The excitation wavelength of laser was 808 nm.

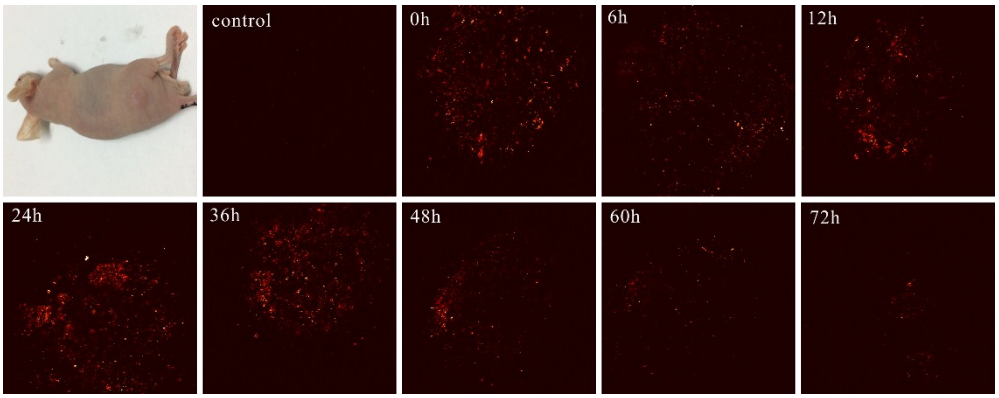

Fig. S11 *In vivo* photoacoustic imaging of HeLa-tumor-bearing mice treated with 100 μL Ag_2S QD@PC_{10}ARGD (0.025% w/w PC_{10}ARGD, 1 mg mL^{-1} Ag ion). The excitation wavelength of laser was 744 nm.

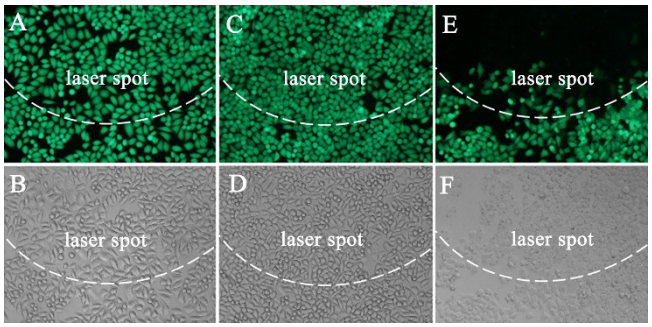

Fig. S12 Photothermal therapy effect of Ag_2S QD@polypeptide hybrid nanogels. (A, B) HeLa cells, (C, D) HeLa cells incubated with Ag_2S QD@PC_{10}A (0.25 mg mL^{-1}), (E, F) HeLa cells incubated with Ag_2S QD@PC_{10}ARGD (0.25 mg mL^{-1}) that were irradiated with 810 nm laser at a power density of 2.5 W cm^{-2} for 10 min. The dashed curves indicate the region exposed by NIR laser.

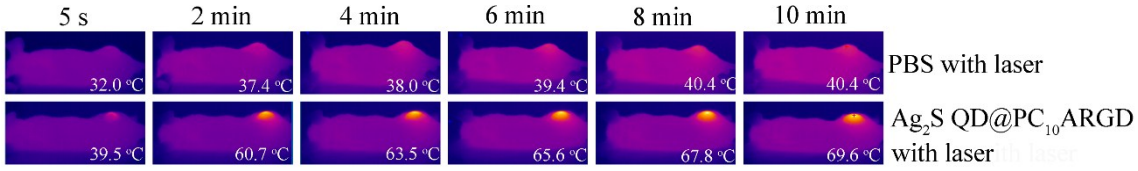

Fig. S13 Infrared thermal images of HeLa-tumor-bearing mice by intratumorally injected with 100 μL PBS or Ag_2S QD@PC_{10}ARGD (0.025% w/w PC_{10}ARGD, 1 mg mL^{-1} Ag ion) after exposure to NIR irradiation (810 nm, 2.5 W cm^{-2}, 10 min) for 10 min. The data of temperature in the figure is the highest temperatures of the tumor sites.

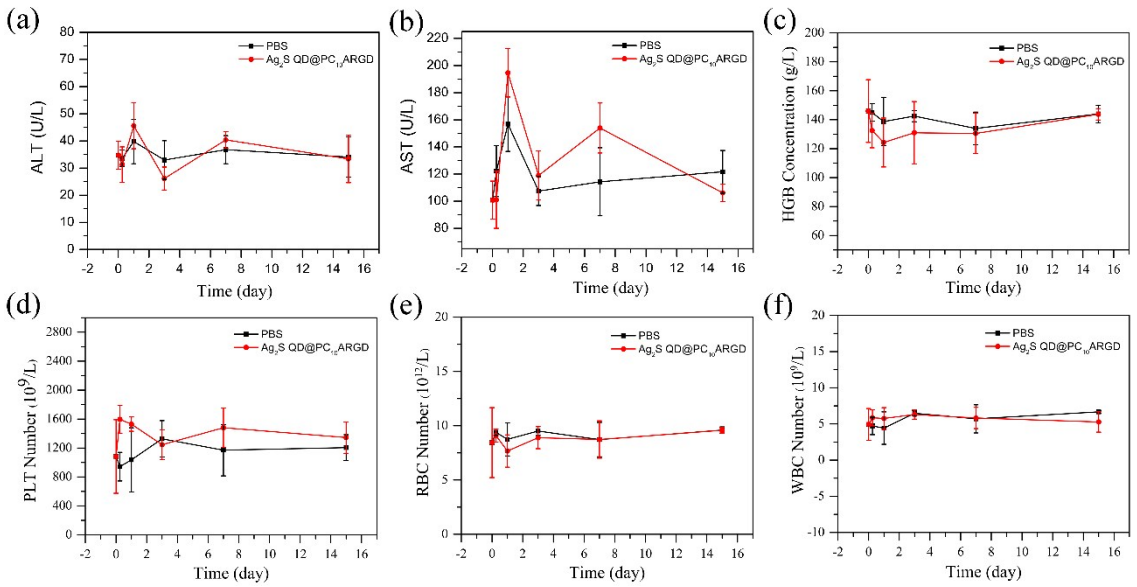

Fig. S14 The hematology analysis and serum biochemical assay of female BALB/c mice. The data were collected at different time point of 0, 0.25, 1, 3, 7, and 15 days after treated with 100 μL Ag_2S QD@PC_{10}ARGD (0.025% w/w PC_{10}ARGD, 1 mg mL^{-1} Ag ion). Related serum biochemcal assay included alanine aminotransferase (ALT, a), aspartate aminotransferase (AST, b). Related hematology analysis included haemoglobin (HGB, c), platelets (PLT, d), red blood cells (RBC, e), white blood cells (WBC, f). Mice treated with PBS were used as control group. Error bars present mean and standard deviation of four mice.

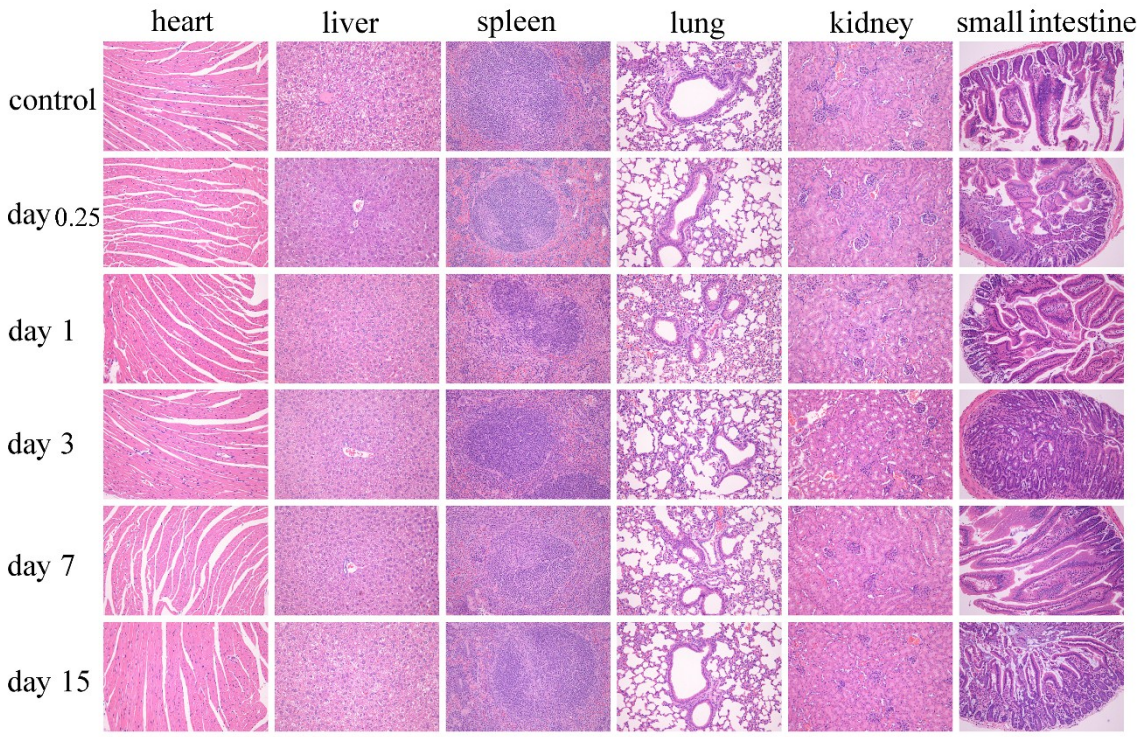

Fig. S15 H&E staining results of the major organs of mice treated with 100 μL Ag_2S QD@PC_{10}ARGD hybrid nanogels (0.025% w/w PC_{10}ARGD, 1 mg mL^{-1} Ag ion). No noticeable abnormality was observed in the heart, liver, spleen, lung, kidney, small intestine.