Supporting Information

NIR-Controlled HSP90 Inhibitor Release from Hollow Mesporous Nanocarbon for Synergistic Tumor Photothermal Therapy Guided by Photoacoustic Imaging

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1. Materials and Characterization

1.1 Materials

Gambogic acid (GA, ≥97%), Distearoyl phosphoethanolamine-polyethylene glycol (DSPE-PEG, Mw=5000 Da), Cy 5 carboxylic acid (Cy5, 95%) and Tetrapropoxysilane (TPOS, 97%) were obtained from Aladdin (Shanghai, China). Resorcinol (AR, ≥99.5%), methanal (37.0~40.0%), sodium hydroxide (AR, ≥96.0%), dimethyl sulfoxide (AR, ≥99.0%), trichloromethane (AR, ≥99.0%), ammonium hydroxide ($NH_3 \cdot H_2O$, 25 wt %) and ethanol (CP, ≥95.0%) and were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Rabbit anti-GAPDH antibody [EPR16891] - loading control (ab181602) and HSP90 (C45G5) rabbit mAb were purchased from Abcam. Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Laboratoeise. Annexin V-FITC apoptosis detection kit was obtained from Beyotime Institute of Biotechnology (Nanjing, China). Phosphate buffered saline (PBS, 1 X), penicillin-streptomycin, and trypsin-EDTA solution, calcein acetomethoxyl (calcein AM) /propidium iodide (PI) apoptosis detection kits were obtained from Sigma-Aldrich Co. LLC. (Shanghai, China). Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS) was purchased from Gibco BRL (Grand Island, NY). All reagents were used as received without any further purification.

1.2 Instruments and Characterization

The morphology and structure of the samples were investigated by field emission scanning electron microscope (FESEM, Ultra 55) operated at 3 kV and Transmission electron microscope (TEM, Tecnai G2 20 TWIN). The size and ζ-potential of particles were measured on a Zetasizer Nano ZS90 analyzer at 25 ^oC (Malvern Instrument Ltd, UK). The pore size distribution and the pore volume were collected at 77 K by an ASAP2020 volumetric adsorption analyzer (Micromeritics, USA). Perkin-Elmer Lambda 750 spectrophotometer was hired to get the UV-Vis-NIR absorption spectrum. FT-IR spectrum was recorded on a Thermo-Fisher Nicolet 6700 Fourier transform infrared spectrometer with KBr pellets. Infrared thermal imaging camera thermographic system could take Thermal image with an accuracy of 0.1 ^oC (Infra Tec, VarioCAM research, German). The XploRA laser Raman spectrometer (HORIBA JobinYvon, France) reccorded raman spectra of samples with excitation of 638 nm. Cytotoxicity assay was acquired on a Epoch 2 Microplate Spectrophotometer, which is a enzyme-linked immunosorbent assay reader (BioTek Instruments Inc., USA). The green and red fluorescense of treated cells were collected under a Nikon C2+ laser scanning confocal microscope (Nikon, Japan). Photoacoustic images and ultrasonic imaging were conducted on a

high resolution PAI system with excitation range from 680 to 970 nm (VevoLAZR, FujiFilm VisualSonics, Inc., USA). Beckman Coulter Gallios was used to explored the apoptosis and necrosis status at 37 °C. 808 nm consecutive NIR laser (Changchun New Industries Optoelectronics Technology Ltd., China) was used to test the Photothermal effects of samples. Cytotoxicity assay was acquired on a enzyme-linked immunosorbent assay (ELISA) reader (Epoch 2 Microplate Spectrophotometer, BioTek Instruments Inc., USA). Flow cytometry analysis was operated at 37 °C on a Beckman Coulter Gallios flow cytometer to measure the uptake by HepG2 cells and the cell apoptosis .

1.3 Statistical analysis

All the results are presented as the mean ± standard deviation (S.D.). Differences between groups were analyzed using Student's t-test. Differences among more than two groups were analyzed using a one-way analysis of variance, and the Bonferroni post hoc test was used to analyze differences between any two groups. $P < 0.05$ was considered to indicate a statistically significant difference.

2. Experimental section

2.1 Calculation of the the drug loading content (DLC) and drug loading efficiency (DLE)

8.1 mg of HMCS-PEG and 1 mg GA were added into 3 mL DMSO in a 20 mL centrifuge tube and ultrasound for about 30 minutes. Afterwards, 10 mL DI water was added into the above solution quickly and was sonicatedfor 2 hours, then GA loaded HMCS-PEG nanoparticles could be obtained by centrifugation. The drug loading content (DLC) and drug loading efficiency (DLE) were calculated on the basis of the following formulas: [1]

$$\text{DLC}\,(\%, \text{GA}) = \frac{\text{weight of loaded GA}}{\text{weight of GA loaded nanoparticles}} \times 100\%$$

$$\text{DLE}(\%, \text{GA}) = \frac{\text{weight of loaded GA}}{\textit{the feeding}\ \text{weight of GA}} \times 100\%$$

2.2 Calculation of the photothermal conversion efficiency η of HMCS-PEG

The photothermal conversion efficiency of HMCS-PEG suspension is determined according to previous methods.[2, 3] The detailed calculation was given as below:

$$\eta = \frac{hS\Delta T_{max} - Q_s}{I\left(1 - 10^{-A_{808}}\right)}$$

$$\tau = \frac{m_s C_s}{hS}$$

where h is the heat transfer coefficient, the surface area of the container is S, ΔT_{max} is the temperature variation of the nanoparticle suspensions at the maximum steady state temperature. Q_s is the heat generated by solvent under the NIR-light. I represents laser density (1 W cm^{-2}) of laser used in the experiment, the UV absorbance of the nanoparticles at 808 nm is A_{808}. Where m_s and C_s are the mass and the heat capacity of the solvent (pure water), respectively, τ is the sample system time constant.

2.3 Combination index calculation

The combination index (CI) for the viability of cells equal to 50% were calculated from the equation:

$$CI = \frac{(D)_1}{(Dx)_1} + \frac{(D)_2}{(Dx)_2}$$

The effect of drug combinations could be synergistic (CI < 1), additive (CI = 1), or antagonistic (CI > 1). $(Dx)_1$ and $(Dx)_2$ are the doses of drug 1 and 2 that inhibit cell growth to 50%, and $(D)_1$ and $(D)_2$ are respective doses of drug 1 and 2 in combination therapy to inhibit 50% cell growth .

When the inhibition of cell growth reaches 50%, the dosage of GA is about 3.58 μg mL^{-1} (GA group) and the dosage of HMCS-PEG nanoparticles is about 50.12 μg mL^{-1} (HMCS-PEG+Laser group), and for HMCS-PEG-GA+Laser group, the dosage of HMCS-PEG-GA is about 16.55 μg mL^{-1} (containing 1.50 μg mL^{-1} GA and 15.05 μg mL^{-1} HMCS-PEG nanoparticles, DLC=15%). So the CI value of HMCS-PEG-GA is calculated as 0.72.

$$CI = \frac{1.50}{3.58} + \frac{15.05}{50.12} = 0.719 \approx 0.72$$

2.4 Synthesis of HMCS-PEG-GA-Cy5

HMCS were modified by mixing DSPE-PEG and DSPE-PEG-NH_2 with mass ratio (9:1) in $CHCl_3$ solution ultrasounded for 1h and concentrated in suspension steam to remove $CHCl_3$ solution, followed by centrifugation (12000 rpm, 20 min) and washed with deionized water for several times. Subsequently, 5 mg Cy5 dyes, 15 mg EDC and 15 mg NHS were added into the solution and stirred for 24 h. Then centrifugation with distilled water for several times to remove excess EDC, NHS and free dyes.

2.5 Study on HMCS-PEG-GA ability to reduce cells' thermal resisitance

To study the ability of reduce the resistances of cells to heat stresses of HMCS-PEG-GA or GA under mild temperature, HepG2 cells were seeded in 96-well plate for 24 h. Then remove the supernatants and the equivalent GA of free GA and HMCS-PEG-GA with two different concentration group (0.5, 5μg/mL) and (1, 10 μg/mL) were added to HepG2 cells for varying incubated time (0, 1, 2, 4h) at 43 ^{o}C, then cultured at 37 ^{o}C for a total incubation time of 24 h.And the cell viability was measured with CCK-8 assays.

2.6 Photothermal effects on cell systems

HepG2 cells were seeded in 96-well plate for 24 h, with $1x10^4$ each well. Then, the culture medium was removed, and HMCS-PEG-GA were added into the well for 4 h, then the cell systems were exposed to NIR laser for 5 min. And the photothermal images were captured by an infrared imaging camera.

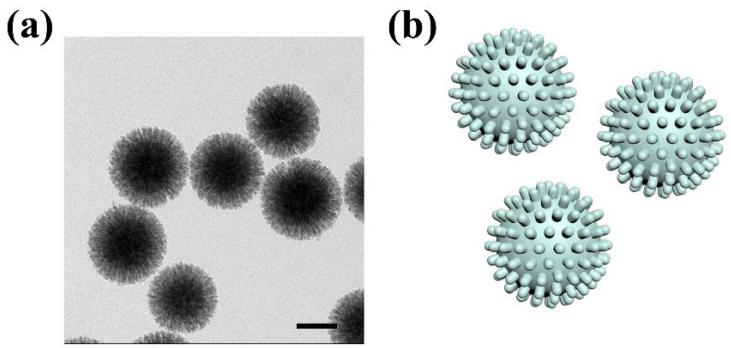

Fig. S1. (a) TEM image of SiO_2 template with solid core and radial spines, (b) 3D model of SiO_2 template. The scale bar is 100 nm.

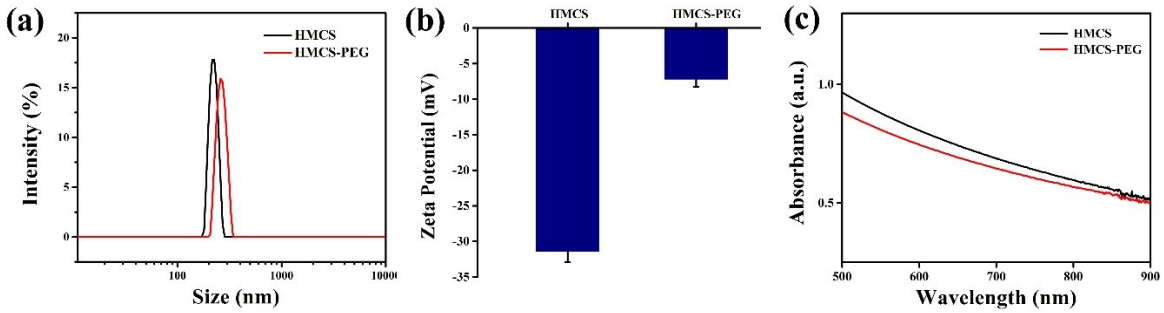

Fig. S2. (a) DLS diamgram, (b) ζ-potential data and (c) UV absorption of HMCS before and after PEG modification.

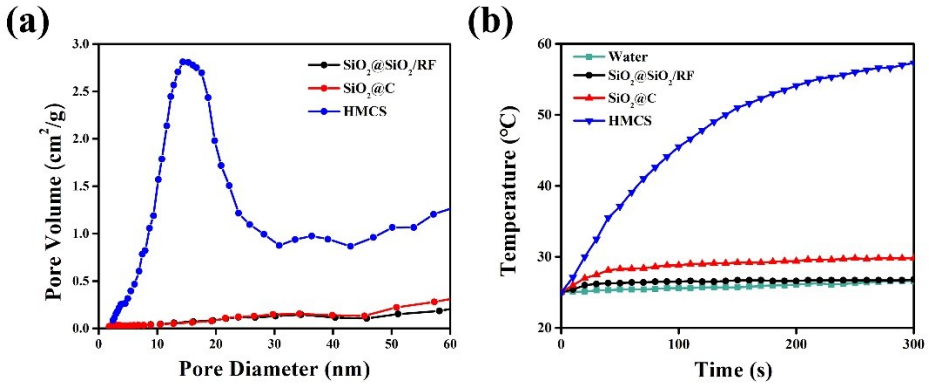

Fig. S3. (a) Nitrogen adsorption-desorption isotherms of pore volume distribution curves and (b) photothermal effects of SiO_2@SiO_2/RF, SiO_2@C and HMCS.

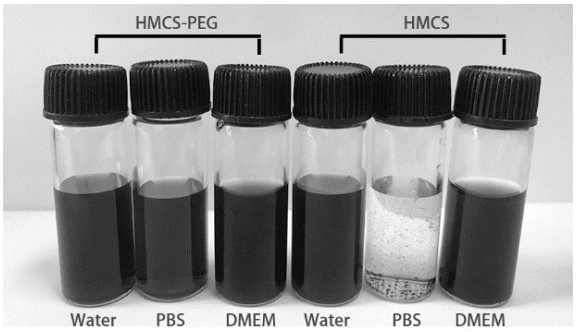

Fig. S4. Photographs of HMCS and PEG modified HMCS in different solution medium.

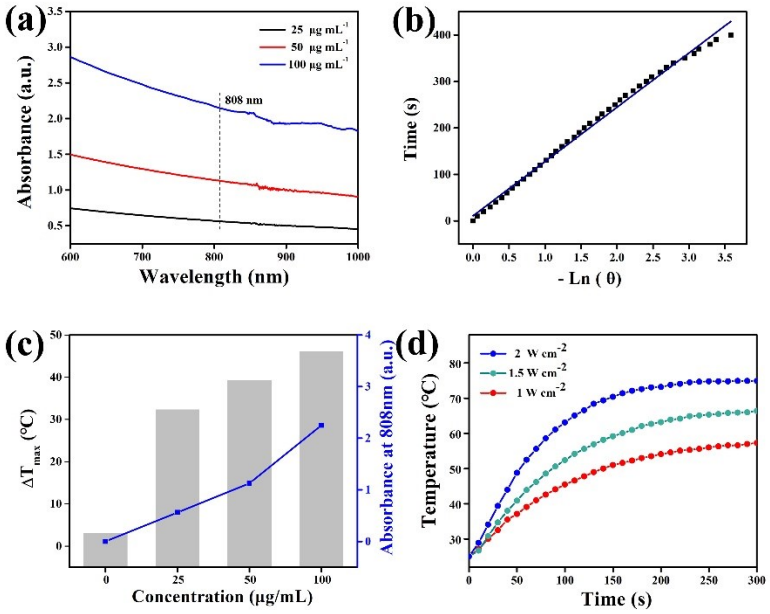

Fig. S5. (a) The UV absorbance of HMCS-PEG with different concentration, (b) linear relationship of time versus -lnθ obtained from the cooling periods, (c) a summary of the maximum temperature change of the nanoparticle suspensions during laser exposure, together with their relative absorbance at 808 nm, (d) photothermal performance of HMCS-PEG dispersion irradiated with different power densities.

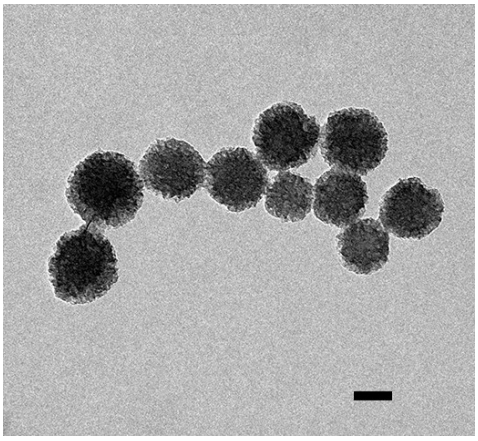

Fig. S6**.** TEM images of HMCS-PEG-GA. The scale bar is 100 nm.

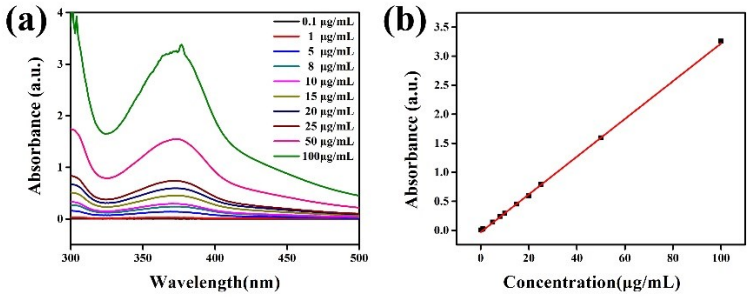

Fig. S7. (a) The UV-vis spectra of different concentrations of GA in 10% DMSO aqueous solution, (b) standard curve of GA in mixture of DMSO and water at the ratito of 1:9.

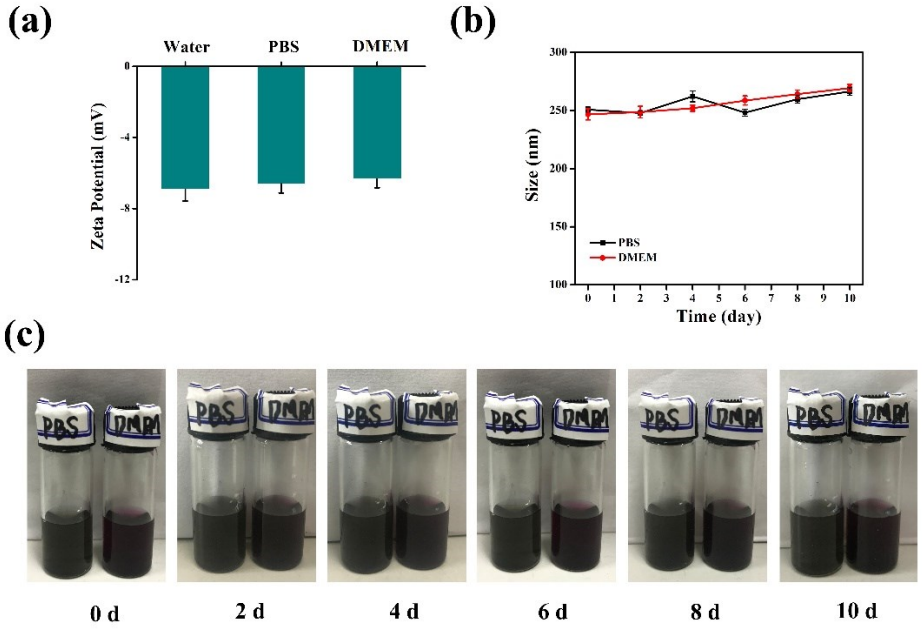

Fig. S8. (a) ζ-potential data of HMCS-PEG-GA in water, PBS and DMEM, (b) the curves of diameter changing of HMCS-PEG-GA with time within 10 days, (c) photographs of HMCS-PEG-GA in PBS and DMEM for 10 days.

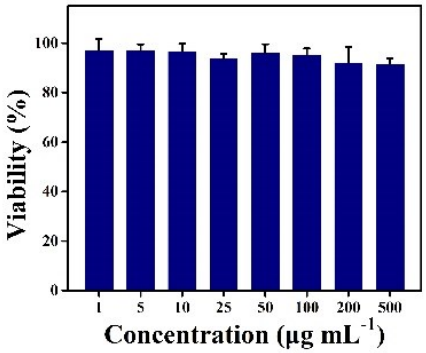

Fig. S9. Cell viability of HepG2 cells incubated with HMCS-PEG nanoparticles for 24 h with different concentrations. Data are means ± s.d. N = 6.

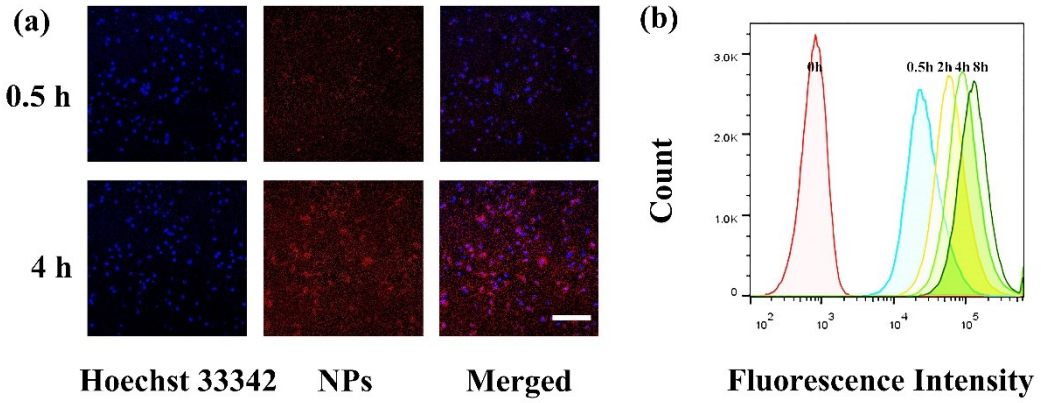

Fig. S10. (a) CLSM images of HepG2 cells incubated with HMCS-PEG-GA-Cy5 (shorted as NPs, 50 μg mL^{-1}) at 37 °C for 0.5 and 4 h (the fluorescence of Hoechst 33342 (blue), NPs (red) and merged images from left to right), (b) cellular uptake of HMCS-PEG-GA-Cy5 in HepG2 at 0, 0.5, 2, 4 and 8 h incubation time. The scale bar is 50 μm.

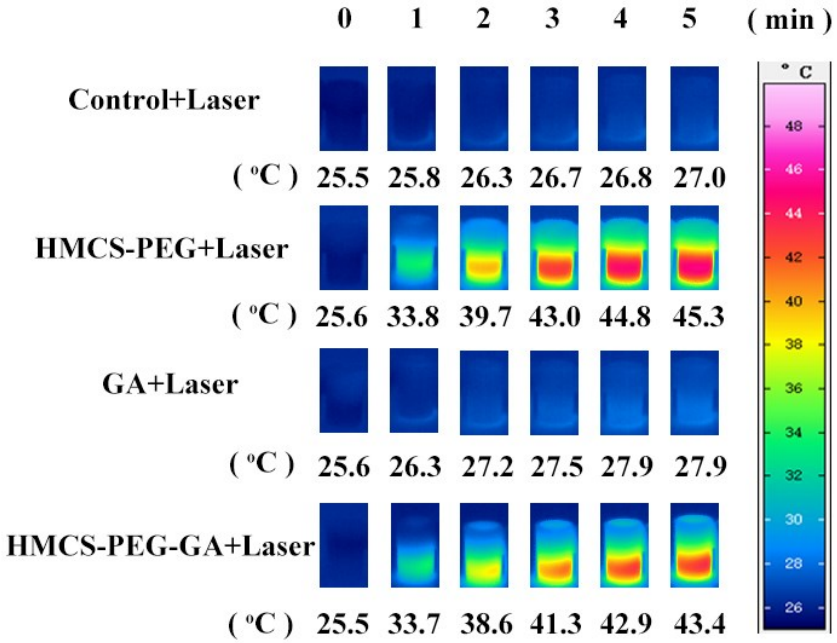

Fig. S11. The photothermal images of cell culture system after 808 nm irradiation with different treatments (control+Laser, HMCS-PEG-GA+Laser, GA+Laser and HMCS-PEG-GA+Laser).

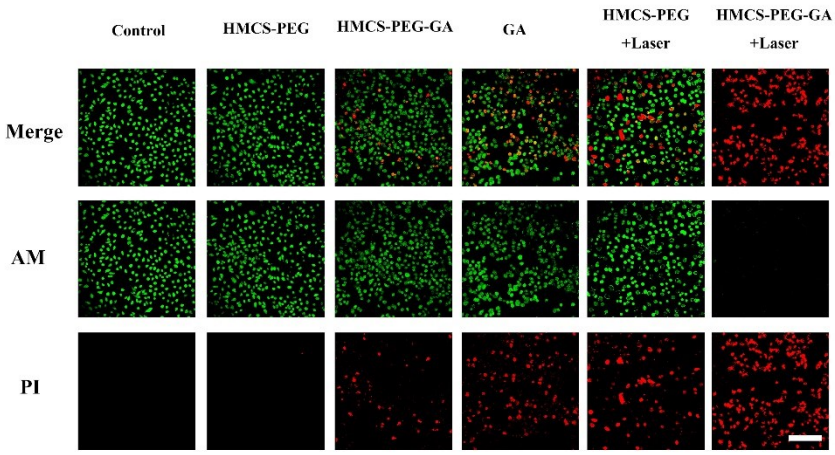

Fig. S12. Confocal fluorescence images of Calcein AM (green, live cells) and PI (Propidium iodide) (red, dead cells) co-stained HepG2 cells treated by HMCS-PEG, HMCS-PEG-GA, GA, HMCS-PEG + NIR laser, and HMCS-PEG-GA + NIR laser at concentration of 50 μg mL^{-1}. The scale bars are100 μm.

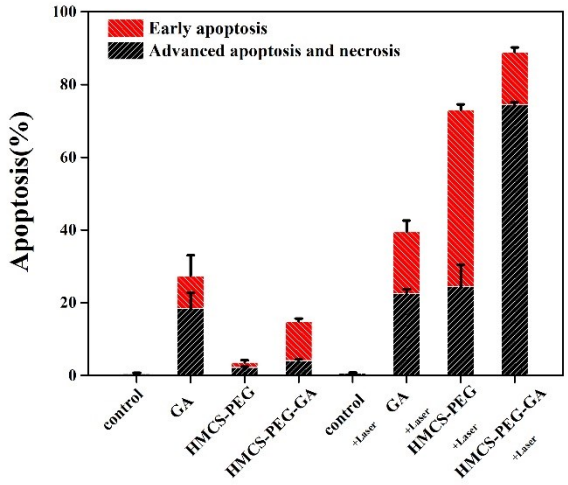

Fig. S13. Flow cytometry analyses of cell apoptosis rate after different treatment. For the 808 nm laser treatment, the power intensity is 1W cm^{-2} and the time is 300s.

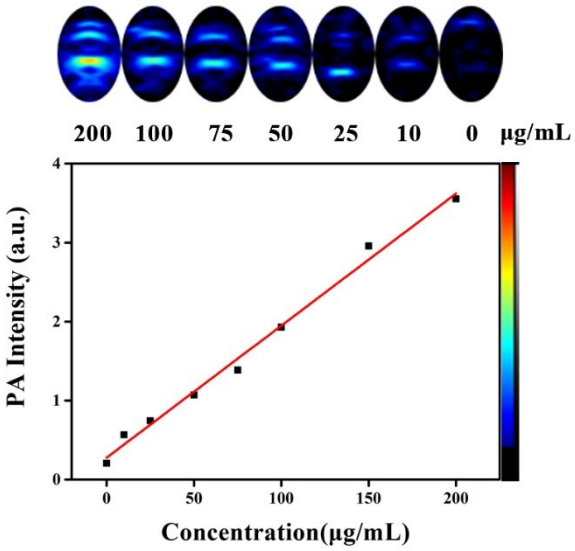

Fig. S14. PA signal intensity of HMCS-PEG-GA with different concentrations (0, 10, 25, 50, 75, 100 and 200 μg/mL) in water.

Table S1 Surface area and pore volume of HMCS, HMCS-PEG and HMCS-PEG-GA determined by Barrett-Joyner-Halenda (BJH) methods.

Nanoparticles	Surface area (m^2/g)	Pore volume (cm^3/g)
SiO_2@SiO_2/RF	42.5	0.35
SiO_2@C	107.4	0.34
HMCS	826.7	1.96
HMCS-PEG	449.2	1.49
HMCS-PEG-GA	242.2	1.03

References

[S1] Lv, S. X.; Wu, Y. C.; Cai, K. M.; He, H.; Li, Y. J.; Lan, M.; Chen, X. S.; Cheng, J. J.; Yin, L. C., High Drug Loading and Sub-Quantitative Loading Efficiency of Polymeric Micelles Driven by Donor-Receptor Coordination Interactions. Journal of the American Chemical Society 140 (2018),1235-1238.

[S2] Roper, D. K.; Ahn, W.; Hoepfner, M., Microscale heat transfer transduced by surface plasmon resonant gold nanoparticles. J Phys Chem C 111 (2007),3636-3641.

[S3] Tian, Q.; Hu, J.; Zhu, Y.; Zou, R.; Chen, Z.; Yang, S.; Li, R.; Su, Q.; Han, Y.; Liu, X., Sub-10 nm Fe3O4@Cu(2-x)S core-shell nanoparticles for dual-modal imaging and photothermal therapy. J Am Chem Soc 135 (2013),8571-7.