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Supporting Information

Self-Sacrificial Template-Induced Modulation of Conjugated Microporous Polymer Microcapsules and Shape-dependent Enhanced Photothermal Efficiency for Ablation of Cancer Cells

Jing Tan, Jiaxun Wan, Jia Guo*, Changchun Wang

State Key Laboratory of Molecular Engineering of Polymers, Collaborative Innovation Center of Polymers and Polymer Composite Materials, Department of Macromolecular Science, and Laboratory of Advanced Materials, Fudan University, Shanghai 200433, P. R. China

* E-mail: guojia@fudan.edu.cn

Materials and methods

Materials

Methylacrylic acid (MAA), *N,N'*-methylenebisacrylamide (MBA), 2,2-azobisisobutyronitrile (AIBN), acetonitrile(AN), sodium acetate (NaOAc), ethylene glycol (EG), anhydrous ethanol (EtOH), toluene, triethylamine (TEA), methanol, acetone were purchased from Shanghai Chemical Reagents Company and used as received. Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), trisodium citrate dehydrate, 1,4-dibromobenzene were purchased from Aladdin Industrial Corporation. *Trans*-dichlorobis(triphenylphosphine)Palladium(II) were purchased from Energy Chemical Company. Copper(I) iodide (CuI) was purchased from Adamas Reagent Company. 1,3,5,-Triethynylbenzene was purchased from TCI Shanghai Chemical Company. Deionized water was used in all experiments.

Synthesis of PMAA microspheres

The crosslinked PMAA microspheres were prepared by using reflux-precipitation polymerization method.[S1] Typical synthetic procedures were as follows for 20%-crosslinking PMAA microspheres. MAA (200 mg), MBA (50 mg) and AIBN (5 mg) were dissolved in acetonitrile (20 mL) and the solution was transferred to a 50mL of three-neck flask. The reaction mixture was heated to 110^{o}C within 15min and then the reaction proceeded for 1h. The obtained PMAA microspheres were washed with ethanol three times and re-dispersed in ethanol for further use. Under otherwise identical reaction conditions, 50%-crosslinking PMAA microspheres were prepared by increasing the amount of MBA (200 mg). To reduce the size of PMAA microspheres, the feeding amounts of MMA (100 mg) and MBA (18mg) were decreased and the reflux precipitation polymerization was carried out under otherwise identical conditions.

Synthesis of Fe_3O_4@PMAA microspheres

The Fe_3O_4 clusters were prepared through a modified solvothermal reaction.[S2] Typically, $FeCl_3 \cdot 6H_2O$ (4.32 g), NaOAc (4.80 g), and sodium citrate(1.00 g) were dissolved in ethylene glycol (80 mL). The reaction mixture were stirred vigorously for 1 h at 160^{o}C to form a homogeneous dark brown solution and then transferred to a Teflon-lined stainless-steel autoclave (100 mL capacity). The autoclave was heated to 200^{o}C and maintained for 20h. Then the autoclave was cooled to room temperature with water. The product was rinsed with ethanol and water, respectively, and dried in vacuum for further use.

The Fe_3O_4@PMAA magnetic composite microspheres with a well-defined core/shell nanostructure were prepared by using the reflux-precipitation polymerization method.[S3] Typically, Fe_3O_4 nanoclusters (75 mg) were ultrasonically dispersed in acetonitrile (20 mL), and then, MAA (150 mg), MBA (37.5 mg) and AIBN (3.75 mg) were added to the dispersion

of Fe_3O_4 nanocluster. The subsequent polymerization proceeded by the same route as the synthesis of PMAA microspheres, resulting in a uniform PMAA shell around the Fe_3O_4 nanocluster. The brown product was separated from the mixture with applied magnetic field, rinsed with ethanol three times, and dispersed in ethanol for further use. To tune the shell thickness of the resulting microspheres, the feed amount of MAA, MBA and AIBN was accordingly adjusted under otherwise identical conditions.

Synthesis of NCMP microcapsules

NCMP microcapsules with a shell thickness of 40 nm (H-NCMP-40) were prepared as follows. Crosslinked PMAA microspheres (80 mg) were dispersed in a mixture of toluene (15 mL) and triethylamine (15 mL). $Pd(PPh_3)_2Cl_2$(10.5 mg, 0.015 mmol) and CuI (2.8 mg, 0.015 mmol) were used as catalysts and added successively into the dispersion. The yellow solution was stirred for 1 h at room temperature under argon protection. Then 1,3,5-triethynylbenzene (18 mg, 0.12 mmol) and 1,4-dibromobenzene (42 mg, 0.18 mmol) were added and dissolved in the reaction solution. The mixture was kept at 100 ^oC for 24 h under Ar. The yellow product was collected by centrifugation, washed successively with water, methanol, acetone and dichloromethane, respectively, and dried in vacuum for 24 h. To further remove the residual PMAA chains in the CMP shell, the as-synthesized NCMP powder was treated at 350 ^oC for 6 h under Ar protection. The bulk CMPs were synthesized without addition of PMAA templates under otherwise identical conditions.

To tune the shell thickness, 36 mg of 1,3,5-triethynylbenzene and 84 mg of 1,4-dibromobenzene were mixed with 240 mg PMAA microspheres. The obtained NCMP microcapsules showed a 30-nm shell (H-NCMP-30). Under otherwise identical conditions, the feeding amount of PMAA microspheres were varied from 160, 120 to 80 mg for preparation of H-NCMP-50, H-NCMP-100 and H-NCMP-140 microcapsules, and flower-like PMAA microspheres (50% crosslinking density) was used as template for preparation of F-NCMP-100.

Synthesis of Fe_3O_4@H-NCMP-100

Firstly, Fe_3O_4@PMAA microspheres (40 mg) were dispersed in a mixture of toluene (15 mL) and triethylamine (15 mL). $Pd(PPh_3)_2Cl_2$(10.5 mg, 0.015 mmol) and CuI (2.8 mg, 0.015 mmol) were used as catalysts and added successively into the dispersion. The brown dispersion was stirred mechanically for 1 h at room temperature under argon protection. Then 1,3,5-triethynylbenzene (18 mg, 0.12 mmol) and 1,4-dibromobenzene (42 mg, 0.18 mmol) were added and dissolved in the dispersion. The mixture was kept at 100 ^oC for 24 h under argon. The brown product was collected by a magnet and washed with water, methanol, acetone and ethanol, respectively, and dried in vacuum for 24 h. To further remove the residual PMAA chains in the CMP shell, the as-synthesized Fe_3O_4@H-NCMP-100 powder was treated at 350 ^oC for 6 h under Ar protection.

In vitro photothermal cell assay

Photothermal cytotoxicity of samples was assessed on Hela cells by using the CCK-8 method. Cells pre-seeded in a 96-well plate for 24 h were incubated with samples of different concentrations (100 and 200 μg/mL) and then irradiated by an 808-nm laser at a power density of 5 W/cm^2 for 5 min. The treated cells were cultured for another 12 h under 37^{o}C within 5% CO_2 atmosphere. After removing supernatant nutrient solution, the cell was incubated in 110 μL of DMEM containing 10 μL CCK-8 solution for 1 h. The absorbance of the suspension was measured at 450 nm on an ELISA reader. Cell viability was calculated by means of the following formula:

$$\text{Cell viability} = \frac{OD_{450\ (sample)} - OD_{450(blank)}}{OD_{450\ (control)} - OD_{450(blank)}} \times 100\%$$

To obtain the fluorescence images of treated cells, the cells were stained with Calcein AM/PI instead of CCK-8 for 30 min, washed with PBS for three times, and then imaged by fluorescence microscope.

Hemolysis Assay

Human blood was friendly provided by Huashan Hospital of Fudan University. The red blood cells (RBCs) were obtained after removing the serum of human blood, blood platelet and white blood cell in the supernatant by centrifugation (2000 r/min, 5min). PBS was used to wash the RBCs for five times. Then we obtained the RBCs precipitate by centrifugation (2000 r/min, 10min). The RBCs were diluted by nine-fold volume of PBS; 0.3 mL of diluted RBCs suspension was mixed with (a) 1.2 mL of NCMPs PBS solution at concentrations of 100, 200, 400, 800, 1600, 3200 μg/mL, (b) 1.2 mL of PBS as a negative control, and (c) 1.2 mL of deionized water as a positive control, respectively. The mixtures were placed in the table concentrator and shocked for 2 h at 37.5 ^{o}C. Then, the 8 samples were centrifuged (2000r/min, 10min), and the absorbance of the supernatants at 542 nm was measured by UV-vis spectroscopy. For the positive control sample, the absorbance was measured by using deionized water as reference solvent. For other samples, the PBS was used as reference solvent. The hemolysis rate was calculated by following formula:

$$Hemolysis\ rate = \frac{Dt - Dnc}{Dpc - Dnc} * 100\%$$

In the formula above, the *Dt* means absorbance for test samples; *Dnc* means absorbance for negative control sample; *Dpc* means absorbance for positive control sample.

Characterization

TEM images were obtained using a JEOL 2100F (JEOL, Japan) transmission electron microscope operated at an acceleration voltage of 200 kV. The sample was prepared by drop-casting the ethanol dispersion onto a copper grid. Thermogravimetric analysis (TGA) was conducted

on Pyris 1 Thermo Gravimetric Analyzer (PE, USA) under a flowing air atmosphere and at a heating rate of 20 ^{o}C/min from 100 ^{o}C to 800 ^{o}C. N_2 adsorption-desorption isotherms were collected at 77 K by an ASAP2020 volumetric adsorption analyzer (Micromeritics, USA). The samples were degassed at 120°C for 12 h before measurement. UV-vis spectra were recorded on a Shimadzu UV-3600 PC spectrometer (Shimadzu Japan).

[S1] S. Jin, Y. J. Pan, C. C. Wang, *Acta Chim. Sinica*, 2013, **71**, 1500.
[S2] J. Liu, Z. K. Sun, Y. H. Deng, Y. Zou, C. Y. Li, X. H. Guo, L. Q. Xiong, Y. Gao, F. Y. Li, D. Y. Zhao, *Angew. Chem., Int. Ed.*, 2009, **48**, 5875.
[S3] W. F. Ma, Y. Zhang, L. L. Li, Y. T. Zhang, M. Yu, J. Guo, H. J. Lu, C. C. Wang, *Adv. Funct. Mater.*, 2013, **23**, 107.

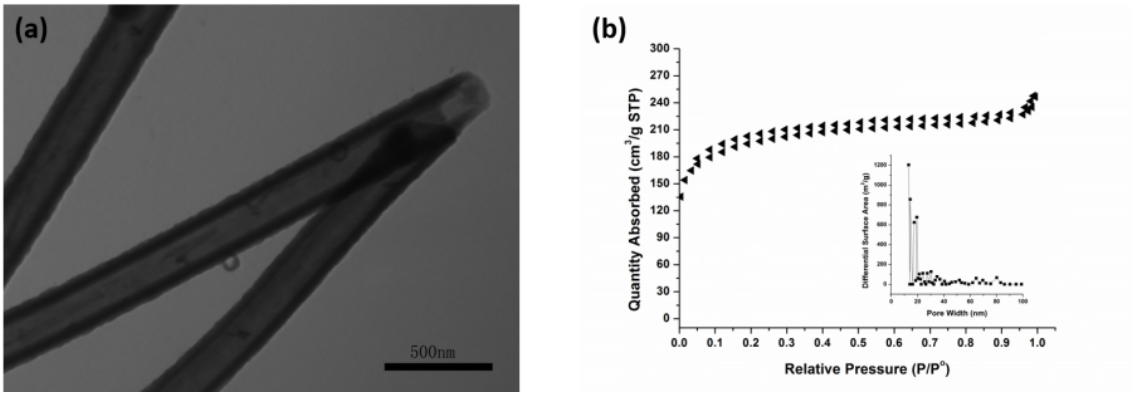

Figure S1. (a) TEM image and (b) N_2 sorption isotherm and pore size distribution (inset) of the bulk CMPs synthesized without using PMAA templates.

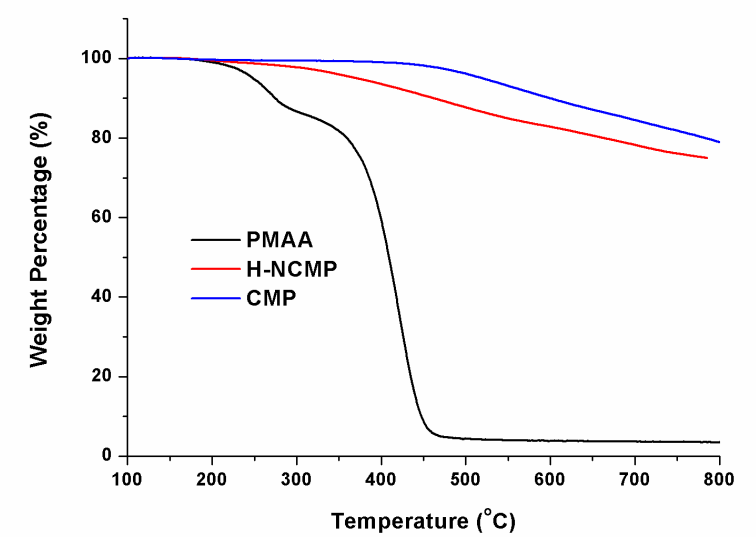

Figure S2. TGA curves of PMAA microspheres, H-NCMP microcapsules and bulk CMP solids.

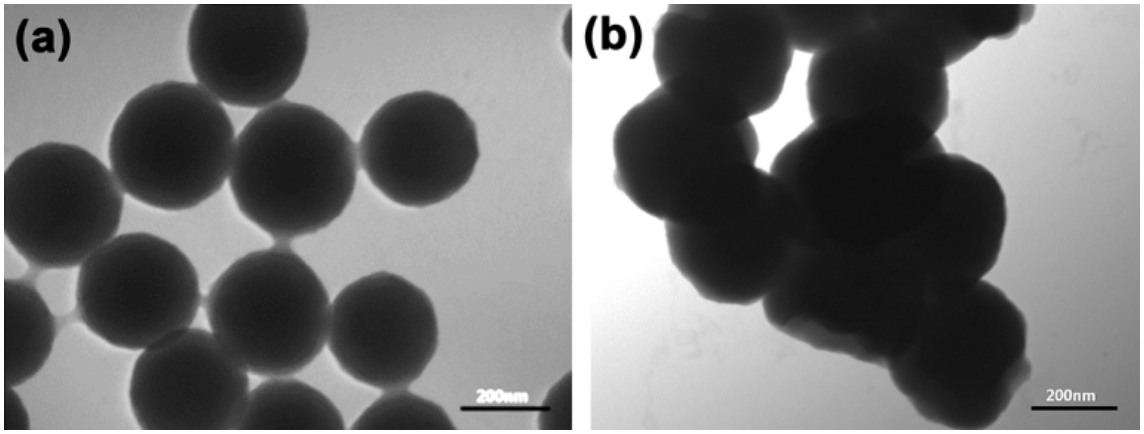

Figure S3. TEM images of DVB-crosslinked PMAA (a) and the corresponding PMAA@CMP microspheres (b).

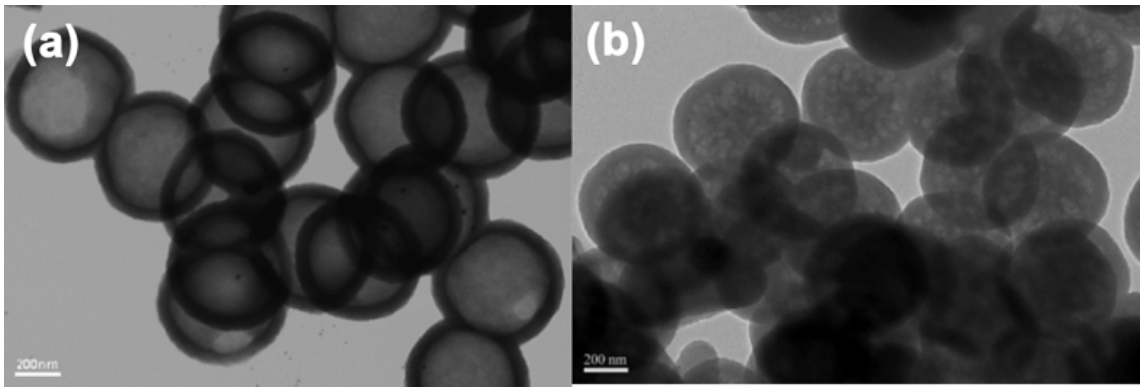

Figure S4. TEM images of the NCMP microcapsules prepared at (a) 130°C and (b) 70°C, respectively.

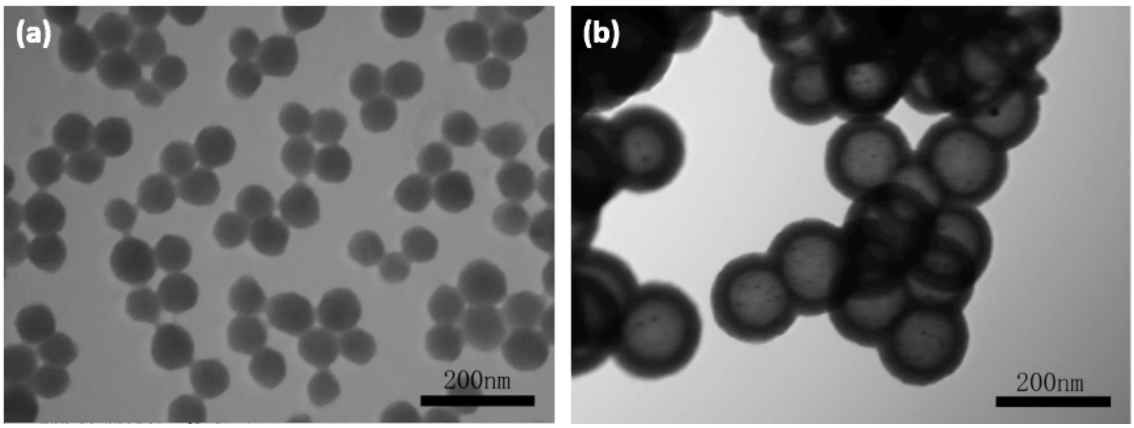

Figure S5. TEM images of (a) the PMAA microspheres (~100 nm) and (b) the corresponding H-NCMPs (~200 nm).

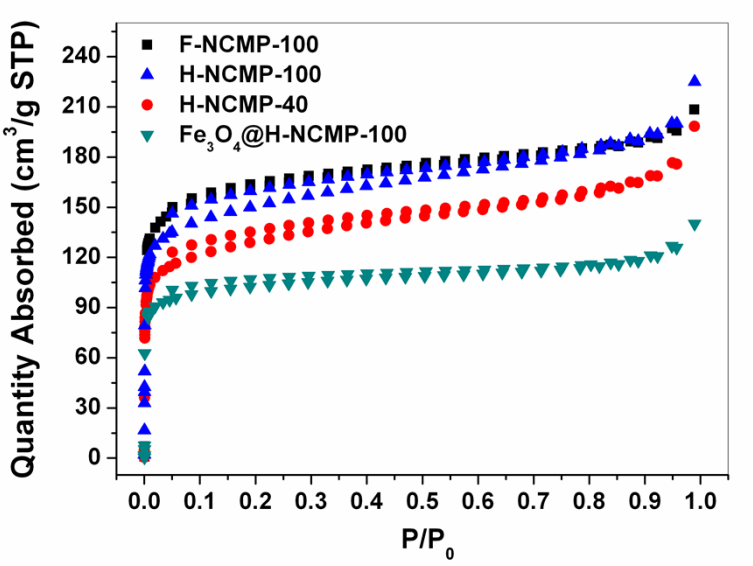

Figure S6. N_2 sorption isotherms of the different NCMPs measured at 77K.

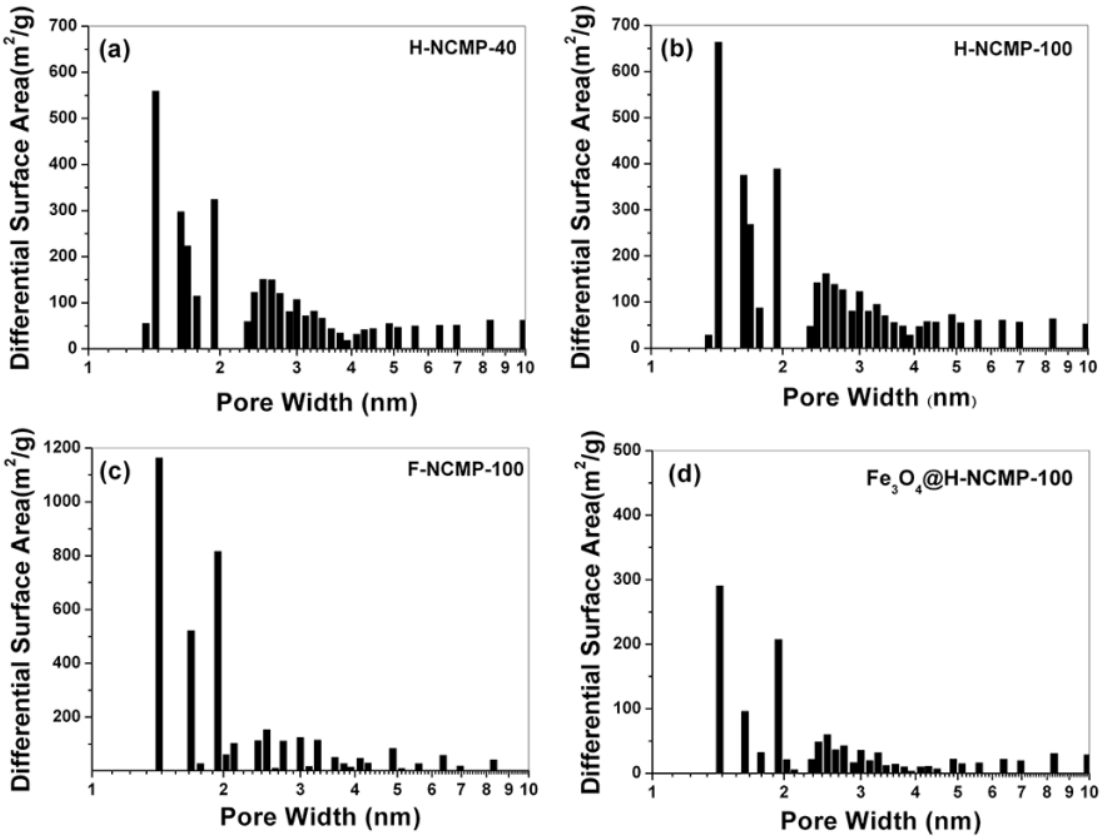

Figure S7. Pore size distributions of (a) H-NCMP-40, (b) H-NCMP-100, (c) F-NCMP-100, and (d) Fe_3O_4@H-NCMP-100, calculated by NLDFT model.

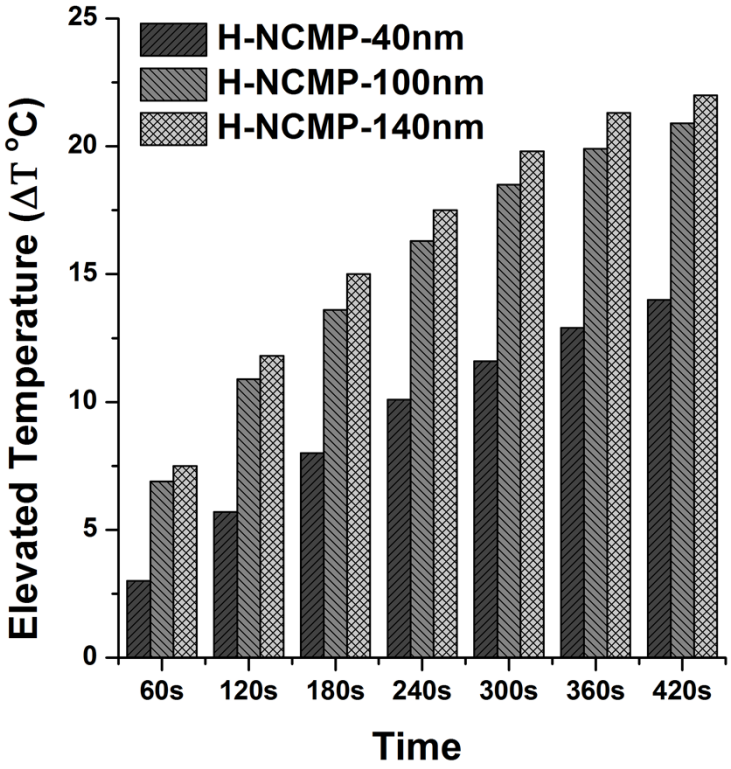

Figure S8. Temperature changes in PBS dispersions of H-NCMPs (200 μg/mL) with the shell thickness of 40, 100 and 140 nm under exposure to 808 laser for 7 min (5 W/cm^2), respectively.

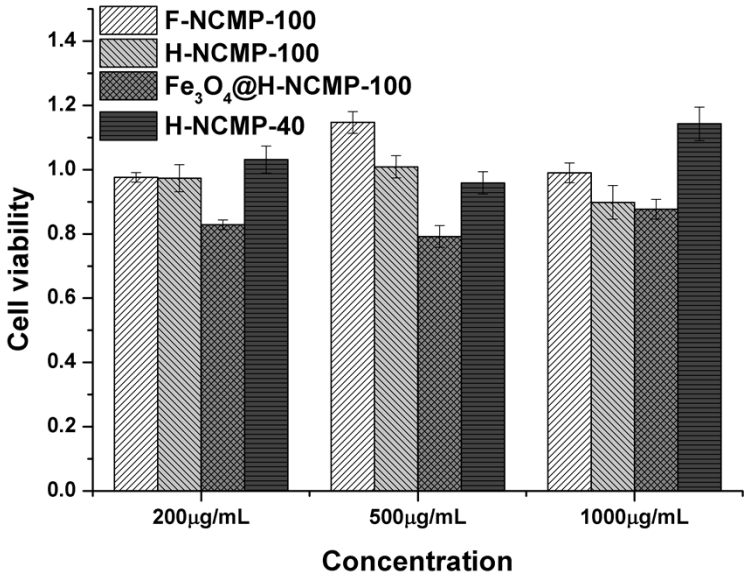

Figure S9. Cell viability of 293T cells (normal cells) incubated with the four kinds of NCMP microcapsules for 24 h.

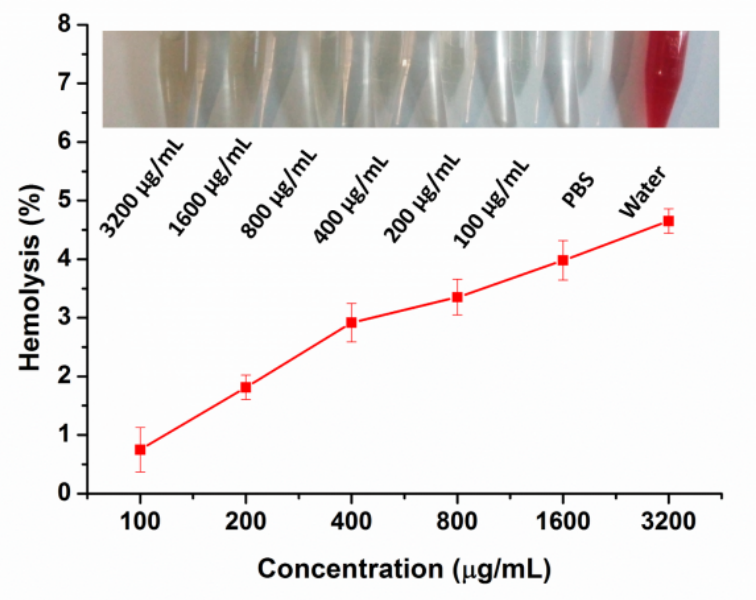

Figure S10. Hemolysis assay of the PBS dispersions of F-NCMPs with the concentrations ranging from 100 to 3200 μg/mL. The mixtures were isolated to quantitatively detect hemoglobin by UV-vis spectroscopy in the supernatant, which are also displayed in the photographs (inset).