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Ultra-small bimetallic phosphide for dual-modal MRI imaging guided photothermal ablation of tumor

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Experimental section

Materials

Nickel(II) acetylacetonate (95%), cobalt(II) acetylacetonate (99%), and oleylamine (>50%) were purchased from Sigma-Aldrich; Octyl ether (90%) and trioctylphosphine (TOP, 97%) were purchased from Macleans; Poly(vinylpyrrolidone) (PVP, molecular weight is 58,000 g mol^{-1}) was purchased from Aladdin Reagent Company; Dichloromethane, acetone, and ethanol were purchased from Xilong Chemical.

Synthesis of NiCoP

According to the method reported in the literature,[1] the reaction is carried out through the Schlenk wire technique after slight modification under an argon atmosphere. $Ni(acac)_2$ (1.0 mmol), $Co(acac)_2$ (1.0 mmol), oleylamine (5.0 mL), octyl ether (10.0 mL), and trioctyl phosphine (4.0 mL) were added to 200 mL of three-necked flask. Then, the flask was placed on a heating mantle and a thermocouple was inserted to adjust the reaction temperature. The mixture was purged with argon at 110 °C for 30 minutes to remove moisture or oxygen. Next, the temperature was raised to 230 °C and held for 90 minutes. Afterwards, the temperature was increased rapidly to 320 °C at a heating rate of 40 °C/min; 5.0 mL of TOP were injected immediately after heating, and then heated at 320 °C for 4.5 h. After that, the black product was isolated by precipitation with acetone. The product (NiCoP) was redispersed in cyclohexane, sonicated, and centrifuged again with acetone for 3 times at least.

Surface modification with PVP

The surface modification of NiCoP is based on the reported reference.[2] The dried product

was dissolved in 50 mL of dichloromethane. Then, 0.40 g of polyvinylpyrrolidone was added, and the resulting mixture was stirred at room temperature for 8 h. After multiple centrifugation, the uniform NiCoP/PVP solution in water is obtained.

Characterization

The structure performance of nanoparticles was characterized by Bruker D8 ADVANCE X-ray diffractometer, using Cu Kα (λ = 1.5418 Å) as the radiation source, and the operating voltage and current were 40.0 kV and 40.0 mA, respectively. The FEI Tecnai G2S-Twin high-resolution transmission electron microscope with an accelerating voltage of 200 kV was used to observe the morphology of the nanoparticles. The composition of the sample was measured by a field emission scanning electron microscope (FE-SEM, S-4800, Hitachi) equipped with a Bruker XFlash 4010 EDX detector. Fourier transform infrared spectra (FT-IR) were carried out on PerkinElmer 580B infrared spectrophotometer. Thermo Scientific iCAP 6000 inductively coupled plasma emission spectrometer (ICP-OES) was used for elemental analysis. X-ray photoelectron spectroscopy (XPS) is measured on VG ESCALAB MK II electron spectrometer. The absorbance of the sample was measured on a Shimadzu UV-3600 ultraviolet-visible-near infrared spectrophotometer. A Malvern instrument Zetasizer Nano ZS90 was used to measure the zeta potential and hydrated diameter of the sample.

Photothermal performance of NiCoP/PVP

1.0 mL of NiCoP/PVP solutions with different concentrations were put into the colorimetric dish, and the temperature probe was inserted into the solution to record the temperature change irradiated by 808 nm laser (1.0 $W \cdot cm^{-2}$, 10 min). In addition, NiCoP/PVP solution

with the fixed concentration was continuously irradiated by 808 nm laser with different power densities (0.5, 1.0, 1.5 W·cm^{-2}) for 10 min, and the temperature of NiCoP/PVP solution was recorded using a temperature probe every 10 seconds. Pure water is set as the blank control group.

Photothermal stability of NiCoP/PVP

NiCoP/PVP solution (1.0 mL) was irradiated by 808 nm continuous laser (1.0 W·cm^{-2}) for 10 min, and then naturally cooled to room temperature. The above process is repeated five times.

Photothermal conversion efficiency of NiCoP/PVP

The photothermal conversion efficiency (PCE) of NiCoP/PVP is calculated according to the following formulas [3]:

$$\eta = \frac{hS(T_{max} - T_{surr}) - Q_{Dis}}{I(1 - 10^{-A808})} \times 100\% \quad (1)$$

$$hS = \frac{\Sigma m_i C_{p,i}}{\tau_s} \quad (2)$$

$$t = \tau_s \times (-\ln\theta) \quad (3)$$

$$\theta = \frac{T - T_{surr}}{T_{max} - T_{surr}} \quad (4)$$

The content represented by each item is shown in the following table:

T_{max}	Equilibrium temperature
T_{surr}	Ambient temperature
Q_{Dis}	Heat loss due to absorption of light by the container
I	Power density of 808 nm continuous laser

A_{808}	NIR Absorption of NiCoP/PVP Aqueous Solution at 808 nm
τ_s	Sample system time constant (225.7 s)
h	Heat transfer coefficient
S	Irradiated area

Therefore, $\eta = \frac{hS\,(T_{max} - T_{surr}) - Q_{Dis}}{I(1 - 10^{-A808})} \times 100\% = 43.5\%$

Cytotoxicity test

4T1 cells were seeded in a 96-well cell culture plate (10^4 cells per well) in a Roswell Park Memorial Institute-1640 (RPMI-1640) containing 10% fetal bovine serum albumin (FBS) and 1% penicillin/streptomycin in the culture medium and cultured at 37 °C and 5 % CO_2 for 24 h. Then, after the culture medium was removed, NiCoP/PVP with different concentrations were added to the culture medium, and the cells were cultured for 24 h. After removing the culture medium, the holes were washed with phosphate buffer, 10 μL of CCK-8 solution was added to each hole, and then the plate was incubated for 3 hours. The absorbance at 450 nm was measured by a microplate reader, and the cell viability was calculated from the average value by six parallel holes.

***In vitro* photothermal performance**

4T1 cells were seeded in a 96-well plate (5×10^4 cells per well) for 24 h, and then incubated with different concentrations of NiCoP/PVP. After incubating for 4 h, the cells were washed twice with PBS, and the cells were irradiated with 808 nm NIR laser (1.0 W·cm^{-2}) for 10 min. Then, the cells were cultured for another 24 h. Thereafter, a standard CCK-8 assay was performed to determine the survival rate of the cells.

Meanwhile, we used live/dead cell staining to evaluate the photothermal property of

NiCoP/PVP. In short, 4T1 cells were placed on a 24-well cell culture plate (4×10^4 cells per well). After overnight incubation, the culture medium was removed and the cells were washed three times with phosphate buffer. NiCoP/PVP solution (200 $\mu g \cdot mL^{-1}$) was added to the culture medium and incubated for 6 hours. Then, the cells were irradiated by 808 nm laser with a power density of 1.0 $W \cdot cm^{-2}$ for 10 minutes. After incubation for 8 hours, 4T1 cells were washed several times with phosphate buffer and stained with calcein acetyloxy methyl ester and propidium iodide. The cells were observed through a fluorescence microscope. Living cells are green, and dead cells are red.

Hemolysis analysis

Blood samples obtained from mice were diluted with PBS (5 mL) and centrifuged at 1200 rpm to separate red blood cells from the mixture. After washing for several times, the red blood cells were diluted with 20 mL of PBS to obtain the suspension. The diluted red blood cell suspension was divided into eight groups and mixed with the following substances: (+) deionized water as positive control, (-) PBS as negative control, and NiCoP/PVP suspensions with concentrations of 12.5, 25, 50, 100, 200, and 400 $\mu g \cdot mL^{-1}$. The mixture was vortexed and cultured at 37 °C for 4 hours, then centrifuged at 12000 rpm. Subsequently, the supernatant was added to the 96-well plate, and the absorbance was measured by an enzyme-labeled instrument. The hemolysis percentage was calculated by the following equation: hemolysis rate (%) = $(A_{sample} - A_{control(-)}) / (A_{control(+)} - A_{control(-)}) \times 100\ \%$.

***In vitro* and *in vivo* T_1/T_2 MRI**

T_1/T_2 MR contrast signals of NiCoP/PVP (0, 0.10, 0.21, 0.41, 0.83 and 1.65 mM) were measured with a clinical MRI scanner (GE Discovery MR750 3.0 T). *In vivo* T_1/T_2 MRI was

carried out after intravenous or intratumor injection of NiCoP/PVP (5 mg/kg, 100 μL).

***In vivo* PTT**

Female balb/c mice were purchased from the Experimental Animal Center of Jilin University (Changchun, China). All the animal experiments are conducted according to the rules of the Institutional Animal Care and Use Committee of Jilin University (IACUC). The tumor model was established by subcutaneous injecting 4T1 cells into the right armpit of the mice. When the tumor volume reaches approximately 100 mm^3, the mice were used for *in vivo* experiments. The tumor-bearing mice were randomly divided into four groups. i) control, ii) 808 nm laser, iii) NiCoP/PVP, and iv) NiCoP/PVP + 808 nm laser. After 24 h of tail vein injection, the tumor sites of mice in group ii) and group iv) were irradiated with 808 nm laser for 10 min to the tumor site. During the treatment process, a near-infrared thermal imaging camera was used to monitor the temperature changes at the tumor site. After treatment, the tumor volume and body weight of the mice from each group were measured every other day. After 14 days of treatment, the mice were euthanized, and the tumor mass was weighed after dissection to evaluate the treatment effect.

Biodistribution of NiCoP/PVP

NiCoP/PVP solution (5 mg/kg, 100 μL) was injected into tumor-bearing mice through the tail vein. After 24 h, the main organs (heart, liver, spleen, lungs, kidneys) and tumors were taken out and weighed. Then, the heart, liver, spleen, lungs, kidneys, and tumors of mice were soaked in aqua regia. Finally, the resulting solution was used to analyze the distribution of NiCoP/PVP in mice by electrolytic coupled plasma mass spectrometry.

Histological staining

The mice were dissected to obtain the tumors, heart, liver, spleen, lungs, and kidneys. Then, fixed them with 4% paraformaldehyde, respectively. Afterwards, the tissue samples were embedded in paraffin, sectioned and stained with hematoxylin-eosin (H&E). The stained tissue sections were observed under the optical microscope.

TUNEL staining

The mice in each experimental group were sacrificed, the tumor tissues were taken out, fixed with 4% paraformaldehyde, and stained with TUNEL to evaluate and compare the photothermal damage of tumor.

Supporting Figures

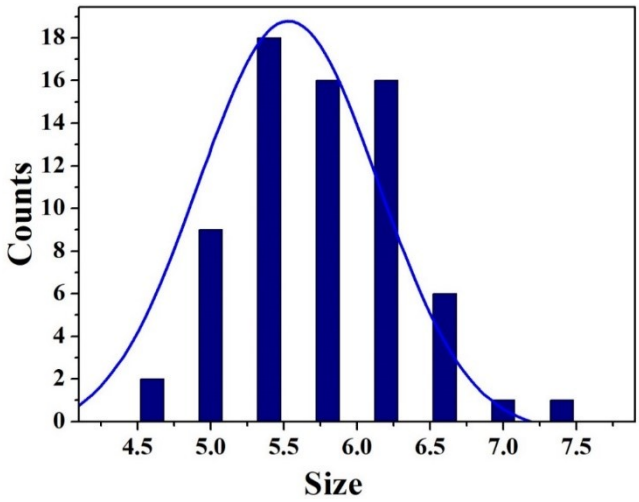

Figure S1. Size distribution histogram of NiCoP.

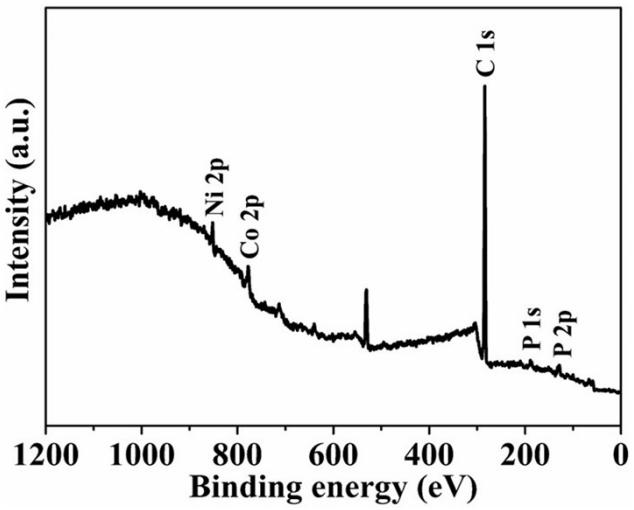

Figure S2. XPS of NiCoP.

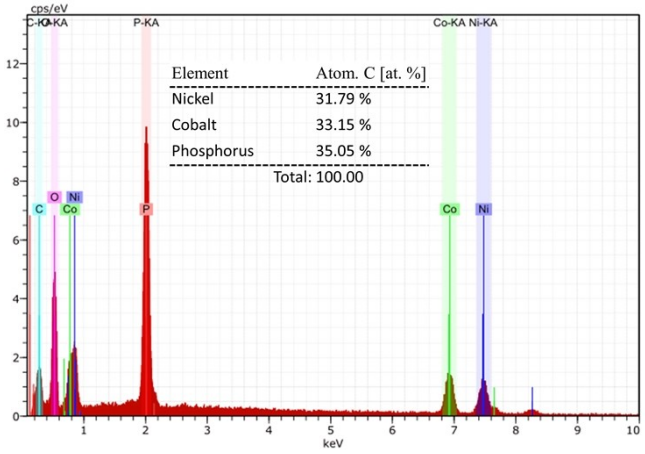

Figure S3. EDX of NiCoP.

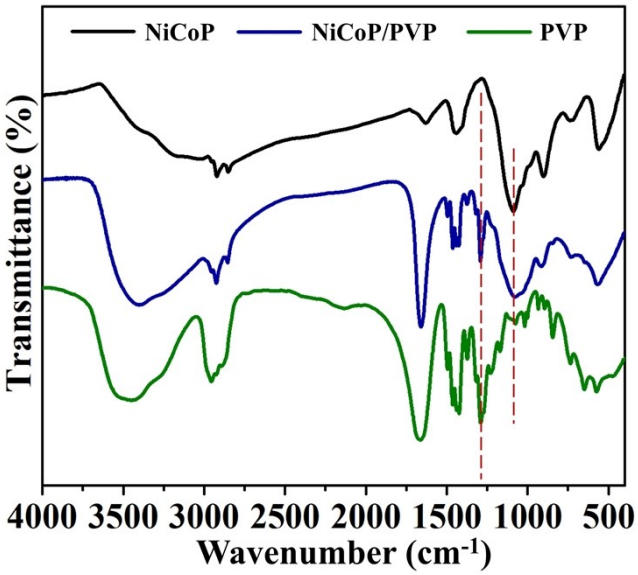

Figure S4. FT-IR spectra of NiCoP, NiCoP/PVP, and PVP.

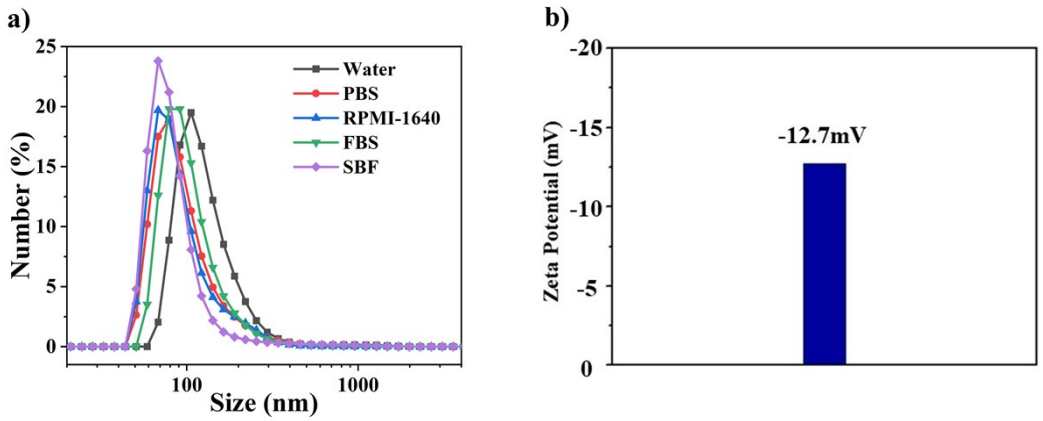

Figure S5. (a) Hydrodynamic size of NiCoP/PVP in water, PBS, RPMI-1640, FBS, and SBF. (b) Zeta potential of NiCoP/PVP in water.

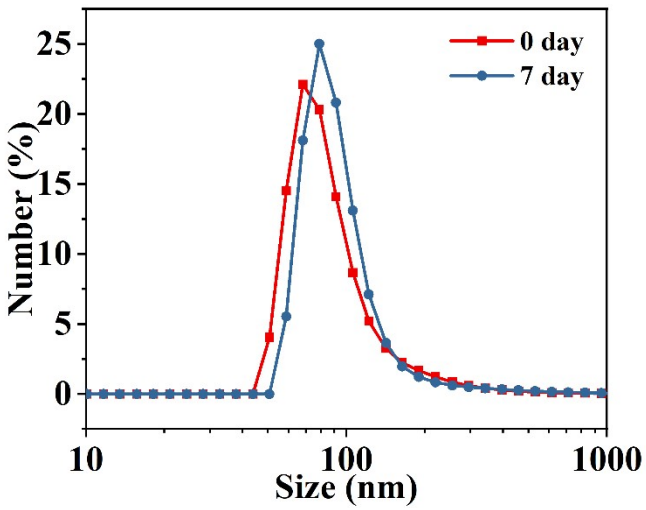

Figure S6. Hydrodynamic size of NiCoP/PVP in deionized water at 0 day and 7 day.

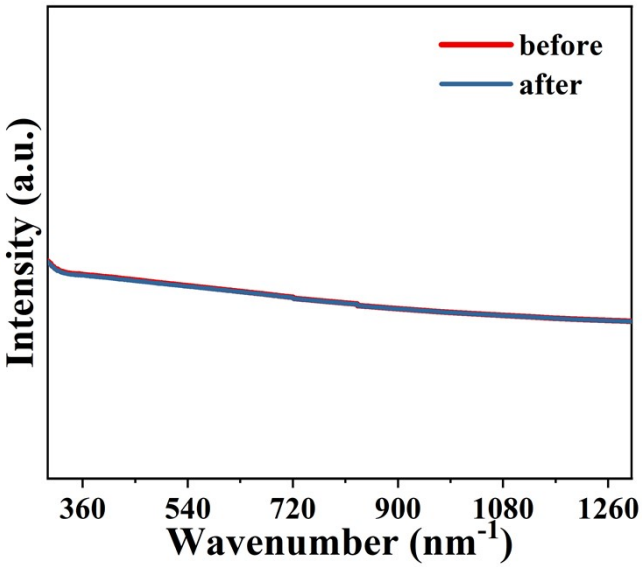

Figure S7. Vis-NIR absorption spectra of NiCoP/PVP before and after several heating cycles.

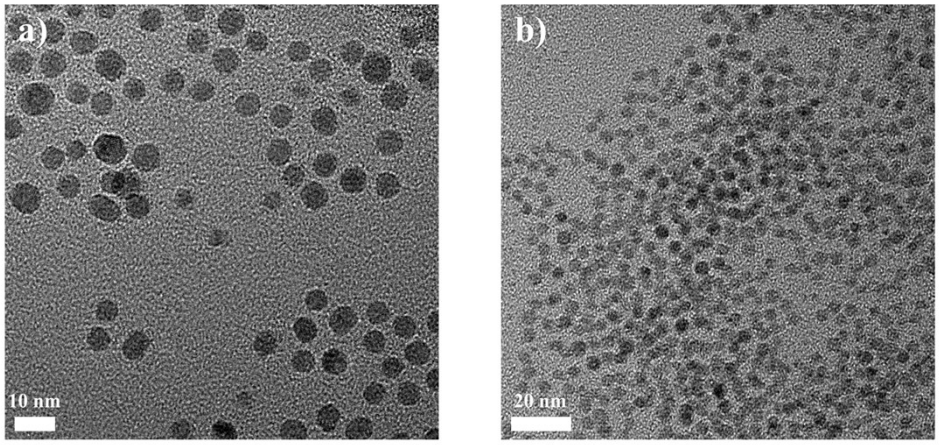

Figure S8. TEM images of NiCoP/PVP at (a) high and (b) low magnification after several heating cycles.

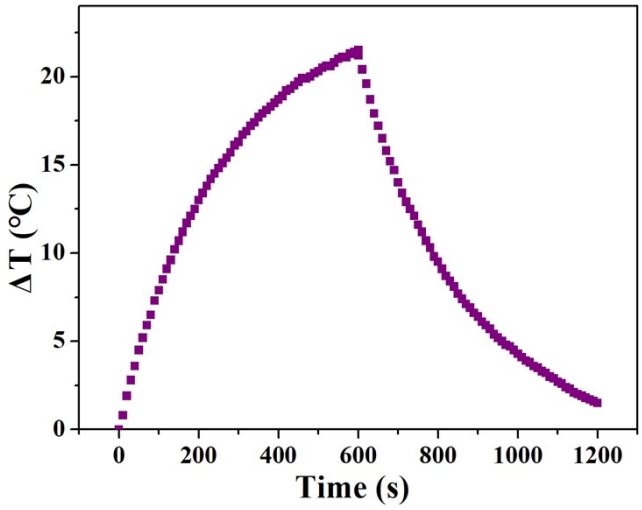

Figure S9. Heating and cooling curve of NiCoP/PVP aqueous solution under 808 nm laser irradiation for 10 min and then stop.

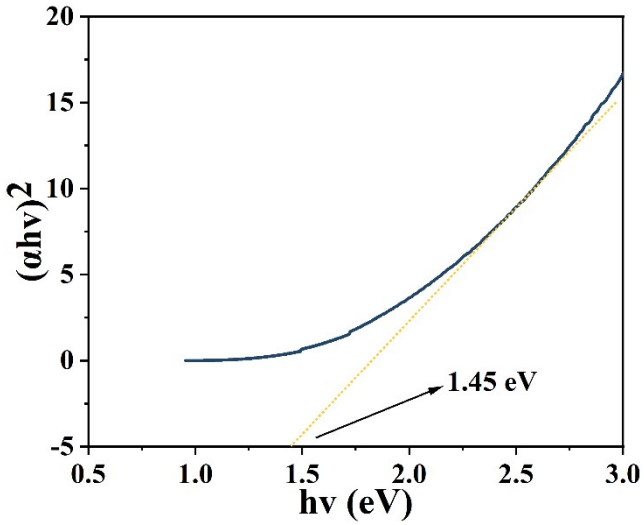

Figure S10. The corresponding optical band gap (Eg) of NiCoP/PVP (1.45 eV), estimated based on the Tauc plot [$(\alpha h\nu)^2 = k(h\nu - Eg)$].

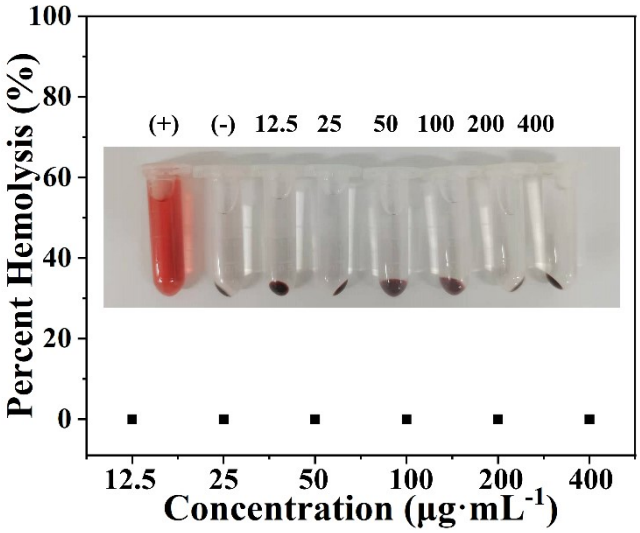

Figure S11. Hemolysis rate of NiCoP/PVP at different concentrations.

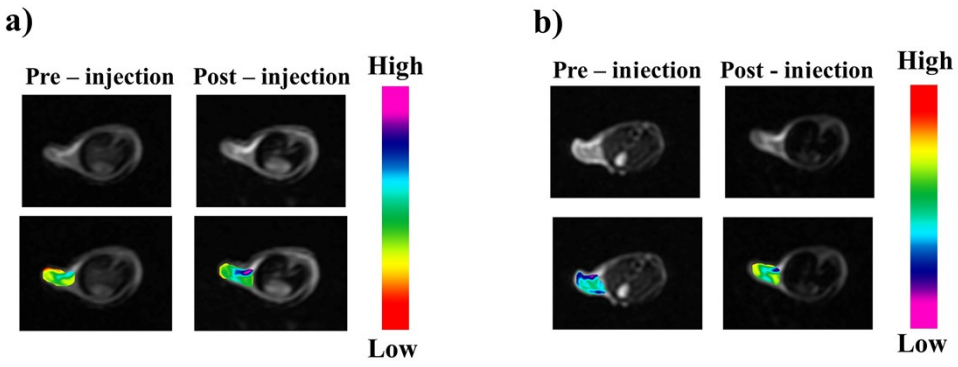

Figure S12. (a) T_1- and (b) T_2-weighted MRI of the mice after intratumoral injection *in vivo*.

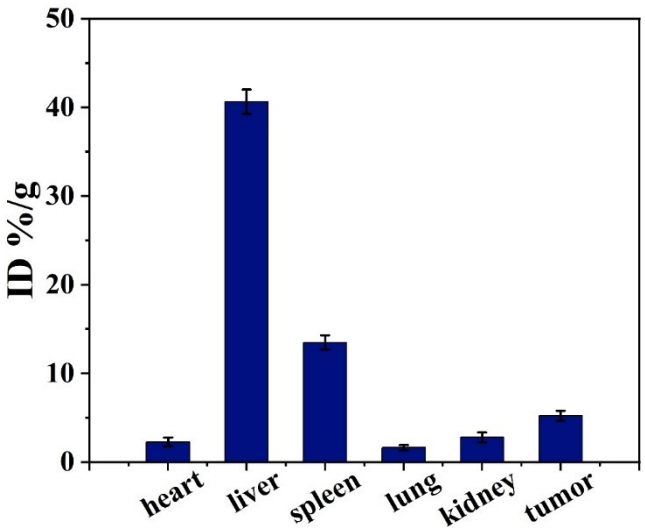

Figure S13. Biodistribution of NiCoP/PVP in tumor and major organs at post-injection of 24 h.

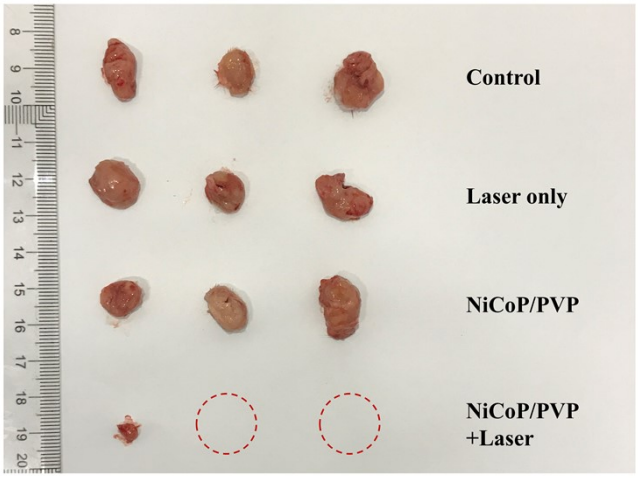

Figure S14. Photographs of the excised tumors from representative euthanized mice after various treatments.

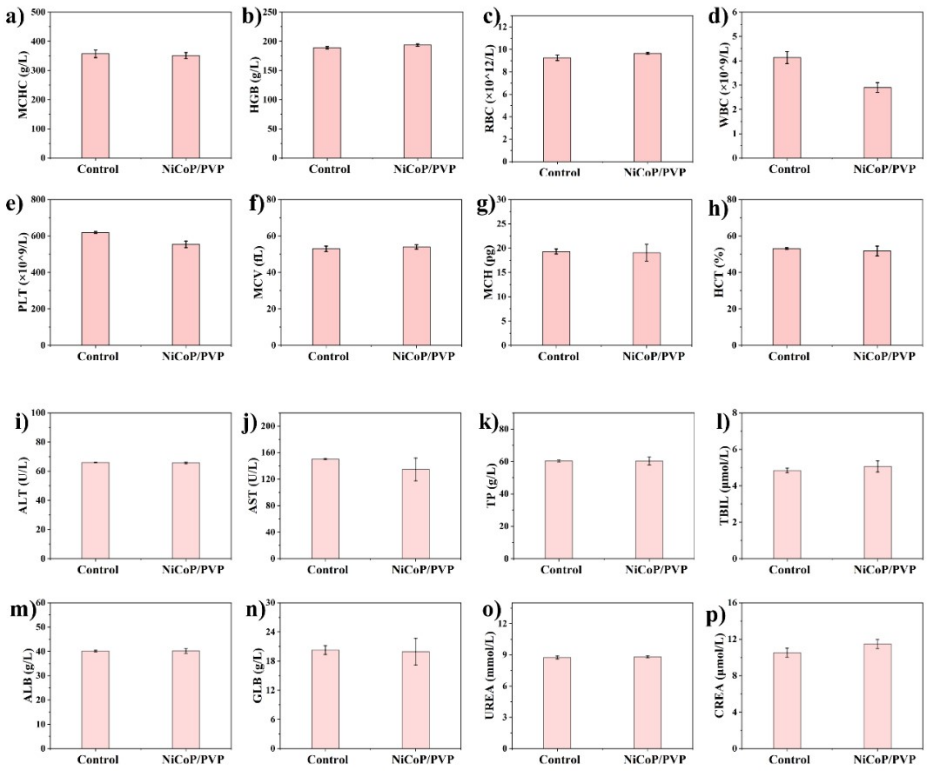

Figure S15. Blood analysis. (a-h) Hematological index and (i-p) biochemical blood analysis of the mice after intravenous injection of NiCoP/PVP at 30 d.

References

1. D. R. Liyanage, S. J. Danforth, Y. Liu, M. E. Bussell and S. L. Brock, *Chem. Mater.* 2015, **27**, 4349-4357.

2. L. Dong, G. Ji, Y. Liu, X. Xu, P. Lei, K. Du, S. Song, J. Feng and H. Zhang, *Nanoscale* 2018, **10**, 825-831.

3. P. Lei, R. An, P. Zhang, S. Yao, S. Song, L. Dong, X. Xu, K. Du, J. Feng and H. Zhang, *Adv. Funct. Mater.* 2017, **27**, 1702018.