Electronic Supplementary Material (ESI) for RSC Advances.
This journal is © The Royal Society of Chemistry 2020

Supporting Information

Self-assembled $CeVO_4$/Au Heterojunction Nanocrystals for Photothermal/Photoacoustic Bimodal Imaging-Guided phototherapy

Junrong Wang, [a] Yubo Hu, [b] Junyang Chen, [b] Cong Ye [*a]

[a] *Department of Obstetrics and Gynecology, China-Japan Union Hospital of Jilin University, Changchun, Jilin 130033, china.*

[b] *Department of Anesthesiology, China-Japan union hospital of Jilin university, Changchun, Jilin 130033, china.*

*Corresponding author. E-mail: zhanggs576@163.com (C. Ye)..

1.1 Materials and Reagents

All of the chemicals used were of analytical grade and were used without further purification. Sodium hydroxide (NaOH), ammonium metavanadate (NH_4VO_3), Hydrogen tetrachloroaurate (III) ($HAuCl_4 \cdot 3H_2O$), Sodium borohydride ($NaBH_4$), were purchased from Aladdin. Oleic acid (OA), $Ce(NO_3)_3 \cdot 6H_2O$, were purchased from Science and Technology Parent Company of Changchun Institute of Applied Chemistry. Tetrahydrofuran (THF), cyclohexane were purchased from Beijing Chemical Works. 2,2-Dimethoxy-2-phenyl acetophenone (DMPA) was purchased from Tokyo Chemical Industry. Thiol-polyethylene glycol with different chemical groups (HS-PEG_{1000}-OH) were purchased from PegBio Co., Ltd (Jilin, China).

1.2. Characterization

The X-ray diffraction (XRD) patterns were tested with a D8 Focus diffractometer (Bruker) with the use of Cu Kα radiation (λ = 0.15405 nm). Transmission electron microscopy (TEM) was recorded using an FEI Tecnai G2 S-Twin with a field emission gun operating at 200 kV. Fourier transform Infrared spectra (FT-IR) were measured on a Vertex Perkin–Elmer 580BIR spectrophotometer (Bruker) with the KBr pellet technique. The UV-Vis adsorption spectral values were measured on a U-3310 spectrophotometer (Hitachi). The X-ray photoelectron spectra (XPS) were taken on a VG ESCALAB MK II electron spectrometer using Mg Kα (1200 eV) as the excitation source. Inductively Coupled Plasma (ICP) was taken on an ICAP 6300 of Thermo scientific. Dynamic light scattering (DLS) and Zeta potential were obtained by using a Malvern instrument Zetasizer Nano system. NIR laser and infrared thermal imager (E40, FLIR) were used to obtain the infrared thermal images. MSOT in Vision 128 small animal imaging system (iThera Medical GmbH, Munich, Germany).

1.3. Statistical Analysis

Statistical analysis was performed with the Statistical Program for Social Sciences software (SPSS, Chicago, IL, USA) as needed. All data were expressed as means (standard deviation and a statistically significant difference were considered to be present at $p < 0.05$. Except as mentioned, all assays were repeated in triplicate in three independent experiments.

1.4. Cell Culture

The HeLa cells line and L929 cells line were cultured in DMEM and MEM culture medium, respectively. And they were supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) streptomycin, and 1% (v/v) penicillin at 37 °C in 5% CO_2.

1.5. Photothermal effect of $CeVO_4$ and $CeVO_4$/Au in aqueous solution

The aqueous $CeVO_4$ and $CeVO_4$/Au solution (0.1 mL) was placed into 96-well plates separately at different

concentrations (100, 250 and 300 μg·mL^{-1}) and was exposed to the NIR laser irradiation (NIR, 1.0 W·cm^{-2}) for 5 min. Simultaneously, an infrared camera (NEC, with an accuracy of 0.1 °C) was used to measure the real-time temperature, and the change of temperature was recorded every 15 s. And, the photothermal effect of aqueous $CeVO_4$ and $CeVO_4$/Au solution with the same concentration of 300 μg·mL^{-1} and different power density (0.5, 1.0 and 1.5 W·cm^{-2}) was also measured in the same way. The photothermal stability of $CeVO_4$/Au was measured by cycle irradiation. The aqueous $CeVO_4$/Au solution (300 μg·mL^{-1}) was exposed to NIR laser irradiation with a power density of 1.5 W·cm^{-2} for 6 min. Then, the laser was shut off, and the solution was cooled down naturally for another 12 min. The above procedure was repeated for three times and the temperature was recorded by the infrared camera.

1.6. Photothermal Conversion Efficiency of $CeVO_4$ and $CeVO_4$/Au in Aqueous Solution

To examine the photothermal behavior, the aqueous solution of $CeVO_4$ and $CeVO_4$/Au with concentration of 0.3 mg·mL^{-1} (1 mL) was irradiated using an NIR laser (BWT Beijing Ltd., China) with a power density of 1.0 W·cm^{-2} over a period of 1200 s, then the NIR laser was shut off. Simultaneously, an infra-red camera was used to measure the real-time temperature, and the change in temperature was recorded every 15 s.

The photothermal conversion efficiency (η) of $CeVO_4$ and $CeVO_4$/Au can be calculated according to the eq1

$$\eta = \frac{\mathrm{hS}(T_{\max} - T_{\mathrm{surr}}) - Q_{dis}}{I(1-10^{-A_{808}})} \quad \textbf{(1)}$$

The T_{max} (K) means the equilibrium temperature; T_{surr} (K) is ambient temperature. The Q_{dis} (W) is heat loss from light absorbing by the container, and it is calculated to be approximately equal to 0 mW. I (W/cm^2) represents incident laser power density; A_{808} is the absorbance of samples at NIR. Where h (W/(cm^2·K)) means heat transfer coefficient, S (cm^2) represents the surface area of the container, the hs is calculated from the Fig S4. The hS is calculated using the following eq 2

$$\tau_s = \frac{\mathrm{m}_D c_D}{hs} \quad \textbf{(2)}$$

Where τs is the sample system time constant, m_D and c_D are the mass (1 g) and heat capacity (4.2 J/(g·°C)) of the solvent. Thus, according to the calculation, the heat conversion efficiency (η) of the samples is listed in the table.

	Tmax-Tsurr	A_{808}	τs	η

$CeVO_4$	11.9	0.4960	581.51	11.65 %
$CeVO_4$/Au	17.2	0.9405	488.65	17.32 %

1.7. Extracellular $\cdot O_2^-$ detection

For the extracellular $\cdot O_2^-$ generation test, a 1, 3-diphenylisobenzofuran (DPBF) probe was employed to detect the ROS. 10 μL of DPBF (10 mg·mL^{-1}) solution was added to 3 mL of $CeVO_4$ or $CeVO_4$/Au (65 μg·mL^{-1}) aqueous solution. Then the mixtures were irradiated under NIR laser (1.0 W·cm^{-2}) for different times (0, 5, 10, 20, and 30 min). Then, the samples were centrifuged at 10000 rpm for 10 min. Afterwards, the absorption of DPBF at 420 nm was recorded.

1.8. Extracellular ·OH detection

For the extracellular ·OH generation text, $CeVO_4$ and $CeVO_4$/Au (0.2 mg) were added in 3 mL of methylene blue (MB) solution. Then, the mixture solution was stirred in darkness for 30 min to attain absorption-desorption equilibrium. Afterwards, the samples were exposed to the NIR laser (1.0 W·cm^{-2}). After irradiation for different times (0, 5, 10 and 20 min), the MB aqueous solution was centrifuged to remove $CeVO_4$ and $CeVO_4$/Au. The absorbance at 660 nm was measured by UV−vis spectroscopy.

1.9. Cell compatibility

L929 cells were cultured in MEM supplemented with 1% (v/v) penicillin/streptomycin, and 10% (v/v) fetal bovine serum (FBS) at 37 °C under 5% CO_2. In total, 8000 cells were seeded into 96 well plates and incubated with different concentrations (50, 100, 150, and 200 μg·mL^{-1}) of $CeVO_4$ and $CeVO_4$/Au dispersed in MEM for 24 h. Relative cell viabilities were detected by the standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

1.10. Cellular uptake

HeLa cells were seeded in 6-well plate at a density of 1×10^5 cells per well and cultured overnight. Then the medium was replaced with fresh culture medium containing rhodamine B-labeled $CeVO_4$ or $CeVO_4$/Au (300 μg·mL^{-1}), respectively. After incubation for 4 h, the cells were washed with PBS several times and fixed with 4% paraformaldehyde for 10 min. The cell nuclei were dyed with DAPI. Then the medium was removed and rinsed with PBS several times. The cellular uptake was observed under dark-field scattering microscopy.

1.11. *In vitro* cytotoxicity evaluation

The Hela cells were seeded in 96-well culture plates with a density of 8000 cells/well. After being incubated overnight, the cells were treated with $CeVO_4$ or $CeVO_4$/Au at serial concentrations of 50, 100, 150 and 200 μg

mL^{-1} for 4 h (37 °C, 5% CO_2), followed by removing the medium containing non-internalized NCs, the cells were washed once with PBS, and then irradiated for 5 min with NIR laser (1.0 $W \cdot cm^{-2}$) in the fresh culture medium. Then, the cells were further incubated for 24 h at 37 °C under 5% CO_2. At last, the cell viability was evaluated using MTT assay. The MTT assay of NIR laser-triggered phototherapy with $CeVO_4$ or $CeVO_4$/Au at 200 $\mu g \cdot mL^{-1}$ with different power density was also performed under the same condition.

1.12. Intracellular ROS detection

For *in vitro* ROS detection, ROS production was detected by a cell permeable dye 2′,7′-dichlorofluorescin diacetate (DCFH-DA), which is non-fluorescent and could be easily oxidized to green fluorescent 2′,7′-dichlorofluorescein (DCF) by intracellular ROS. Briefly, Hela cells were seeded into 24-well culture plates at a density of 0.2 $\times 10^5$ cells per well and incubated at 37 °C in 5% CO_2 for 24 h. Afterward, Hela cells were incubated with 400 µL of $CeVO_4$ or $CeVO_4$/Au (200 µg mL^{-1}) at 37 ℃ for 4 h. Thereafter, the culture media were replaced by fresh culture media and exposed to a NIR laser (1.0 W cm^{-2}) for 5 min. Then, the ROS probe was added to each well and the mixture was incubated for 20 min at 37 ℃. Finally, the cells were washed repeatedly with PBS. The fluorescence was routinely detected by exciting at 485 nm and measuring emission at 545 nm with an inverted fluorescence microscope system(Nikon Ti–S).

1.13. *In vivo* phototherapy

U14 tumor-bearing Balb/c mice with an average tumor volume of 100 mm^3 were randomly assigned into six groups: (a) PBS (control group), (b) NIR laser (0.5 $W \cdot cm^{-2}$) for 5 min, (c) $CeVO_4$ injection alone, (d) $CeVO_4$/Au injection alone, (e) $CeVO_4$ injection + NIR laser irradiation (0.5 $W \cdot cm^{-2}$) for 5 min, (f) $CeVO_4$/Au injection + NIR laser irradiation (0.5 $W \cdot cm^{-2}$) for 5 min. All the groups were intravenously injected with the same volume of PBS, $CeVO_4$ or $CeVO_4$/Au (100 µL, 20 $mg \cdot kg^{-1}$) solution. The tumor sizes and weight of mice were measured every 2 d. Tumor volume = length×$width^2$/2, relative tumor volume was calculated as V/V0 (V0 was the corresponding tumor volume when the treatment was initiated). These mice were sacrificed at day 14 to harvest the major organs and tumor for histological analysis.

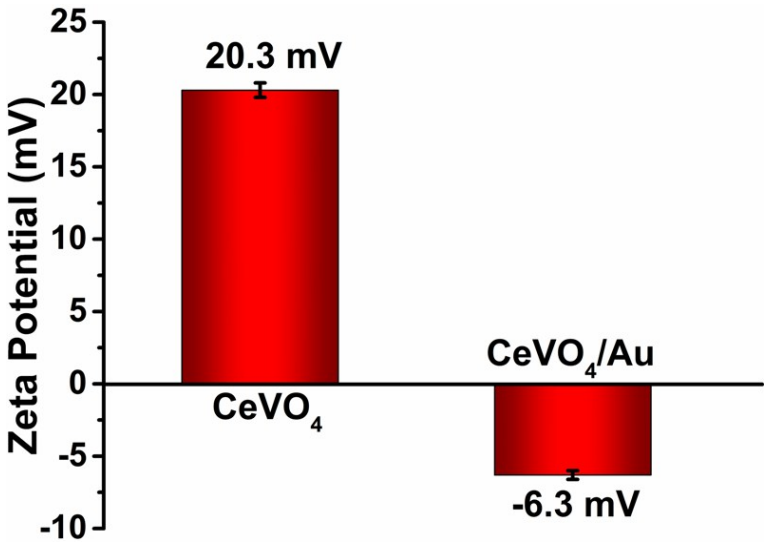

Fig. S1 The zeta potential of $CeVO_4$ and $CeVO_4$/Au.

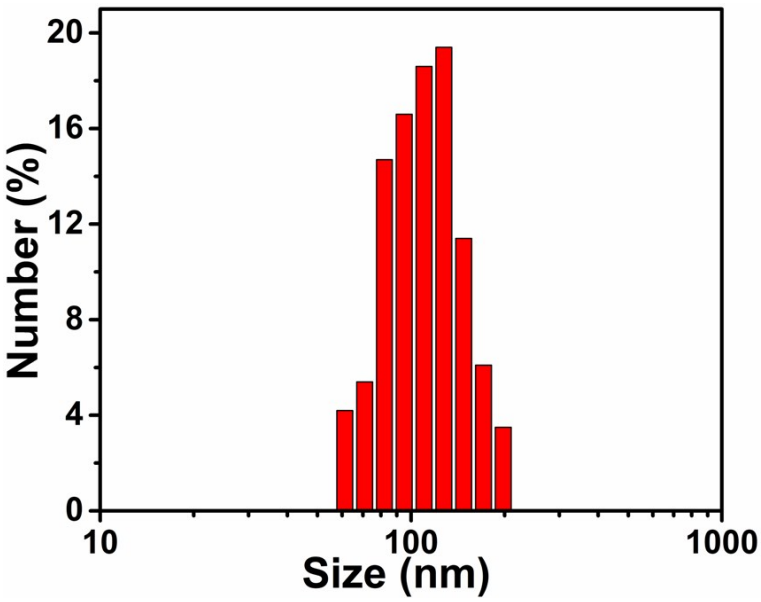

Fig. S2 Hydrodynamic size of $CeVO_4$/Au.

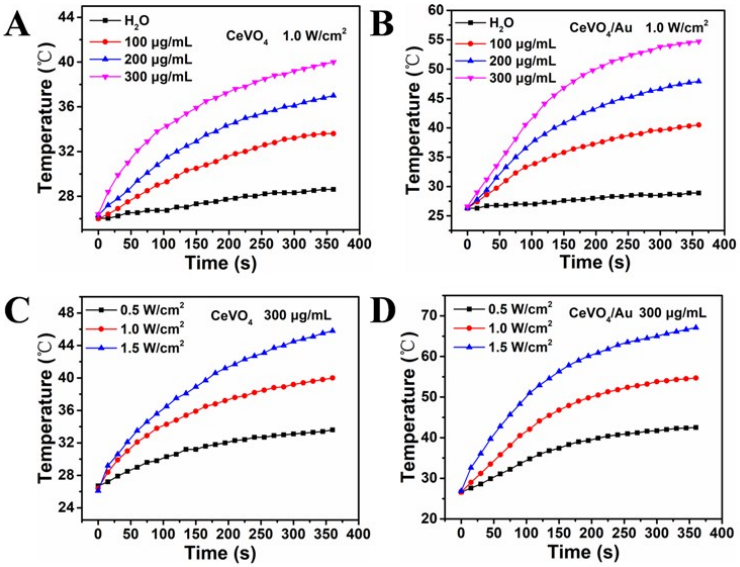

Fig. S3 Photothermal activity of $CeVO_4$ (A) and $CeVO_4$/Au (B) with different concentration under 1.0 W·cm^{-2} of NIR laser irradiation for 5 min. Photothermal activity of $CeVO_4$ (C) and $CeVO_4$/Au (D) with concentration of 300 μg·mL^{-1} under different power density of NIR laser irradiation for 5 min.

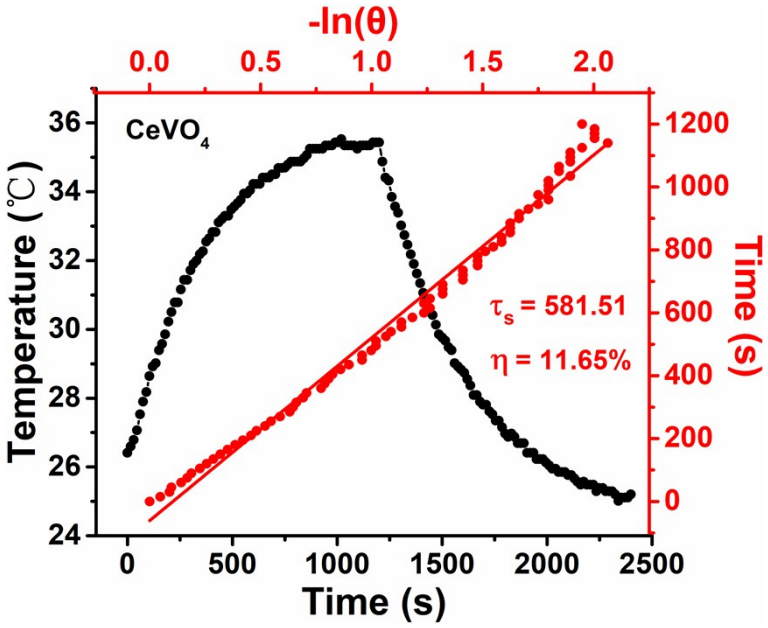

Fig. S4 The temperature change of $CeVO_4$ aqueous solution (300 μg·mL^{-1}, 1 mL) response to 1.3 W·cm^{-2} of NIR laser on and off and Linear time data versus -lnθ obtained from the cooling period of $CeVO_4$ aqueous solution.

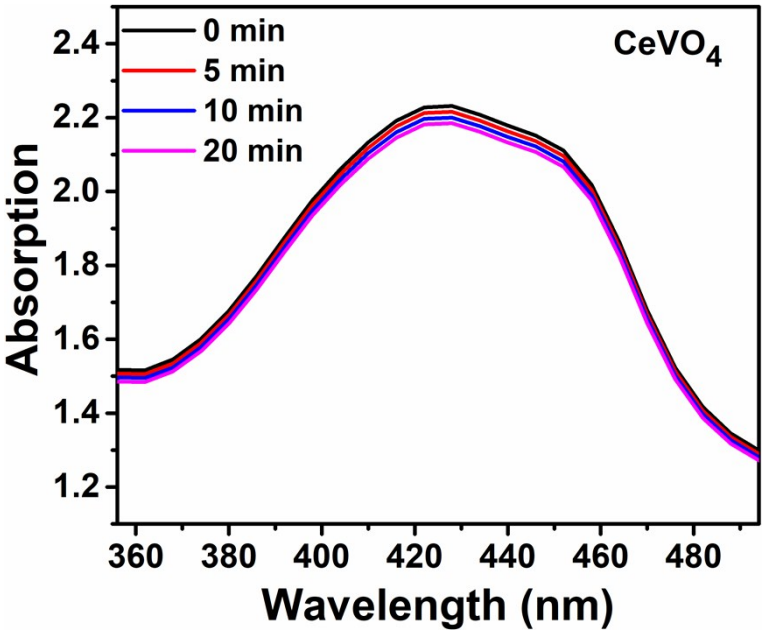

Fig. S5 Depletion of DPBF due to $\cdot O_2^-$ generation: 65 μg·mL of $CeVO_4$ with NIR laser (1.0 W·cm^{-2}) irradiation.

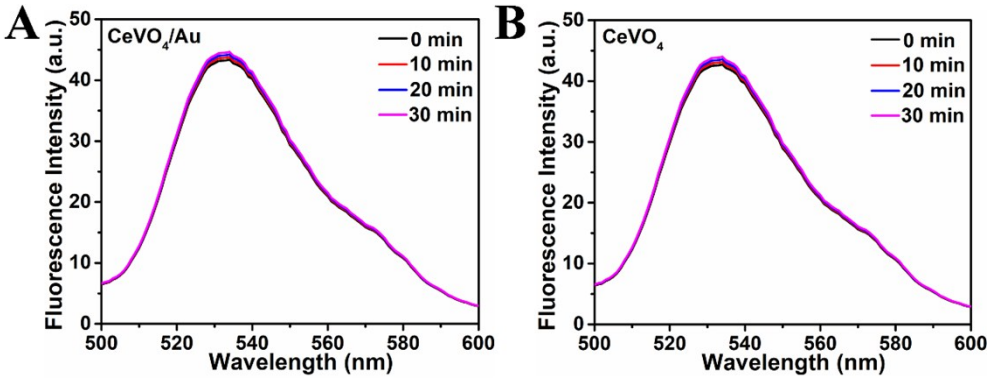

Fig. S6 The fluorescence intensity of SOSG in the presence of $CeVO_4$ and $CeVO_4$/Au with NIR laser (1.0 W·cm^{-2}) irradiation

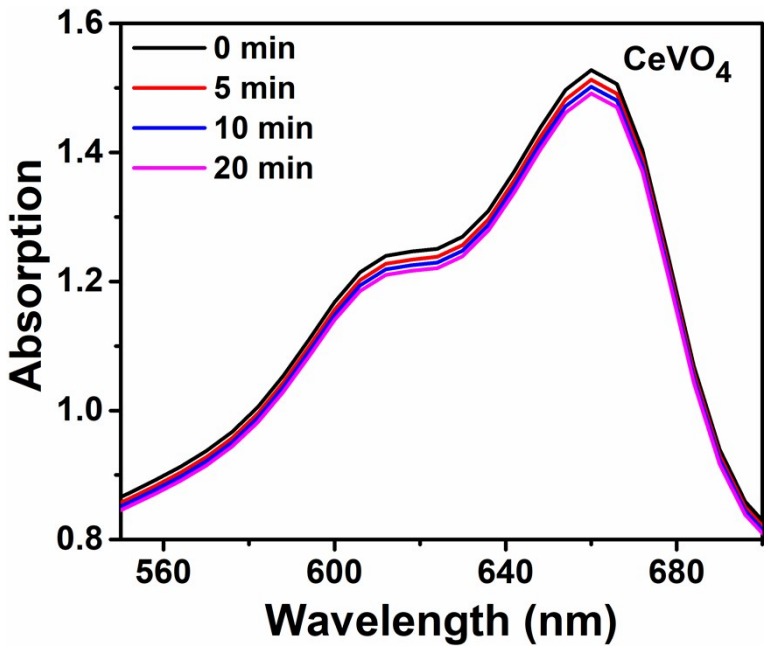

Fig. S7 Depletion of MB due to ·OH generation: 65 μg·mL of $CeVO_4$ with NIR laser (1.0 W·cm^{-2}) irradiation.

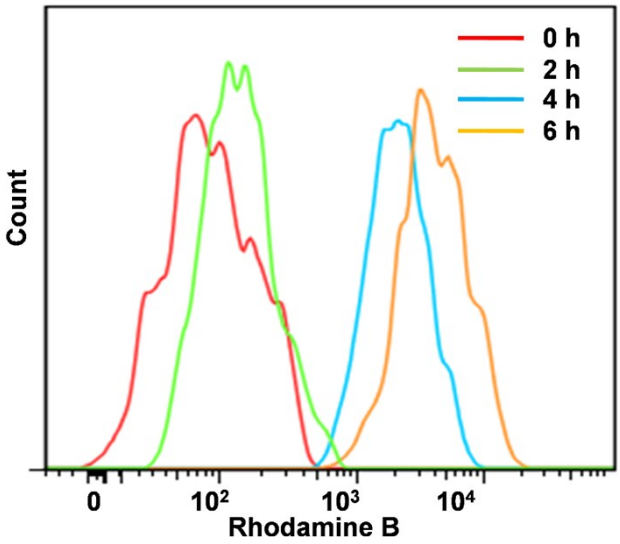

Fig. S8 The intensity of red fluorescence from rhodamine B by different treatments (Hela cells treated with $CeVO_4$/Au NCs -RhB at different times, 0 h, 2 h, 4 h and 6 h) was detected by flow cytometry

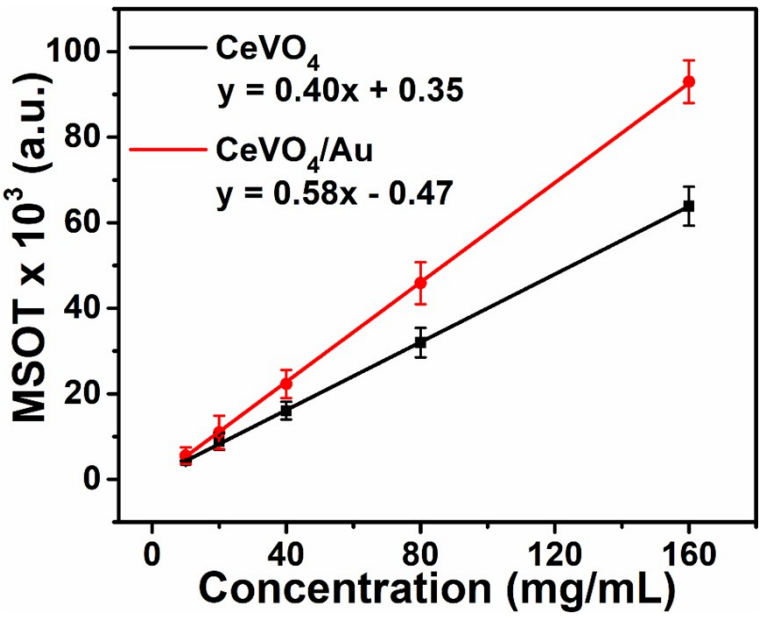

Fig. S9 Linear relationship between PA signal intensity and concentrations of $CeVO_4$ and $CeVO_4$/Au, respectively.

Table S1. The blood panel and serum biochemical index parameters for the control group and $CeVO_4$/Au treated group. Serum biochemical date including blood urea nitrogen (BUN) levels, serum creatinine (CRE) and liver function markers such as Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP). Blood levels of White blood cells (WBS), Red blood cells (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), were also measured.

	Reference range	Untreated control	1 day	7 day	14 day
ALT(IU)	40~170	50.34±3.45	57.88±3.99	54.52±4.44	58.77±3.25
AST(IU)	67~381	100.68±3.02	109.41±5.09	110.56±10.07	121.25±30.92
ALP(IU)	108~367	130.11±4.79	121.26±5.12	115.25±7.43	131.97±4.51
BUN(mmol/L)	7~31	10.38±1.01	10.22±0.19	9.76±0.38	10.34±0.25
CRE(umol/L)	18~44	30.04±2.43	29.51±2.36	30.56±0.69	31.45±5.26
WBC (10^9/L)	5.69~14.84	10.12±1.33	10.98±2.21	11.23±0.98	10.87±1.23
RBC (10^{12}/L)	8.16~11.69	9.23±1.12	9.34±2.01	8.89±1.34	9.11±0.87
HGB (g/L)	124~189	133.78±9.32	130.42±10.88	145.67±10.77	143.22±13.42
HCT (%)	43~67	50.34±4.55	51.43±3.32	54.31±0.99	55.56±4.32
MCV (fL)	50.8~64.1	55.77±1.23	56.87±2.12	58.53±3.12	59.22±2.34
MCH (pg)	13~17.6	15.43±0.67	15.11±0.45	15.44±0.76	15.98±1.21
MCHC (g/L)	239~331	267.89±10.12	245.54±14.64	287.53±10.89	243.43±13.55
PLT (10^9/L)	476~1611	805.42±21.32	823.54±20.13	810.43±27.45	815.45±21.09