Supporting Information

Self-assembled CeVO$_4$/Au Heterojunction Nanocrystals for Photothermal/Photoacoustic Bimodal Imaging-Guided phototherapy

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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 (NH₄VO₃), Hydrogen tetrachloroaurate (III) (HAuCl₄·3H₂O), Sodium borohydride (NaBH₄), were purchased from Aladdin. Oleic acid (OA), Ce(NO₃)₃·6H₂O, 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-PEG₁₀₀-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% CO₂.

1.5. Photothermal effect of CeVO₄ and CeVO₄/Au in aqueous solution

The aqueous CeVO₄ and CeVO₄/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 (\(\eta\)) of CeVO\(_4\) and CeVO\(_4\)/Au can be calculated according to the eq1

\[
\eta = \frac{hS(T_{\text{max}} - T_{\text{surr}}) - Q_{\text{dis}}}{I(1 - 10^{-\sigma A_{808}})}
\]  

(1)

The \(T_{\text{max}}\) (K) means the equilibrium temperature; \(T_{\text{surr}}\) (K) is ambient temperature. The \(Q_{\text{dis}}\) (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

\[
\tau_s = \frac{m_Dc_D}{hS}
\]  

(2)

Where \(\tau_s\) is the sample system time constant, \(m_0\) and \(c_0\) 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 (\(\eta\)) of the samples is listed in the table.

| \(T_{\text{max}}-T_{\text{surr}}\) | \(A_{808}\) | \(\tau_s\) | \(\eta\) |
1.7. Extracellular ·O$_2^-$ detection

For the extracellular ·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 test, 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·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 μg·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 ×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 °C 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 °C. 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·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·cm\(^{-2}\)) for 5 min, (f) CeVO\(_4\)/Au injection + NIR laser irradiation (0.5 W·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·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/V\(_0\) (V\(_0\) 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 CeVO₄ (A) and CeVO₄/Au (B) with different concentration under 1.0 W·cm⁻² of NIR laser irradiation for 5 min. Photothermal activity of CeVO₄ (C) and CeVO₄/Au (D) with concentration of 300 μg·mL⁻¹ under different power density of NIR laser irradiation for 5 min.
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 $-\ln \theta$ obtained from the cooling period of CeVO$_4$ aqueous solution.

**Fig. S4**
Fig. S5 Depletion of DPBF due to ·O₂⁻ generation: 65 μg·mL of CeVO₄ with NIR laser (1.0 W·cm⁻²) irradiation.
Fig. S6 The fluorescence intensity of SOSG in the presence of CeVO₄ and CeVO₄/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.
The intensity of red fluorescence from rhodamine B by different treatments (HeLa cells treated with CeVO₄/Au NCs - RhB at different times, 0 h, 2 h, 4 h and 6 h) was detected by flow cytometry.
Fig. 59 Linear relationship between PA signal intensity and concentrations of CeVO₄ and CeVO₄/Au, respectively.
Table S1. The blood panel and serum biochemical index parameters for the control group and CeVOs/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|