Supplementary Information

BSA-templated ultrasmall Ag/Gd_2O_3 as a self-enabled nanotheranostics for MR/CT/PA tri-modality imaging and photothermal therapy

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Chemicals

Bovine serum albumin (BSA) was purchased from Solarbio (China). $GdCl_3 \cdot 6H_2O$ was obtained from Sigma Aldrich (USA). Fetal bovine serum (FBS) and trypsin were purchased from Gibco (USA). DMEM was provided Kaiji Biotechnology (China). Phosphate buffered saline (PBS，1×，pH 7.40) was purchased from HyClone (USA).

Characterizations

Transmission electron microscopy (TEM) images was recorded on JEOL JEM-2100 (JEOL Ltd., Japan). Dynamic light scatting (DLS) data was got from a Nano-ZS90 Zetasizer (Malvern, UK). X-ray photoelectron spectroscopy was realized on an instrument (AXIS SUPRA, Kratos). Fourier transform infrared (FT-IR) spectra was got from iS50 (Thermo Nicolet Corporation,USA). The UV-Visible spectrum was obtained on a UV-3010 spectrophotometer (Hitachi, Ltd., Japan). Circular dichroic (CD) spectra were collected on a J-810 (JASCO, Japan). X-ray photoelectron spectroscopy (XPS) spectra were obtained on a PHI Quantera-II SXM (Ulvac-PHI, Japan). The elemental contents were measured on an Inductive Coupled Plasma Emission Spectrometer (Varian, USA). The T_1 relaxation times and corresponding MR images were acquired on a 7T MR scanner (Bruker Pharmascan, Germany). Computed tomography (CT) imagings were obtained on a small animal PET/SPECT/CT scanner (Siemens, Germany). Photoacoustic imaging (PA) imagings were obtained on a small animal imaging system MultiSpectral Optoacoustic Tomography (iThera Medical, Germany).

***In vitro* photothermal effect**

Typically, 1 mL of Ag/Gd_2O_3@BSA aqueous suspension was placed in a vial at a series of Ag concentrations (0, 1.25,2.5, 5, 10, 20, 40 mM) and was exposed to an 808 nm NIR laser (Beijing Viasho Technology Co., China) at 1 $W \cdot cm^{-2}$ for 10 min. During the irradiation, the temperature was measured by the infrared thermal camera (Fotric 225).

To evaluate the photothermal conversion efficiency (η), the temperature variations of Ag/Gd_2O_3@BSA aqueous solution (Ag concentration 10 mM) were tested under 808 nm laser irradiation (1 $W \cdot cm^{-2}$) for 10 min. After arriving at the max temperature, the laser was turned off for natural cooling to room temperature. η can be calculated according to the following Equation[1]:

$$\eta = \frac{hS(T_{Max} - T_{Surr}) - Q_{Dis}}{I\left(1 - 10^{-A808}\right)} \quad \cdots\cdots (1)$$

$$\tau_S = \frac{m_D C_D}{hS} \quad \cdots\cdots (2)$$

$$t = -\tau_S \ln\left(\frac{T_{RT} - T_{Sum}}{T_{Max} - T_{Sum}}\right) \quad \cdots\cdots (3)$$

Where h is the heat transfer coefficient; S represents the surface area; T_{max} and T_{surr} mean the max temperature triggered by laser and ambient temperature; Q_{Dis} is the heat loss of light in solvent; I indexes laser intensity (1 $W \cdot cm^{-2}$); A_{808} means the 808 nm absorbance of Ag/Gd@BSA; m_D means the solution mass (1.0 g); C_D means the heat capacity of pure water (4.2 $J \cdot g^{-1}$); t means the cooling time; T_{RT} means the real-time temperature in the cooling period.

In this case, the photothermal conversion efficiency (η) of Ag/Gd_2O_3@BSA aqueous solution (Ag concentration 10 mM) is calculated as 47.4%.

Biosafety assay

Cytotoxicity of Ag/Gd_2O_3@BSA nanoparticles was investigated through a standard MTT assay on C6 cells. The cell viability was acquired by triple independent experiments. Cytotoxicity of Ag/Gd_2O_3@BSA nanoparticles was also investigated through a standard MTT assay on 3T3 cells.

For histopathological analysis, the major organs were harvested from nude mice. Then, the organs were embedded by paraffin, sectioned, and subjected to Hematoxylin and Eosin (HE) staining.

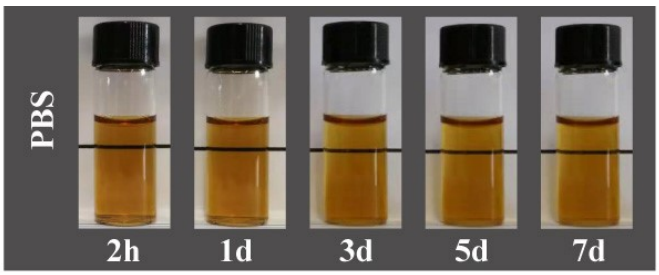

Figure S1 Digital photographs of Ag/Gd@BSA dispersed in PBS at different time point.

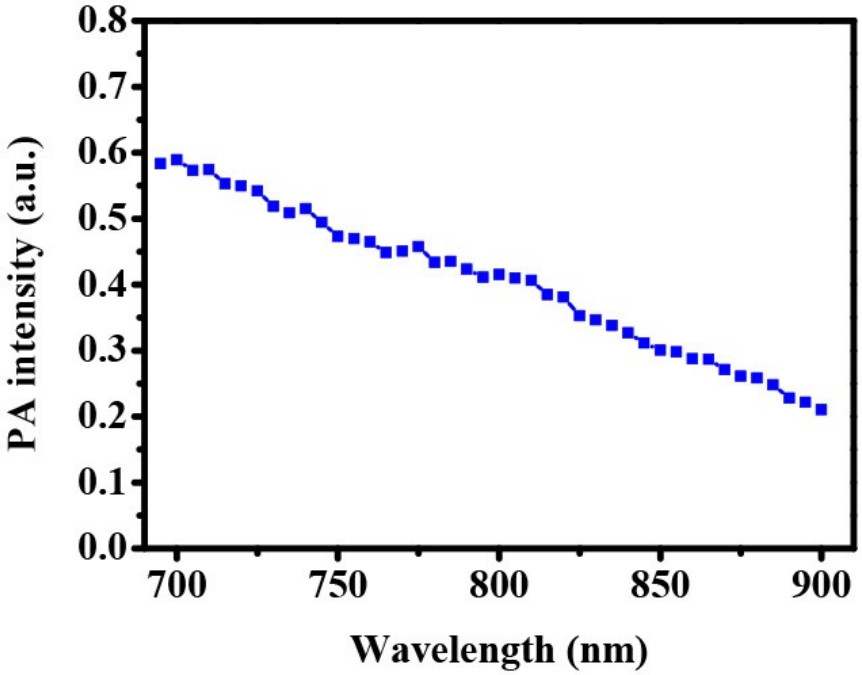

Figure S2 PA signal intensity of Ag/Gd@BSA over a 700-900 nm NIR range.

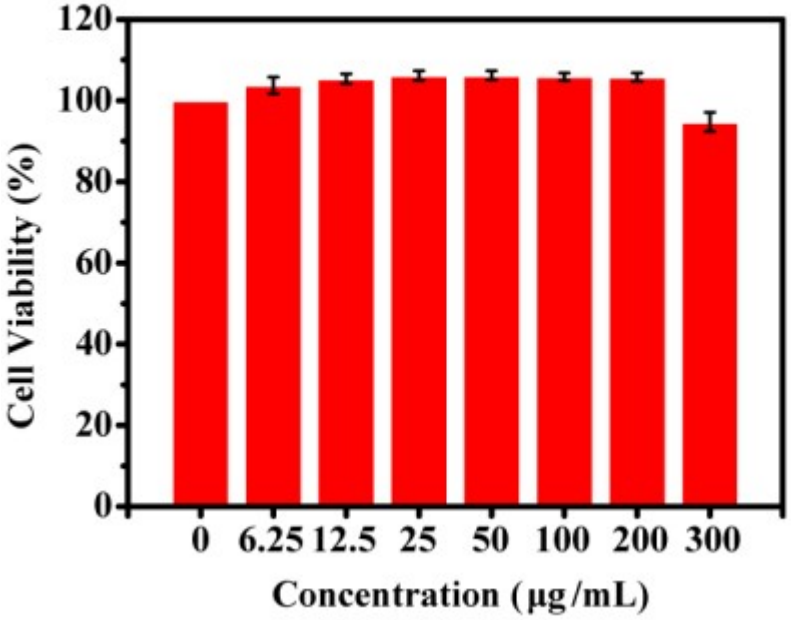

Figure S3 Viability of C6 cells upon incubation with Ag/Gd@BSA for 24 h.

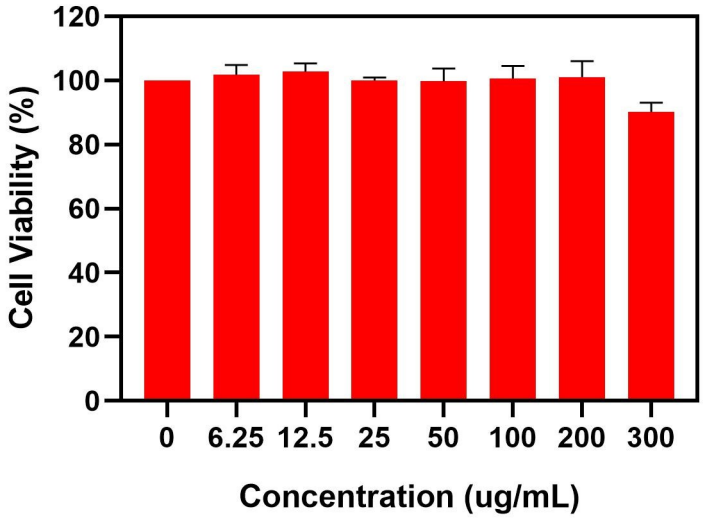

Figure S4 Viability of 3T3 cells upon incubation with Ag/Gd@BSA for 24 h.

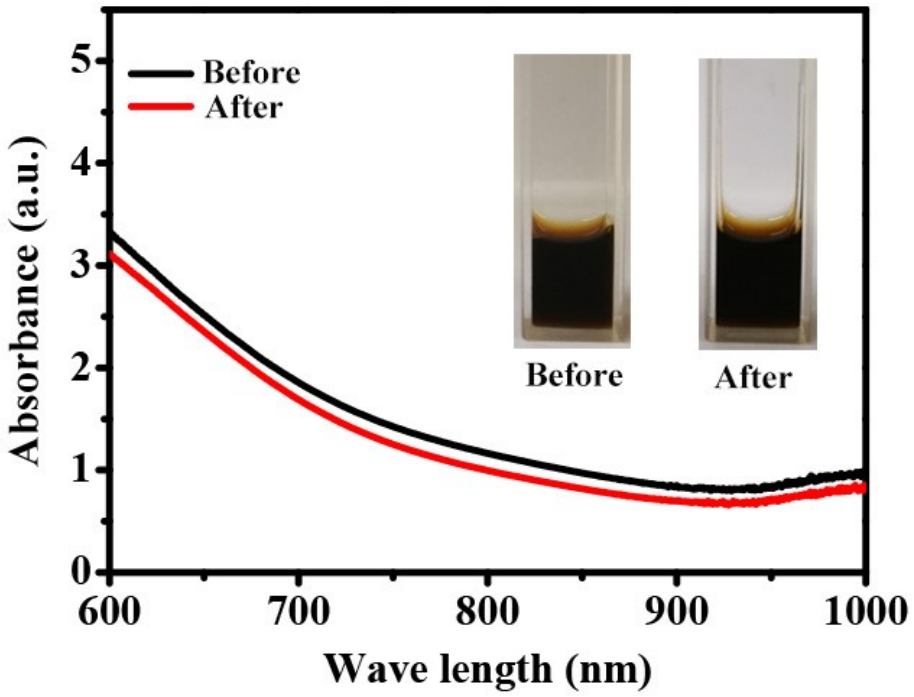

Figure S5 UV-vis spectrum variation before and after irradiation.

References

1. **S. L. Li, Q. Y. Deng, Y. C. Zhang, X. Z. Li, G. H. Wen, X. Cui, Y. P. Wan, Y. W. Huang, J. X. Chen, Z. H. Liu, L. D. Wang and C. S. Lee, *Adv. Mater.*, 2020, 32, 8.**