NIR light response CO nanodonor for the enhanced EPR effect in photothermal cancer treatment

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1. Experimental Details

Chemicals and instruments

N,N-Dimethylformamide (DMF), ethyl alcohol absolute, dimethyl sulfoxide (DMSO), acetone and poly(vinylpyrrolidone) (PVP, MW=30000) were all procured from Sinopharm Chemical Reagent (Shanghai, China). Evans blue, sodium iodide (NaI) and Pd(II) acetylacetonate ($Pd(acac)_2$, 99%) were procured from Aladdin (Shanghai, China). Dulbecco's minimum essential medium (DMEM) and fetal bovine serum (FBS) were procured from Gibico (USA). 3-[4,5- dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) were procured from Amresco (USA). Annexin V-FITC/PI apoptosis detection kit and were procured from Beyotime (Shanghai, China). All cells were from Cell repository of Shanghai Chinese academy of sciences (Shanghai, China). Hematoxylin and eosin (H&E) stain kit was from Yisheng Biological Technology (Shanghai, China). All agents were used without further purification. CO probe was synthesized according to previous report. [1]

Transmission electron microscope (TEM) images were measured with JEM-2100 microscopy (Hitachi, Japan). The UV-Vis-near IR absorption spectra of PdNS-CO was taken on a Cary 5000 spectrophotometer (Varian, USA) in ethanol. Cellular fluorescence images were detected using a Nikon Ti-E-A1R laser scanning confocal microscope (CLSM). Inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700) was used to obtain quantitative measurement of the distribution of PdNS-CO. Fluorescence spectra were recorded on Cary Eclipse fluorescence spectrophotometer (Varian, USA). Dynamic Lighting Scatter (DLS) were detected using MALVERN NANO-ZS90 laser particle size analyzer (Malvern, UK). The Fourier transform infrared (FTIR) spectrum was obtained from a Fourier Transform Infrared Microscope (HYPERION 2000, Germany) by using DMF as the solvent. All of the fluorescence spectra were obtained using a Cary Eclipse fluorometer (Varian, USA).

The preparation of PdNS-CO

In a facile synthesis of palladium nanosheets, Pd(II) acetylacetonate ($Pd(acac)_2$, 50.0 mg), poly(vinylpyrrolidone) (PVP, MW=30000, 160.0 mg) and NaI (75.0 mg) were mixed together with N,N-dimethylformamide (10 mL) and ultrapure water (2 mL) in a pressure vessel. By gentle stirring for 30 minutes, a homogeneous yellow solution was obtained. After being charged with CO (99.99%) to 2.5 bar, the vessel was heated from room temperature to 100°C in 30 min and kept at this temperature for another 4 h with stirring before it was naturally cooled to room temperature. Finally a dark blue solution was resulted, and the deep colloidal products were precipitated by acetone, separated via centrifugation. Then washed three times by an ethanol-acetone (V/V=1/8) mixture and re-dispersed in ethanol for further use.

Photothermal effect measurement

To study the photothermal effect of the PdNS-CO induced by the near infrared SPR absorption, 1 mL saline and 1 mL aqueous solution containing 14 ppm PdNS-CO were irradiated by an 808 nm laser (0.5 W) for 12 min. During the irradiation, thermographic images and the temperature profile were recorded by an infrared camera for every minute. At the meantime, different concentrations (3.5, 7, 14 and 28 ppm) of PdNS-CO but same light source intensity (0.5 W), and different light source intensity (0.25, 0.5, 0.75 and 1 W) doses but same concentration were also recorded. Moreover, recycling performance of PdNS-CO was also studied. 1 mL aqueous solution containing 14 ppm PdNS-CO were irradiated by an 808 nm laser (0.5 W) for 12 min and the temperature

was recoded. After it cooled naturally, the solution was irradiated for another 12 min. The study was ended until four cycles. During all of the photothermal effect measurement, the laser was 15 cm upon the solutions.

Photothermal conversion efficiency

To measure the photothermal conversion efficiency (η) of PdNS-CO (14 ppm), its temperature change was recorded as a function of increased irradiation time by 808 nm laser (0.75 W/cm^2) until the PdNS-CO aqueous solution reached a steady state temperature. Its η value was calculated by the following formula: [2]

$$\eta = \frac{hs(T_{Max} - T_{Surr}) - Q_s}{I(1-10^{-A_{808}})} \times 100\%$$

Where h is heat transfer coefficient, s is the surface area of the container. The maximum steady temperature (T_{Max}) was 80 °C and T_{surr} =23.5 °C was the environmental temperature. Q_s is heat dissipated from the light absorbed by the container itself, which was determined independently to be 3.5 mW using a container containing pure water. [3] The incident laser power (I) is 0.75 W/cm^2 and the absorbance of the PdNS-CO at 808 nm (A_{808}) is 2.619.

A dimensionless parameter θ is calculated as followed:

$$\theta = \frac{T - T_{Surr}}{T_{Max} - T_{Surr}}$$

A sample system time constant τ_s can be calculated as

$$t = -\tau_s \ln(\theta)$$

According to Figure S5, τ_s was determined and calculated to be 13 min.

$$hs = \frac{m_D C_D}{\tau_s}$$

In addition, m_D is 1 g and C_D is 4.186 J/g °C. Thus, hs is calculated to be 5.37 mW/°C. Thus, the η value of the PdNS-CO was 40.1%.

CO release detection by using a CO fluorescent probe

In order to detection the existence of CO, a colorimetric and fluorescent probe system was used, which is based on the well-known Pd^0-mediated Tsuji–Trost reaction. To construct the CO probe system, two molecules was composed, an allyl chloroformate functionalized 3-benzothiazolyl-7-hydroxycoumarin (BTHC-CO) as the CO signalling molecule and $PdCl_2$ as an additive. To be specific, 10 mM BTHC-CO and 20 mM $PdCl_2$ was added to PBS buffer (pH=7.4, with 10% DMSO, v/v), the CO fluorescence detection system was finally completed.

For a typical CO fluorescence detection study, 3 mL solution containing BTHC-CO (5 μM) and $PdCl_2$ (10 μM) in PBS buffer (pH=7.4, with 10% DMSO, v/v) was placed in a quartz cuvette, and the PdNS-CO dispersed in ethanol (4.67 μg) were added. Then, the cuvette was exposed to an 808 nm laser for 1 min (1 W).And the fluorescent spectra were recorded upon every minute. For fluorescence measurement, λ_{ex} = 462 nm, slit width: d_{ex} = 5 nm, d_{em} = 5 nm.

In vitro cytotoxicity and PTT anticancer studies

To study the in vitro dark toxicity of PdNS-CO, A549 cells were grown in DMEM containing 10% inactivated FBS and 1% penicillin streptomycin. The same culture media were used in subsequent biological studies and all cells were maintained in a humidified incubator at 37 °C with 5% CO_2. PdNS-CO were washed by an ethanol-acetone mixture and re-dispersed in DMEM for next use. Healthy A549 cells were seeded in DMEM containing 10% FBS, were grown in a 5% CO_2 atmosphere at 37 °C in 96 well plates. And the cell density was 5 × 10^6 cells/well. After incubation for 24 h, the cells were treated with PdNS-CO in various concentrations (0, 20, 40, 60, 8 and 100 μg/mL). After incubation for another 24 h, cell viability was measured by the standard MTT assay.

To study the in vitro PTT activity of PdNS-CO, PdNS-CO were washed by an ethanol-acetone mixture and re-dispersed in DMEM for next use. A549 cells were seeded into 96 well plates at a density of 5×10^6 cells/well and incubated for 24 h in incubator. Subsequently, the cells were treated with PdNS-CO in various concentrations (0, 5, 10, 15 20 μg/mL). After incubation for 4 h, cells were exposed to a 0.25 W 808 nm laser for 12 min. And the no treatment as the control. After incubation for another 24 h, cell viability was detected by the standard MTT assay.

In vitro photothermal triggered CO release from PdNS-CO

A549 cells were seeded into 6 well plates at a density of 5×10^6 cells/well and incubated for 24 h in an incubator. Subsequently, the cells were treated with PdNS-CO (20 μg/mL) for 4 h. Afterwards, the CO prober was added ([CO probe] = 5 μM; [$PdCl_2$] = 10 μM). 1 h later, the cells were irradiated by an 808 nm laser (0.25 W) for various time and the in vitro fluorescence was obtained using CLMS immediately.

In vivo photothermal therapy

Female BALB/c mice (3-4 weeks old) were purchased from the Comparative Medicine Centre of Yangzhou University. All animal experiments were carried out in accordance with regulations of the Nanjing Committee for the Use and Care of Laboratory Animals, and all experimental protocols were approved by the Animal Ethics Committee of Nanjing Normal University. Female 4T1 tumor-bearing BALB/c mice were divided into four treatment groups (control, 808 nm laser irradiation only, only PdNS-CO-treated, PdNS-CO-treated with 808 nm laser irradiation) to test the effect of PdNS-CO phototherapy in vivo. The fresh PdNS-CO were injected to the mice via tail veins at the dose of 10 mg/kg at day 1, 3, 5, 7, 9, 11 and 13. The tumor was irradiated by an 808 nm laser (0.25 W, 8 min) post the injection of drugs for 6 h. The body weight and the tumor volume were recorded for 14 days. The tumor size of each group was measured everyday using a skinfold caliper, and tumor volume was calculated using the following equation: V (tumor volume) =a (the maximum diameter of tumor) × b (the minimum diameter of tumor) × b/2. The relative tumor volume was calculated as V/V_0, which V_0 and V stand for the tumor volume on the first day and on the day of treatment. Finally, the mice were sacrificed and their tumors and major organs (liver, kidneys, spleen, heart, brain, and lung) were taken out for the assessment using H&E staining for histological analysis.

ICP-MS was used to obtain quantitative measurement of the accumulation of PdNS-CO. Tumours from above mice were weighted and completely digested by 2 mL of aqua regia at room temperature overnight. Subsequently, the solution was diluted to 1 L using 0.5% HCl and 2% HNO_3. Samples were filtered to remove any undigested residues, and then the Pd content was analysed by ICP-MS. Quantification was carried out by external five-point calibration with an internal standard correction and the percentage of injected Pd dose per gram of tissue (mg/g) was calculated.

Determination CO from PdNS-CO up-regulating blood vascular permeability in normal mouse skin and tumour bearing mice.

Evans blue dye at a dose of 10 mg/kg was infused through the tail vein to four mice. After 10 min test samples (100 μL each) were injected intradermally into the dorsal skin of mice (the injected concentration of PdNS-CO is 3.5 μg/mL). After that, 808 nm (0.25 W) laser was irradiated at the injection site for 8 min. 2 h later, the mice were sacrificed and their blood vascular permeability was imaged. And the amount of extravasated Evans blue in the skin where were was quantified using their formamide extraction (incubated at 37 °C for 24 h).

Female 4T1 tumor-bearing mice were divided into four treatment groups (control, 808 nm laser irradiation only, PdNS, PdNS-CO, PdNS-CO + 808 nm laser irradiation [PdNS-CO] = 10 mg/kg). Evans blue (10 mg/kg) was tail vein injected into the mice. 2 h later, the mice were treated by various drugs through tail veins. The 808 nm laser (0.25 W) was irradiated at the tumor tissue after another 6 h. 2 h later, the mice were sacrificed and the tumors were harvest. And the amount of extravasated Evans Blue in the tumours was quantified after extraction with formamide.

2. Results and discussions

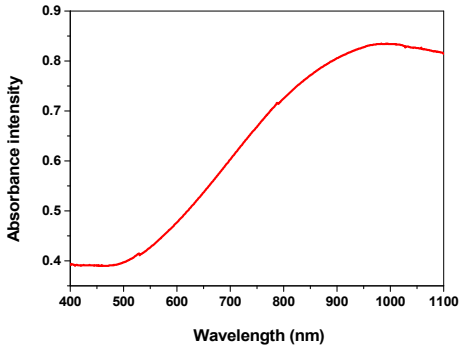

Figure S1. Absorption spectra of PdNS-CO.

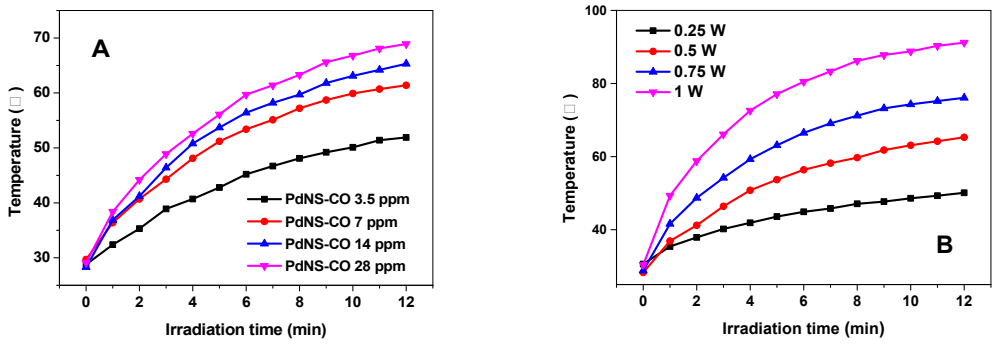

Figure S2. The materials dose (A) and light dose (B) dependent temperature-NIR light irradiation time plots of PdNS-CO.

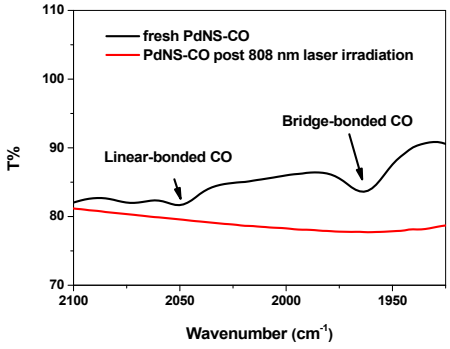

Figure S3. FTIR spectrum of the freshly prepared and 808 nm laser (1 W, 5 min) irradiated PdNS-CO dispersed in DMF.

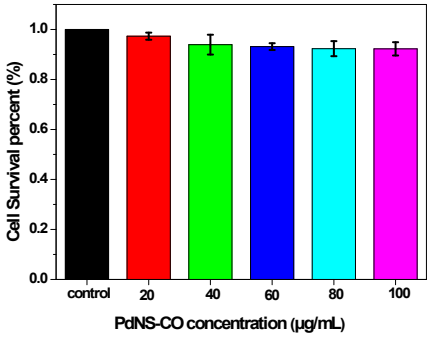

Figure S4. In vitro dark toxicity of PdNS-CO.

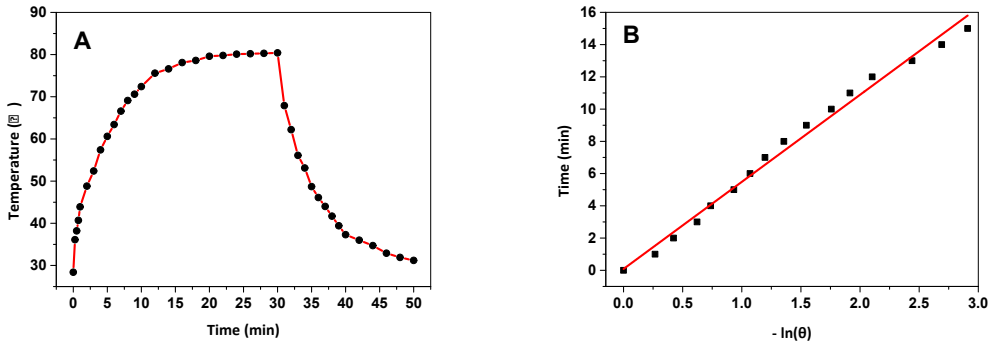

Figure S5. (A) The temporal temperature variation of 1 mL aqueous solution of PdNS-CO. The amount of Pd is 14 μg. The solution was irradiated for 30 min using a 0.75 W cm^2 808 nm laser, and cooled to room temperature under ambient environment. (B) Time constant for heat transfer from the system is determined to be τ_s=13 min by applying the linear time data from the cooling period (after 30 min) versus negative natural logarithm of driving force temperature.

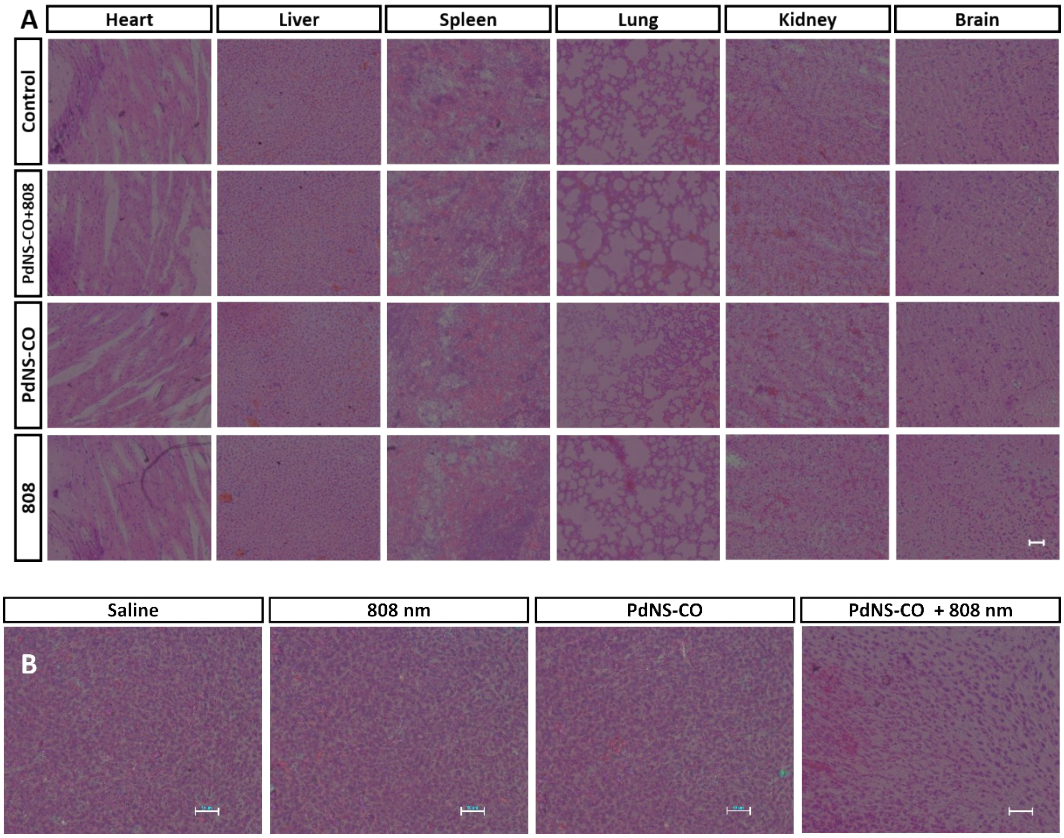

Figure S6. (A) H&E stained histological sections of heart, liver, spleen, lung, brain and kidney from the mice of above groups (Bar = 50 μm) (B) H&E stained tumour sections from the mice of above groups.

Table S1. The result of ICP-MS analysis.

Tumour tissue source	Test result	Pd content in tumour tissue
PdNS-CO treated mice	< 0.1 mg/L	< 0.5 mg/g
PdNS-CO + 808 nm treated mice	0.12 mg/L	0.65 mg/g

References

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