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Prussian blue nanoparticles operate as new generation of photothermal ablation agent for cancer therapy

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1. Reagents

Hydrogen tetra-chloroaurate (III) hydrate ($HAuCl_4 \cdot 4H_2O$), cetyltrimethylammonium bromide (CATB), silver nitrate ($AgNO_3$), and ascorbic acid were obtained from Sinepharm Chemical Reagent CO., Ltd. (China). Unless otherwise stated, reagents such as $FeCl_3 \cdot 6H_2O$, $K_4[Fe(CN)_6] \cdot 3H_2O$ and citric acid were of analytical reagent grade and used as received. Aqueous solutions were prepared with deionized water (18.2 MΩ cm) from a Milli-Q purification system.

2. Instrumentation

UV-vis absorption spectra were recorded on a Cary 4000 UV-vis spectrophotometer (USA) with a quartz cuvette of 1 cm optical path length. Transmission electron microscopy (TEM) was performed with a Tecnai G^2 20 TWIN Transmission Electron Microscope (FEI Co., USA). Photographs were taken by a Canon 600D digital camera (Japan). NIR laser irradiation was performed with a continuous-wave diode NIR laser (T808D2W) with a center wavelength of 808±10 nm and output power of 2 W (Xi'an Minghui Optoelectronic Technology Co., China). The temperature of the solutions was measured by a digital thermometer with a thermocouple probe. The concentration of CTAB-capped Au nanorods was determined with a Perkin-Elmer model 3300 XL inductively coupled plasma-optical emission spectroscope.

3. Experimental section

3.1 Preparation of Prussian blue nanoparticles (PB NPs)

PB NPs were prepared according to the previous literature.[1] Typically, 0.5 mmol citric acid was added into 20 mL $FeCl_3$ aqueous solution (1.0 mM) under stirring at 60 °C. Then, 20 mL $K_4[Fe(CN)_6]$ aqueous solution (1.0 mM) containing the same amount of citric acid was added dropwise into the above solution under stirring at 60 °C. A clear bright blue dispersion formed immediately during the mixing process, which was allowed to cool to room temperature with the stirring continued for another 30 min at room temperature.

To collect PB NPs, equal volume of acetone was mixed with the dispersion. The mixture was then centrifuged at 12500 rpm for 60 min to collect the PB NPs precipitation. The precipitation was washed for 3 times through the above procedure. Finally, the obtained PB NPs precipitation was dried under vacuum at 50 °C for 12 h.

3.2 Preparation of CTAB-capped Au nanorods

CTAB-capped Au nanorods were prepared using the conventional silver ion-assisted seed-mediated method according to previous literature.[2] The Au seed solution was first synthesized by adding 0.25 mL $HAuCl_4$ (0.01 M) into 10 mL CTAB solution (0.1 M) with gentle mixing. Then, 0.6 mL ice-cold $NaBH_4$ solution (0.01 M) was added into the mixture solution, followed by rapid inversion for 2 min. The Au seed solution was kept at room temperature for 2 h before use. To prepare the Au growth solution, 2.0 mL $HAuCl_4$ (0.01 M) and 0.4 mL $AgNO_3$ (0.01 M) were mixed with 40 mL CTAB solution (0.1 M), followed by the addition of 0.32 mL ascorbic acid (0.1 M).

Finally, 0.096 mL of the Au seed solution was added into the Au growth solution. The mixture was gently mixed for 10 s and kept undisturbed at room temperature for 6 h. After discarding free CTAB in the mixture with centrifugation and washing with water for 3 times, the obtained Au nanorods were re-dispersed in a certain volume of deionized water. The concentration of CTAB-capped Au nanorods was determined with a Perkin-Elmer model 3300 XL inductively coupled plasma-optical emission spectroscope.

3.3 Cell culture

HeLa cells were cultured in RPMI 1640 medium (Gibco) containing 10% fetal calf serum, 100 μg/mL penicillin (Gibco) and 100 μg/mL streptomycin (Gibco) in an incubator at 37 °C (5% CO_2).

3.4 Measurement of molar extinction coefficient

Molar extinction coefficients ε (λ) of PB NPs and CTAB-capped Au nanorods were calculated according to the equation:[3]

$$\varepsilon = (AV_{NC}\rho N_A)/(LC_{wt})$$

where ε is the molar extinction coefficient, A is the absorbance at a wavelength λ, V_{NC} (in cm^3) is the average volume of the materials, ρ is the density of the materials assuming that it is the same as the bulk materials, N_A is Avogadro's constant, L is the path length (1 cm), and C_{wt} (in g/L) is the weight concentration of the materials.

Based on the TEM observation, the average volume (V_{NC}) of individual PB nanoparticle (42 nm) and CTAB-capped Au nanorod (r=7.5 nm, L=60 nm) was assumed to be 7.4×10^{-17} cm^3 and 1.06×10^{-17} cm^3, respectively. The density (ρ) of PB

NPs and Au nanorods was 1.8 g/cm^3 and 19.3 g/cm^3, respectively. UV-vis spectroscopy of PB NPs (50 ppm) and CTAB-capped Au nanorods (16 ppm) were tested to achieve the absorbance for the calculation of molar extinction coefficients in the range from 500 nm to 900 nm. Thus, the molar extinction coefficients of PB NPs and CTAB-capped Au nanorods at 808 nm were calculated to be 1.09×10^9 M^{-1} cm^{-1} and 5.24×10^9 M^{-1} cm^{-1}, respectively.

3.5 Measurement of photothermal effect

To study the photothermal effect of PB NPs and CTAB-capped Au nanorods, 3 mL of the aqueous dispersions of the materials with different concentrations in quartz cuvettes (total volume of 4.0 mL) were irradiated by a continuous-wave diode NIR laser with a center wavelength of 808±10 nm and output power of 2 W for 10 min. The temperature of the solutions was measured by a digital thermometer with a thermocouple probe every 10 s.

To study the photothermal stability of PB NPs and CTAB-capped Au nanorods, the PB NPs (50 ppm) and CTAB-capped Au nanorods (16 ppm) aqueous dispersions were continuously irradiated with the 808 nm laser. Typically, the dispersions were first irradiated for 10 min, followed by the cooling of the temperature to the initial value at room temperature. Then, another irradiation cycle was carried out.

3.6 Photothermal effect of PB NPs to HeLa cells

Photothermal cell toxicity of PB NPs was evaluated with HeLa cells (human cervical carcinoma cell line). HeLa cells were first incubated in 6-well plates (2 mL and 5×10^5 cells per well) at 37 ^{o}C for 24 h. Then, the cell culture medium was replaced with 2 mL PB NPs RPMI 1640 medium dispersion (25 ppm). After incubation for another 4 h, cells were exposed to an 808 nm laser with the output of 2 W for 10 min. Finally, the HeLa cells were conventionally stained with calcein AM (calcein acetoxymethyl ester) to verify the photohyperthermic effect of PB NPs on cancer cells under fluorescent microscope.

Conventional MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was also used to study the photothermal cell toxicity of PB NPs. HeLa cells (10^4 cells per well) were first incubated in 96-well plates at 37 °C for 24 h. Then, the cell culture medium was replaced with different dosage of PB NPs RPMI 1640 medium dispersions (0.2 mL per well, from 1.0 ppm to 16.0 ppm). After incubation for another 4 h, cells were exposed to an 808 nm laser with the output of 2 W for 10 min. Finally, the cell viabilities were determined by MTT assay. Results were shown as mean ± standard deviation (SD) (n = 3).

4. Results and discussion

4.1 Photothermal stability of PB NPs and CTAB-capped Au nanorods

To study the photothermal stability, the PB NPs and CTAB-capped Au nanorods aqueous dispersions were continuously exposed to an 808 nm laser. The dispersions were first irradiated for 10 min, followed by the cooling of the temperature to the initial value at room temperature. Then, another irradiation cycle was carried out. 4 cycles were totally carried out. Changes of the PB NPs and CTAB-capped Au nanorods before and after the above 4 cycles were studied.

Colorimetric observation and UV-vis spectroscopy were used to study the photothermal stability. Fig. S1 shows the photographs of PB NPs and CTAB-capped Au nanorods aqueous dispersions before and after the irradiation for 4 cycles. As was can be seen, CTAB-capped Au nanorods dispersion showed obvious change in color after the irradiation process, indicating relatively poor photothermal stability of CTAB-capped Au nanorods. The color of CTAB-capped Au nanorods changed from coffee brown to deep purple which was the typical color of aggregated Au nanorods and nanoparticles. The result indicated that CTAB-capped Au nanorods might aggregate during the irradiation of 808 nm laser. In addition, it had been reported that Au nanorods were not stable and could be melted under the irradiation of 808 nm laser.[4] Significantly, PB NPs showed no apparent change in color (blue) after the irradiation process, indicating good photothermal stability of PB NPs, which was important for the reuse of PB NPs in practical applications.

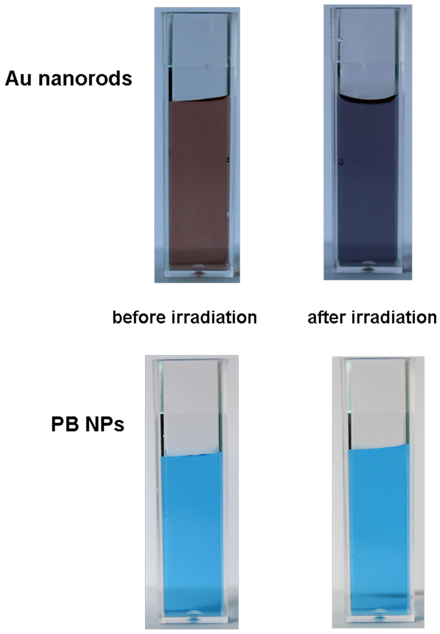

Fig. S1 Photographs of PB NPs (50 ppm) and CTAB-capped Au nanorods (50 ppm) aqueous dispersions before and after the irradiation for 4 cycles.

UV-vis spectroscopy was further used to confirm the photothermal stability. Fig. S2 shows the UV-vis spectroscopy of PB NPs and CTAB-capped Au nanorods aqueous dispersions before and after the irradiation for 4 cycles. As was can be seen, CTAB-capped Au nanorods showed obvious change in UV-vis spectroscopy after the irradiation process, indicating relatively poor photothermal stability of CTAB-capped Au nanorods. The surface plasmon peak at 780 nm decreased dramatically after the irradiation process, indicating the damage of CTAB-capped Au nanorods under NIR irradiation. The surface plasmon peak of CTAB-capped Au nanorods at 500 nm red-shifted to about 530 nm, which was the typical plasmon peak of Au nanoparticles. Significantly, the PB NPs showed much more stable UV-vis spectroscopy after the irradiation process, further demonstrating good photothermal stability of PB NPs.

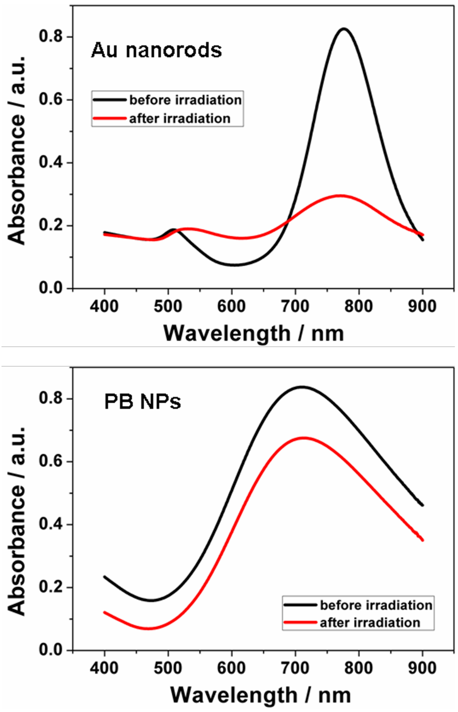

Fig. S2 UV-vis spectroscopy of PB NPs (50 ppm) and CTAB-capped Au nanorods (16 ppm) aqueous dispersions before and after the irradiation for 4 cycles.

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