Supporting Information for

Silica Coating Improves the Efficacy of Pd Nanosheets for Photothermal Therapy of Cancer Cells Using Near Infrared Laser

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Experimental Details

Reagents: $Pd(acac)_2$ (99%) and tetraethoxysilane (TEOS) were purchased from Alfa Aesar. PVP (MW=30000, AR), methylamine, ethanol, N,N'-dimethylformamide (DMF) and tetrabutylammonium bromide (TBAB) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Human hepatoma cells (QGY-7703) was purchased from cell storeroom of Chinese Academy of Science. RPMI 1640 cell culture medium, bovine serum albumin (BSA) and Penicillin-Streptomycin compound were purchased from Hyclone Laboratories Inc. MTT and FITC was purchased from Sigma. The water used in all experiments was ultrapure. All reagents were used as received without further purification.

Synthesis of 41 nm hexagonal Pd nanoplates: Pd(II) acetylacetonate ($Pd(acac)_2$, 50.0 mg), poly(vinylpyrrolidone) (PVP, MW=30000, 160.0 mg) and TBAB (161 mg) were mixed together with N,N-dimethylformamide (10 mL) and water (2 mL). The resulting homogeneous yellow solution was transferred to a glass pressure vessel. The vessel was then charged with CO to 1 bar and heated at 100 °C for 3.0 h before it was cooled to room temperature. The dark blue products were precipitated by acetone, separated via centrifugation and further purified by an ethanol-acetone mixture.

Synthesis of SPNS: 0.5 mL solution of purified Pd nanosheets (1.45 mg/mL, side length 41 nm) was centrifuged and redispersed in 4 mL of water. The colloid was added to 32 mL of ethanolic solution containing 0.05 mL of TEOS, 0.05 mL of methylamine (40 wt %) with stirring. Subsequently, it was allowed to stir for 8 h before being centrifuged and redispersed into ethanol.

Photothermal Effect Measurement of SPNS: To study the photothermal effect induced by the NIR SPR absorption, 1mL aqueous solutions containing different concentration (0, 7.5, 15 and 30 ppm) of SPNS were irradiated by a NIR laser (808nm, 1W) for 10 min. The temperatures of the solutions were monitored by a thermocouple microprobe (φ = 0.5 mm) submerged in the solution in a 1-cm square cuvette. The probe was placed at such a position that the direct irradiation of the laser on the probe was avoided. The tip of the thermocouple was ~5 mm above the bottom of the cuvette.

Synthesis of FSPNS: First, for FITC-APTS conjugate synthesis, 1 mg FITC and 2.2 μL 3-(aminopropyl)triethoxysilane (APTS) were dissolved in 10 mL of absolute ethanol. The reactants were allowed to react in dark for 12 hours under stirring. Then 0.5 mL solution of purified Pd nanosheets (1.45 mg/mL, side length 41nm) was centrifuged and redispersed in 4 mL of water. The colloid was added to 32 mL of ethanolic solution containing 0.05 mL of TEOS, 0.05 mL of methylamine (40 wt %) and 60 μL of FITC-APTS with stirring. Subsequently, it was allowed to stir for 8 h before being centrifuged and redispersed into ethanol.

Surface modification of FSPNS with aminopropyltriethoxysilane (APTES): 0.5 mL solution of FSPNS (1.41 mg/mL) was mixed with 30 μL of APTES and 10 μL of water. The mixture was stirred overnight before centrifugation. The particles were then washed with water at least 5 times.

Quantitative analysis of Pd uptake by cells: The human hepatoma cells (QGY-7703) were incubated separately with media containing the above four different Pd NS samples (i.e., original Pd NS, PEI-modified Pd NS, SPNS and SPNS-NH2 in the same concentration (107 g/mL Pd, total volume 0.5 mL). After 12-hr incubation, the media were removed. The cells were carefully washed for 5 times by PBS before being digested by aqua regia and diluted by ultrapure water for the ICP analysis.

Detection of intracellular uptake of the FSPNS-NH_2 by living cells using fluorescence microscopy or flow cytometry: For fluorescent microscopy analysis, human hepatoma cells were plated in a 24-well plate with a density of ~0.5×10^4 cells/well. After incubation in fresh medium for 24 h, cells were incubated with 0.5 mL of the medium containing 0.27mg/mL

FSPNS and FSPNS-NH_2 for 12 h. The cell medium was removed, and the cells were washed before PBS buffer solution was added. The samples were then analyzed by fluorescence microscope. For flow cytometry studies, the cells were incubated with 0.5 mL of the medium containing 0.54 mg/mL FSPNS and FSPNS-NH_2 for 12 h in a density of ~0.5×10^5 cells/well. The adherent cells were washed and detached from the plate by treatment with trypsin-EDTA. The cells were then resuspended in PBS buffer solution for flow cytometry.

Apoptosis assay: Human healthy liver cells (QSG-7701) were cultured in RPMI 1640 medium in 24-well plates. The cell density was 1×10^5 cells/well. After being seeded for 18 h, the media were replaced by culture media containing different concentration of the original Pd NS (side length 41nm) and SPNS-NH_2. The incubations were carried out at 37 °C in 5% CO_2 atmosphere for 48 h. After incubation for 48 h, cell viabilities were measured by standard MTT assay, a colorimetric assay based on the ability of viable cells to reduce 3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide.

Photothermal killing of cancer cells incubated with various Pd nanosheets. After incubation with various Pd nanosheets (55 μg/mL Pd) for 12 hrs, the cells were washed twice with PBS solutions and then exposed to a 1.4 W/cm^2 808-nm laser for various periods to induce photothermal cell damage. To identify the cell viability, the dead cells were stained with Trypan Blue. Cell viabilities were also measured by the standard MTT assay. Before the MTT assay of the viability, QGY-7703 cells were incubated for 12 hrs with original Pd NS, PEI-modified Pd NS, SPNS and SPNS-NH_2 solutions containing 40 μg/mL Pd, washed twice with PBS and then subjected to irradiation of NIR laser (1.4 W/cm^2, 808nm) for different periods.

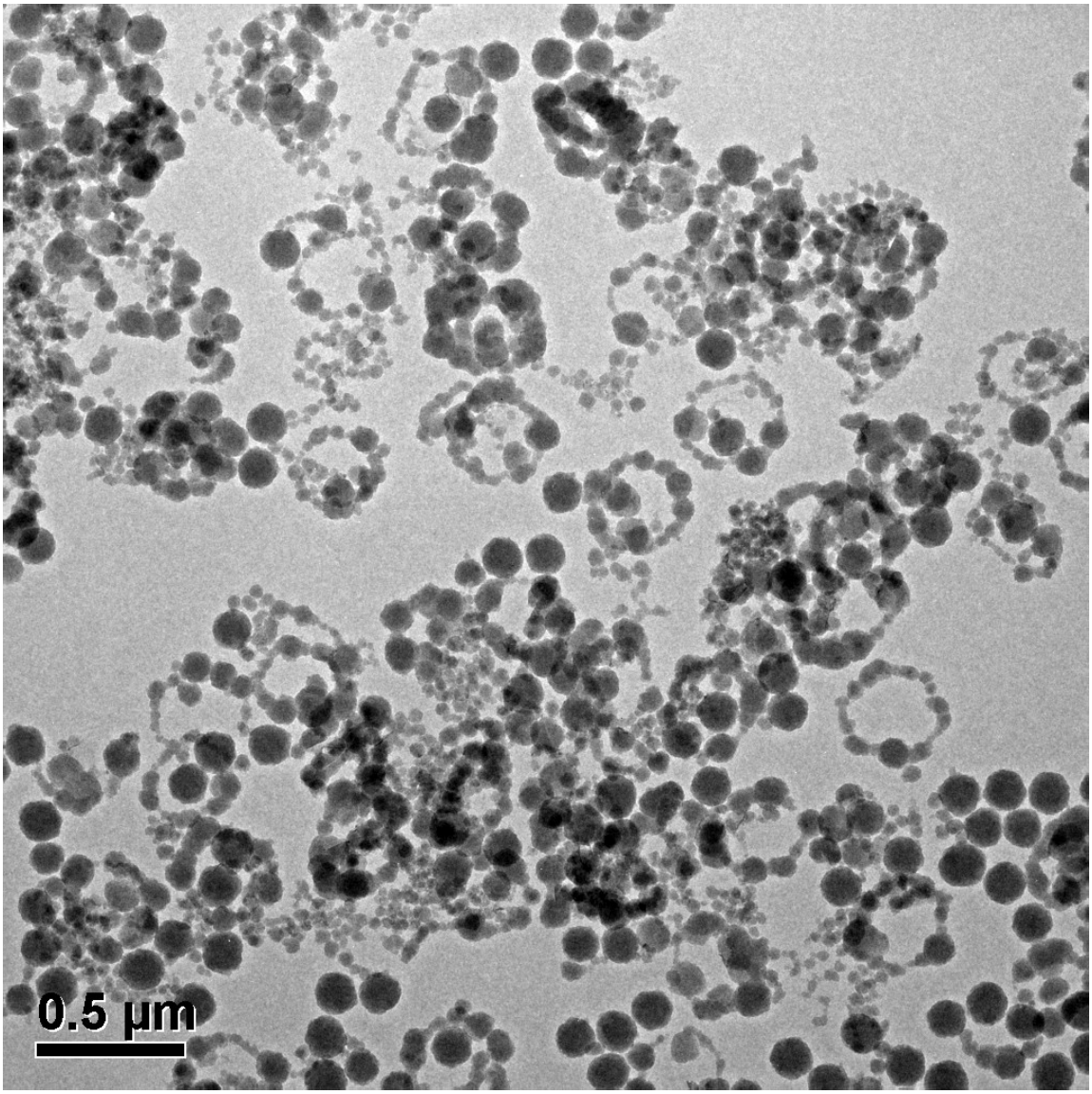

Figure S1. Representative TEM image of the product collected from the reactions in which the SiO_2 coating on Pd nanosheets was catalyzed by ammonia. Detailed conditions: 0.5 mL solution of purified Pd nanosheets (1.45 mg/mL, side length 41 nm) was centrifuged and redispersed in 4 mL of water. The colloid was added to 32 mL of ethanolic solution containing 0.05 mL of TEOS, 0.05 mL of NH_3 (25-28 wt %) with stirring. Subsequently, it was allowed to stir for 8 h before being centrifuged and redispersed into ethanol.

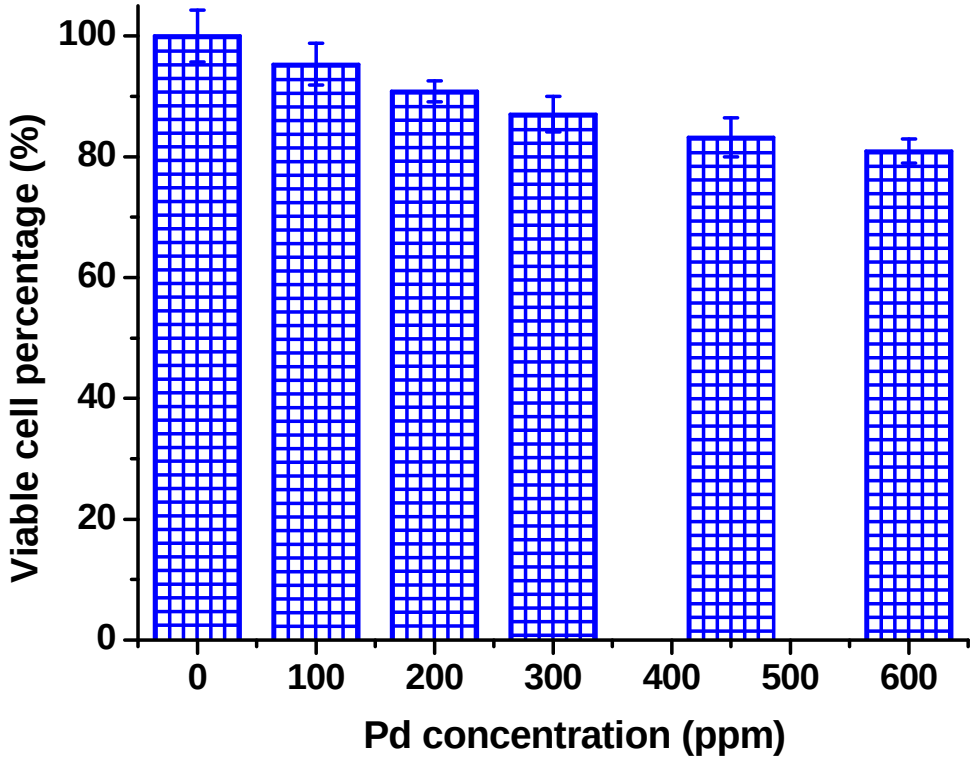

Figure S2. Viability of healthy liver cells incubated for 48 hr with different-concentrations of Pd nanosheets. The cell viabilities were measured by standard MTT assay.

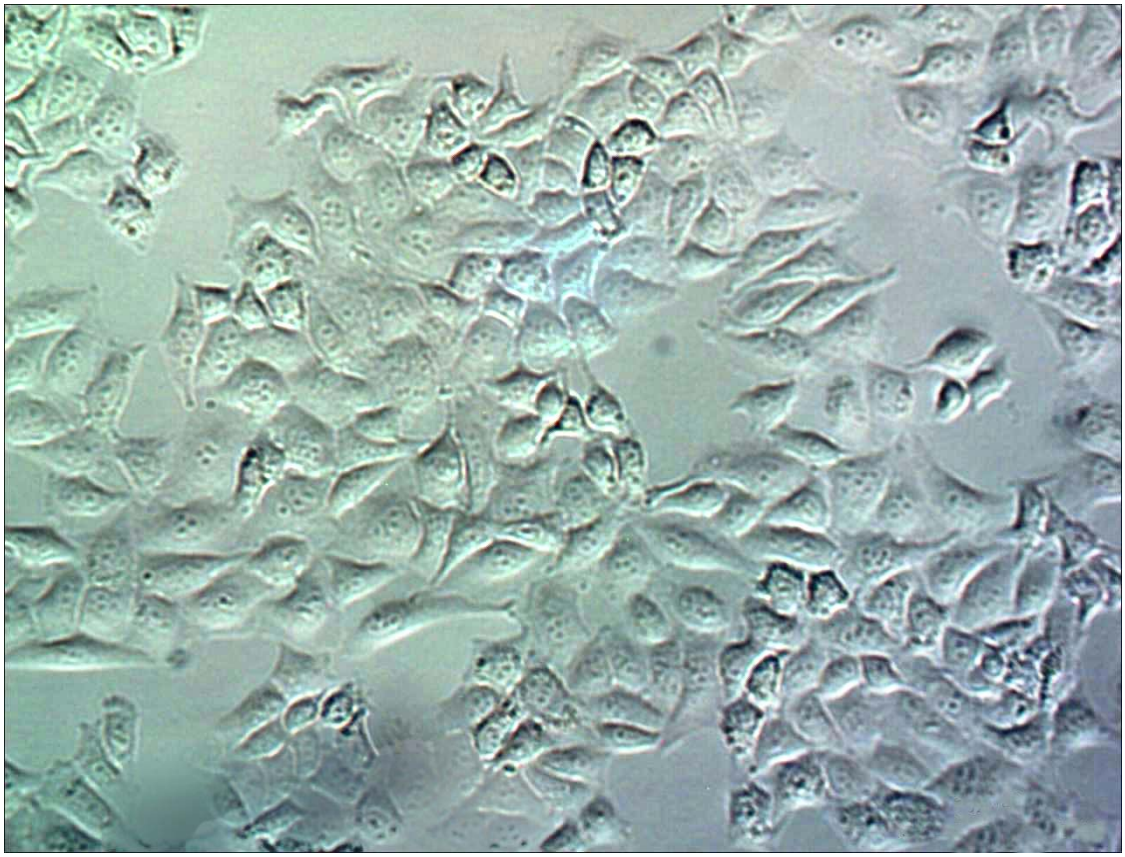

Figure S3. Micrographs of liver cancer cells after 12-hr incubation with 0.5 mL solution of SPNS $–NH_2$ containing 1000 μg ($Pd+SiO_2$)/mL. Trypan blue was used to stain the dead cells before microscopic evaluations.

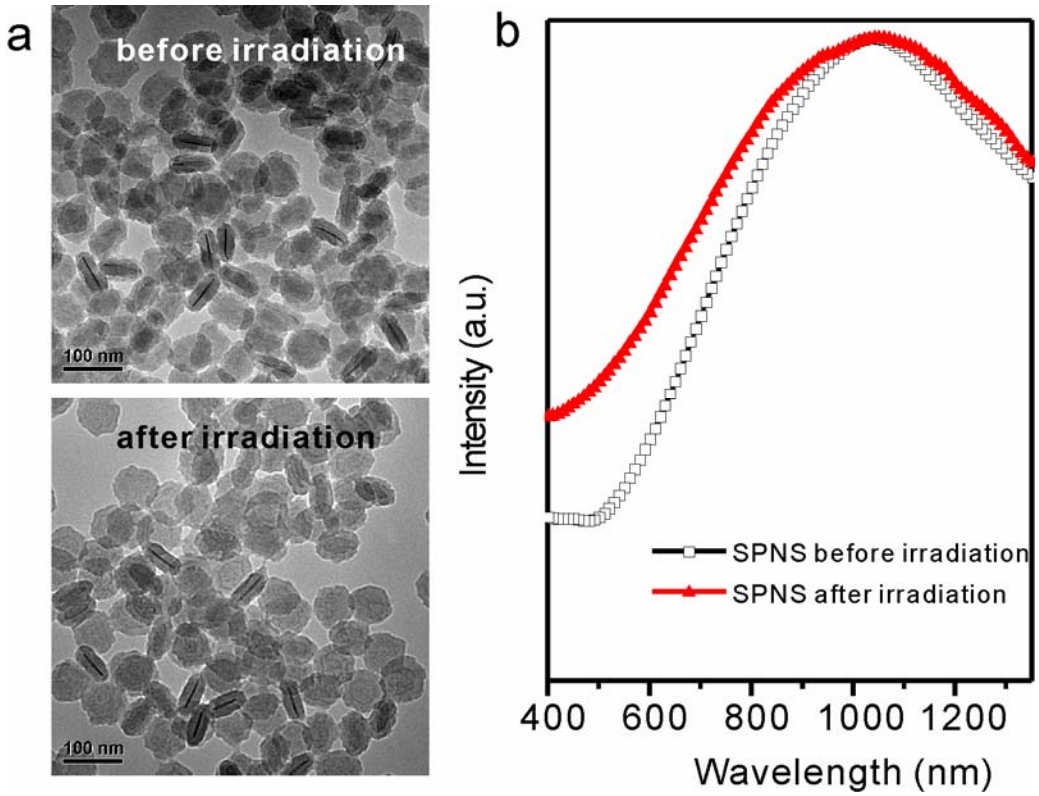

Figure S4. TEM images and UV-vis spectra of SPNP (1 mL, 30 ppm) before and after NIR irradiation (808 nm, 1 W for 10-min).