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Functionalized Ultrathin Palladium Nanosheets as Patches for HepG2 Cancer Cells

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Chemicals and Materials

Palladium acetylacetonate ($Pd(acac)_2$, 98%, STREM Chemicals); acetic acid (AA, Glacial, J.T. Becker); acetone (≥99.5%, ACS Reagent, Macron Avantor); 3-mercaptopropionic acid (MPA, ≥ 99%, Sigma-Aldrich); cysteamine (CA, 95%, Sigma-Aldrich); hydroxyl and amine functionalized SiO_2 sphere (silica-NH_2, 0.54 μm in diameter, Bangs Laboratories Inc.); carbon monoxide (CO, 99.998%, S. J. Smith); phosphate buffered saline without calcium and magnesium (PBS 1×, Corning Cellgro); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (ATCC)

The cell media used in this work was formulated as the following: 1× DMEM (Dulbecco's Modification of Eagle's Medium with 4.5 g/L glucose and L-glutamine without sodium pyruvate, Corning Cellgro), 10% FBS (Fetal Bovin Srum, Gibco) and 1% Pen-Strep (100× Penicillin Streptomycin Solution, Corning).

Synthesis of Palladium Nanosheets

In a typical synthesis, 10 mg of $Pd(acac)_2$ was dissolved in 10 mL of AA. The synthesis mixture was preheated in a 27 ºC water bath, and then bubbled with CO gas for 10 min before the glass vial was capped, sealed with parafilm, and allowed to react in the water bath for another 24 h.

Functionalization of Palladium Nanosheets with 3-Mercaptopropionc Acid (MPA)

The as-synthesized Pd nanosheets were washed with 10 mL of acetone, sonicated (Branson 2510) for 1 min and centrifuged at 6000 rpm for two times. MPA (25 μL) was dissolved in 10 mL

of DI water to disperse the palladium nanosheets in the sonication bath for about 2-3 min. The MPA-functionalized Pd nanosheets were then washed with DI water for five times to remove excess MPA prior to use. The final products redispersed in 5 mL of water.

Functionalizing the Palladium Nanosheets with Cysteamine (CA)

The acetone-washed Pd nanosheets were mixed with 100 mg of CA dissolved in 10 mL of DI water through sonication. About 0.3 mL of acetic acid was added into the solution to provide good colloidal stability. To remove the excess CA and acetic acid, the samples were washed with 5 mL of DI water and centrifuged at 6000 rpm for 10 min. This cleaning process was repeated four more times. The final products were redispersed in 5 mL of water for further use.

Surface Coating of Functionalized Silica Sphere with the Functionalized Pd Nanosheets

In a typical procedure, 3 mg of H_2N-SiO_2 spheres were dispersed in 5 mL of DI water, and the mixture was sonicated for 10 min, followed by the addition of 5 mL of the functional palladium nanosheets. The solution was then allowed to sit on the bench top for 1h. The resulting suspension was centrifuged at 7000 rpm for 10 min and the solid was dispersed in 10 mL of water. The same (or similar) procedure was used for preparation of coating of Pd nanosheets on functionalized HO-SiO_2 spheres. For subsequent SEM characterization, the final product was diluted ten times with water and drop-cast on aluminum foil.

Characterizations

Scanning electron microscopy was carried out on a Hitachi S-4700 microscope. The specimens were prepared by drop casting suspensions of Pd nanosheets or Pd nanosheet-coated silica spheres on a piece of aluminum foil. Lieca SP2 Visible Laser Confocal Microscope used to acquire the fluorescence image of the Pd nanosheet-coated HepG2 cells. Thermal gravimetric analyzer (TGA, TA Instruments, SDT-Q600) and UV-Vis spectrometer (Cary 60, Agilent) were used to measure and calibrate the mass concentration of Pd in water. The surface potential of functionalized Pd nanosheets and silica spheres were measured using Zetasizer Nano ZS from Malvern with the disposable capillary cell (DTS1060). Cell viability was determined by the amount of MTT reagent metabolized using plate reader (Tecan Infinite 200 PRO, Tecan AG, Switzerland).

Determination of the Concentration of Functionalized Pd sheets

An initial mass concentration of 667.5 ppm of as-made Pd nanosheets dispersed in water was determined by TGA. Samples with different concentrations were made by diluting the aforementioned stock suspension of Pd nanosheets with water at 8, 10, 12, 20, and 50 times. The absorption spectrum of the sample at each concentration was measured using the UV-Vis spectrometer (Agilent Cary 60). Absorbance at 420 nm was used as the reference wavelength to determine the calibration curve of absorption. This wavelength was chosen to avoid the interference from the various functional ligands at peak position for Pd nanosheets. Once the calibration curve was established, determination of Pd concentration in various samples was performed by diluting 30 μL of sample solution with 3.99 mL of water prior to the measurements.

Viability Study of HepG2 Cells by MTT Assay

Cellular viability was analyzed with an MTT assay for cellular metabolic activity. In general, HepG2 cells were seeded on a 96-well plate at 5×10^3 cells per well. Cells were incubated for 24 h with functionalized Pd nanosheets at various concentrations. MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, ATCC) was added to the cell culture media, and reduced to formazan dye by metabolically active cells. Formation of the dye was evaluated by measuring the absorbance at 570 nm using plate reader (Tecan Infinite 200 PRO, Tecan AG, Switzerland). The absorbance of the dye was determined by subtracting the value of that of the reference (sheets alone at the same concentration) from that of the sample of cells incubated with Pd nanosheets. The viability was determined by normalizing the absorbance to that of cells cultured in the media without the addition of Pd nanosheets. Error bars represent standard deviation of three replicates. As control experiments, MTT assay was also performed on HepG2 cells using pure CA and MPA ligands.

Fluorescent Imaging of HepG2 Cells Incubated with Pd Nanosheets

HepG2 cells were seeded on 35 mm petri dish with approximately 2×10^4cells and cultured for 3 days. The cells were then incubated with 100 ppm fluorescence labeled functionalized Pd nanosheets for 24 h. The fluorescence labeling of Pd nanosheets (as-made, MPA-, or CA-functionalized) were performed by mixing with fluoresceinamine in DI water under stirring condition (800 rpm) overnight and washed 5 times before the UV-Vis spectrometer measurement. After incubating cells with the fluorescent-labeled Pd nanosheets for 24 h, the media was removed and the cells were washed with 2 mL of 1 ×PBS three times to remove free sheets. The cells were then fixed with formaldehyde (3.7%) for imaging. The fluorescence image of the cells and Pd

nanosheets were taken directly with the samples fixed with formaldehyde on the petri dish using a confocal microscope (Lieca SP2).

Since the Pd nanosheets were either charged or uncharged, the surface concentration of the fluorescent probe should vary due to the different affinity between fluoresceinamine and the different charged surfaces. Thus fluorescent intensity does not give quantitative information on the amount of Pd sheets attached to the cells. However, it does provide visual guidance of the location of cells.

SEM Imaging of HepG2 Cells Incubated with Pd Nanosheets

HepG2 cells were seeded on 35 mm Petri dish with approximately 2×10^4cells and cultured for three days. Cells were then incubated with functionalized Pd nanosheets at 100 ppm for 24 h. The Karnovsky's fixative in a mixture of phosphate-buffered 2% glutaraldehyde and 2.5 % paraformaldehyde was then introduced to the Petri dish and allowed to sit for 1 h. The cells were then transferred into a 1.5-mL centrifuge tube by detaching and collecting the cells from the Petri dish with a 200-μL pipet. Additional Karnovsky's fixative was added into the centrifuge tube to make a suspension (total volume: 0.5 mL). The SEM specimen was prepared through a syringe filter, using the following procedures: 1) Assemble syringe and filter; 2) draw up the suspension of cells and fixative in a 3-mL syringe; 3) slowly drip the syringe contents through. For all succeeding steps, remove syringe filter to draw up next required solution; 4) slowly drip 1-1.5 mL of buffer through; 5) pull up 0.5 mL of 1 or 2% OsO_4 aqueous solution, allowing to sit for 30 min; 6) gently drip 2 mL of water through the filter; 7) dehydrate 3 min with 1.5 mL of 10% (ethanol /water), then repeat this step with 25%, 50%, 75%, 95%, 100%, and 100% again; 8) mix 100% ethanol and hexamethyldisilazane (HMDS) at 1:1 ratio, followed by dripping through assembly

and allowing to sit for 10 min; 9) drip through 1 mL of pure HMDS and let the sample sit for 15 min; repeat this step once; 10) with the whole filter assembly still intact, remove syringe and place under vacuum overnight; 11) mount on stub using a mixture of colloidal carbon and Duco cement. First coat whole stub face, then add more for mounting the filter; and 12) coat with gold-palladium by sputtering for 15 s from the top and then image using SEM.

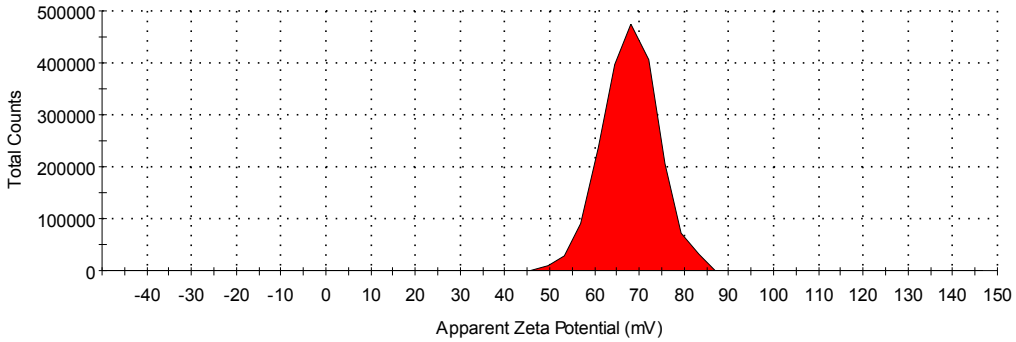

Fig. S1. Measurement of zeta potential for H_2N-silica spheres in acetic acid aqueous suspension.

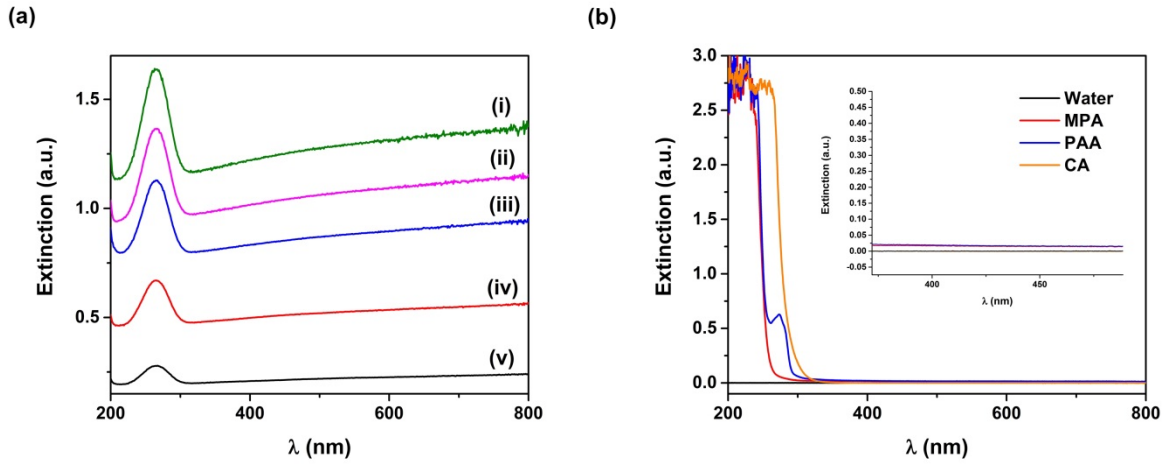

Fig. S2. Extinction of (a) suspensions of Pd nanosheets at different concentrations: (i) 83.4, (ii) 66.7, (iii) 55.6, (iv) 33.4, (v) 13.3 ppm, respectively; and (b) various ligands used for surface functionalization.

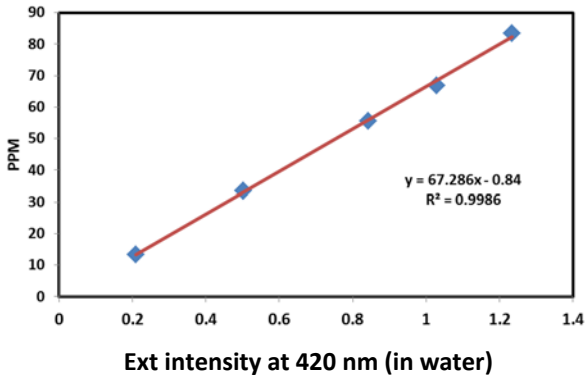

Fig. S3. Calibration curve for determining the Pd nanosheets concentration in DI water. The extinction intensity at 420 nm was used.

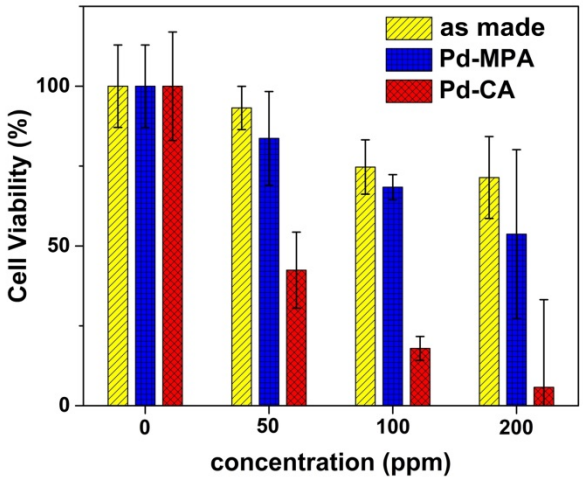

Fig. S4. MTT assay for cell viability study HepG2 cells incubated with as-made, MPA-functionalized and CA-functionalized nanosheets up to 200 ppm.

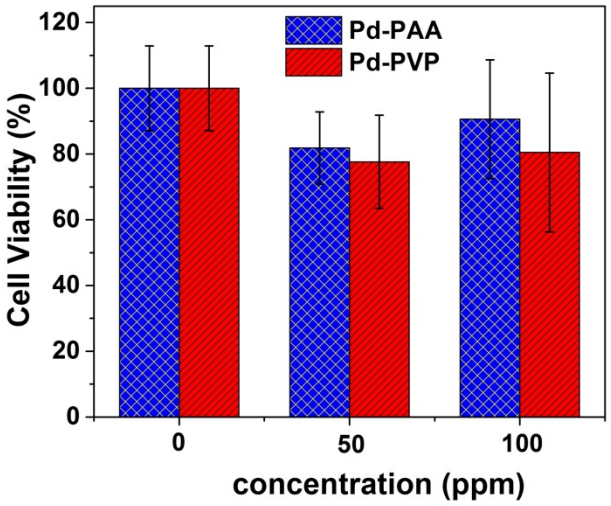

Fig. S5. MTT assay for cell viability of HepG2 cells incubated with polyacrylic acid (PAA) and polyvinylpyrrolidone (PVP) functionalized Pd nanosheets.

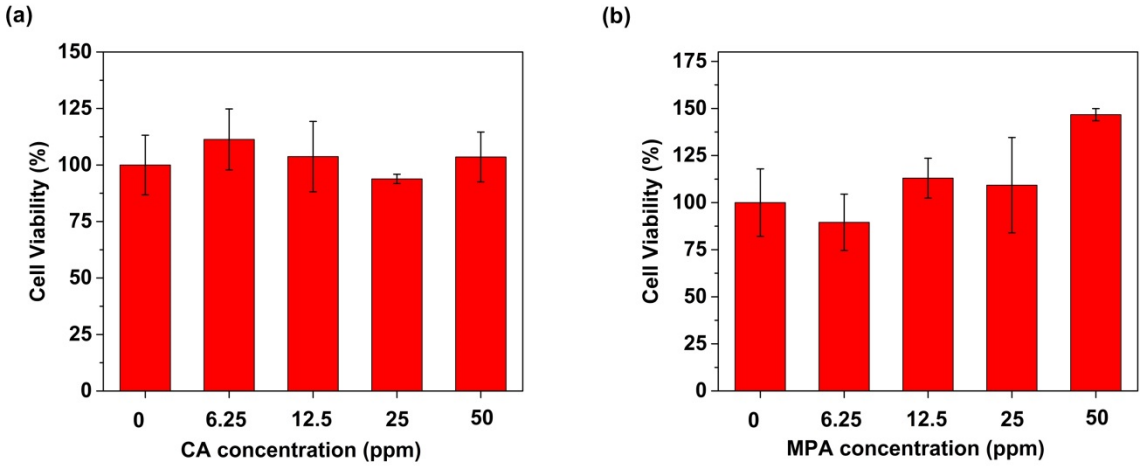

Fig. S6. MTT assay for cell viability of HepG2 cells incubated with (a) CA and (b) MPA, respectively.

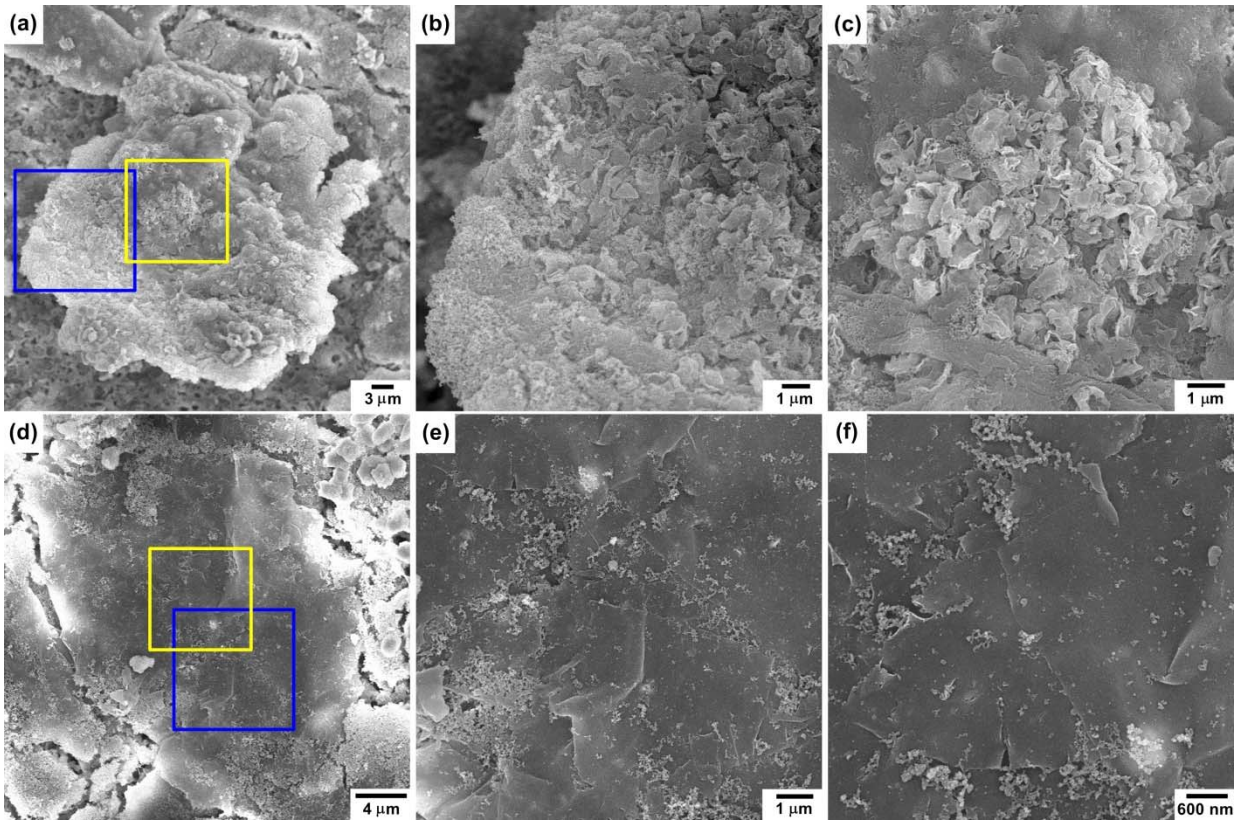

Fig. S7. SEM images of HepG2 cells incubated with 100 ppm Pd nanosheets: (a-c) MPA- and (d-f) CA-functionalized Pd nanosheets, respectively. (b) and (e) show the corresponding enlarged regions in the blue boxes; and (c) and (f) in the yellow boxes.