

Electronic Supplementary Information

Facile fabrication of C₆₀-polydopamine-graphene nanohybrid for single light induced photothermal and photodynamic therapy

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Experimental Section

1. Materials

Dopamine hydrochloride and folic acid were obtained from Acros organic. C₆₀ was purchased from Wuhan University. RPMI 1640 medium and the fetal calf serum were provided from Gibco BRL. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl (MTT) was provided from Sigma. 2, 7-Dichlorofluorescein diacetate (DCF-DA) was obtained from Molecular Probe Inc. Annexin V-fluorescein isothiocyanate (FITC) apoptosis detection kit was obtained from Biosea Biotechnology Co. Ltd.

2. The synthesis of PDA-rGO

Graphite oxide was synthesized by the modified Hummers method. Aqueous suspensions of graphene oxide (GO) was prepared by 30 min sonication of graphite oxide. Polydopamine (PDA) capping was performed by mixing the GO suspensions (0.5 mg/mL) with a buffer solution (1 mg/mL dopamine hydrochloride, 10 mM Tris buffer, pH 8.5) at room temperature. The mixture was stirred vigorously at 60 °C for 24 h. The product (PDA-rGO) was purified by filtering with a 0.45 μm membrane filter and washed several times with deionized water to remove PDA and other reagents.

3. Preparation of C₆₀-PDA-rGO nanohybrid

PDA-rGO (10 mg) and folic acid C₆₀ derivative (20 mg) were dispersed in 20 mL of 10 mM Tris buffer solution (pH = 8.5). The reaction mixture was stirred at room temperature in dark for 24 h. Then the solution was filtered and washed with deionized water for several times to completely removing excess C₆₀ derivative, Followed by drying at 80 °C under vacuum for 12 h.

4. Characterization

FT-IR (Bruker, Tensor 27), and UV-vis spectra (Beijing Purkinje General Instrument Co., Ltd., TU-1901) were used to characterize the functionalized

graphene. TGA was run under a nitrogen flow (40 mL/min) using a Q500 TG instrument (TA Instruments). The samples were heated from room temperature to 400 °C at a ramp rate of 5 °C/min. TEM (JEOL, JEM-2100F) and AFM (Sounding Housing SPA 400) were used to characterize the morphology and structure of the samples.

5. Singlet oxygen generation in aqueous solutions

To measure the singlet oxygen ($^1\text{O}_2$) generation by C_{60} -PDA-rGO, p-nitroso-N, N'-dimethylaniline (RNO) was used as an indicator. Solutions of PDA-rGO, FFA or C_{60} -PDA-rGO were mixed with RNO (50 μM), imidazole (50 μM), and phosphate-buffered saline (PBS, 10 mM, pH = 7.4) and then irradiated by Xe lamp equipped with a band pass filter (400-1100 nm) at the light power density of 2 W/cm^2 for different periods of time. The FFA served as comparison was synthesized by the method mentioned in our previous work. The generation of $^1\text{O}_2$ would result in the reduction of RNO absorption at 440 nm, which reflects the production of $^1\text{O}_2$.

6. Photothermal activity of C_{60} -PDA-rGO nanohybrid

Solutions of PDA-rGO, FFA or C_{60} -PDA-rGO (50 $\mu\text{g}/\text{mL}$) were taken in a 1 mL quartz sample cell. The solution was then illuminated with Xe lamp equipped with a band pass filter (400-1100 nm) for different time periods with a power density of 2 W/cm^2 . The increase in temperature was measured by a thermocouple immersed into suspension.

7. Cell treatment and cytotoxicity assay

HeLa cells were cultured in an atmosphere with 5% CO_2 and at 37 °C provided by a NAPCO CO_2 incubator in RMPI 1640 medium containing 10% heat-inactivated fetal calf serum. Then, the culture medium was replaced by the fresh medium containing 2% fetal calf serum and specific nano-materials. After 12 h of incubation, the cells were washed twice with PBS. For synergistically enhanced anti-cancer effect of the combination of PDT and PTT, the cells were irradiated using a Xe lamp (50 W) equipped with a band pass filter (400-1100 nm) at an output power of 2 W/cm^2 .

After another 24 h incubation, the cell viabilities of samples were determined by the MTT assay. In brief, HeLa cells ($1 \times 10^5/\text{mL}$) were seeded in 96-well plates. 10 μL MTT solutions (final concentration, 0.5 mg/mL) were added and incubated for additional 4 h. The lysis buffer (20% sodium dodecylsulfate in 50% aqueous N, N-dimethylformamide) was added to solubilize the formazan crystal, and absorbance at 570/ 630 nm was measured with a microplate reader (Molecular Devices).

8. In vitro cellular uptake assay

HeLa cells were cultured in 24 well tissue plate at a density of 1×10^5 cells per well. To investigate the targeted uptake of C_{60} -PDA-rGO by HeLa cells, cellular uptake was observed by confocal fluorescence microscopy. FITC was loaded on C_{60} -PDA-rGO by sonicating FITC solution (0.05 mg/mL , 2 mL) with an aqueous suspension of C_{60} -PDA-rGO (2.0 mg/mL , 1 mL) for 30 min to mix them together, followed by stirring in the dark overnight. Unbound FITC was removed by rinsing and centrifugation. As a comparison, GO and PDA-rGO were treated with FITC by the same steps. Then, HeLa cells were exposed to GO-FITC, PDA-rGO-FITC and C_{60} -PDA-rGO-FITC with the final concentration of 50 $\mu\text{g}/\text{mL}$ at 37 °C, respectively. The

cells were harvested and resuspended in PBS after washed for 3 times.

9. Measurement of intracellular ROS accumulation

The fluorescent probe DCF-DA was used to monitor the intracellular accumulation of ROS. After treatment, cells (1×10^6 cells per 3 mL in 6-well plates) were rinsed with D-Hanks solution and 10 μ M DCF-DA was loaded. After 20 min incubation at 37 °C, the cells were harvested after being washed with PBS 3 times. The intracellular ROS accumulation was measured by using a Becton-Dickinson fluorescence-activated cell analyzer while data analysis was performed with Modifit LT 2.0 (Becton-Dickinson). About 1×10^4 cells were counted for each analysis. The fluorescence intensity was quantified with CELLQuest software.

10. Determination of apoptosis

Apoptotic cell death was analyzed by double staining with annexin V-FITC and propidium iodide (PI). Apoptosis of cells was evaluated using an annexin-V FITC apoptosis detection kit. Cells were harvested, washed and incubated at 4 °C for 30 min in the dark with annexin V- FITC and PI, then were analyzed on a FACS Vantage SE flow cytometer (Becton Dickinson).

11. Hoechst 33342/PI staining

After treatment, cells were stained with Hoechst 33342 (1 μ g/mL) and PI (5 μ g/mL) for 20 min at 37 °C. Stained nuclei were observed, using fluorescence photomicroscope (Olympus, Japan).

12. Statistical analysis

Values were reported as means \pm S.D. Statistical comparisons were made by one-way ANOVA to detect significant difference using SPSS 13.0 for windows. $P < 0.05$ was considered to be statistically significant.

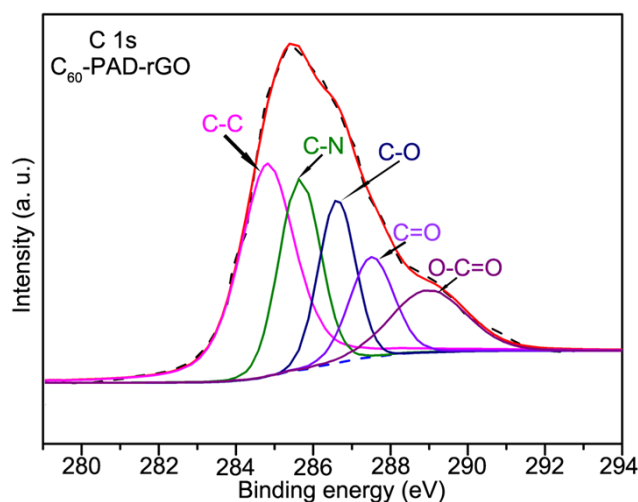


Fig. S1 The C1s spectra and fitted curves of C₆₀-PAD-rGO.

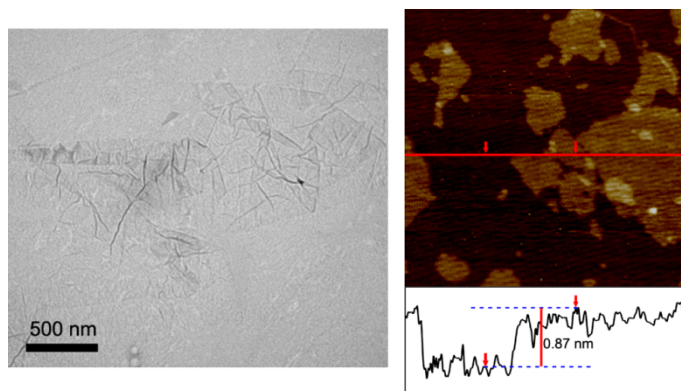


Fig. S2 TEM and AFM images of GO.

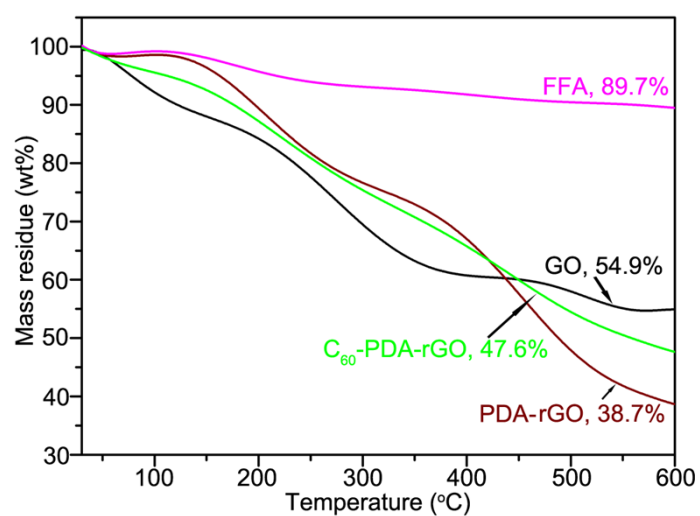


Fig. S3 TG curves of GO, PDA-rGO, FFA and C_{60} -PDA-rGO under N₂ atmosphere.

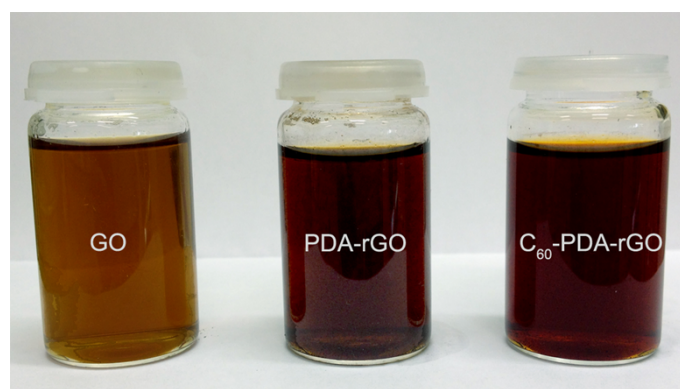


Fig. S4 Photograph of aqueous dispersions GO, PDA-rGO, and C_{60} -PDA-rGO (50 µg/mL) after 3 days.

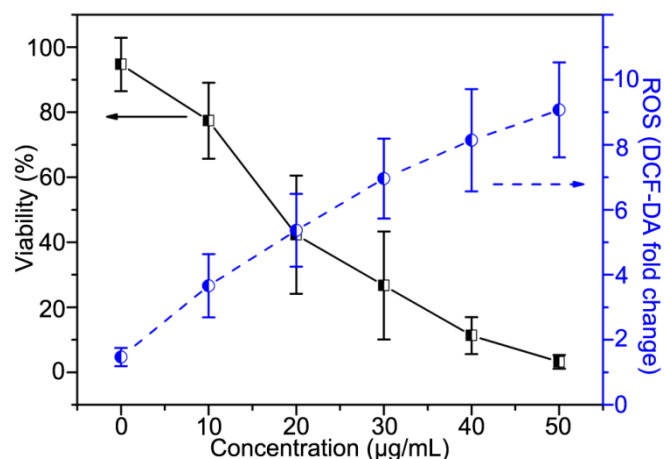


Fig. S5 Concentration-dependent cell viability and intracellular ROS generation of HeLa cells incubated with C_{60} -PDA-rGO. HeLa cells were incubated with nanoparticles for 12 h prior to irradiation (Xe lamp, 2 W/cm², 15 min). Cell viability was measured by the conventional MTT reduction assay. The fluorescent probe DCF-DA was used to monitor the intracellular accumulation of ROS. Data are presented as mean \pm S.D. (n=3).

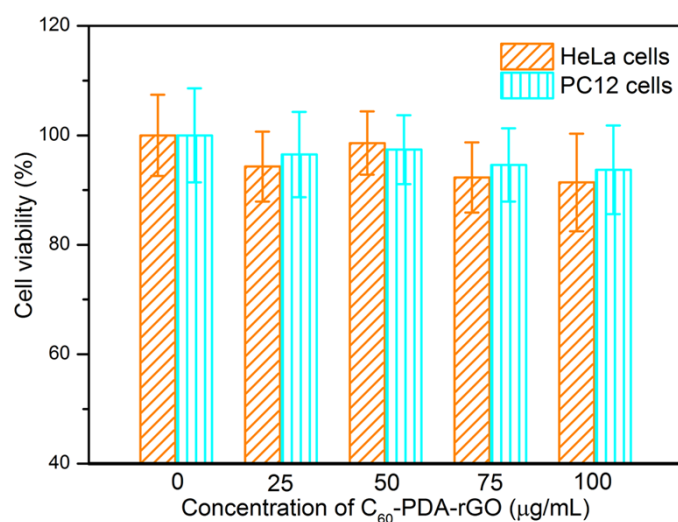


Fig. S6 Cytotoxicity caused by C_{60} -PDA-rGO in dark. HeLa cell and PC12 cell viability was measured by the conventional MTT reduction assay. Data are presented as mean \pm S.D. (n=3).

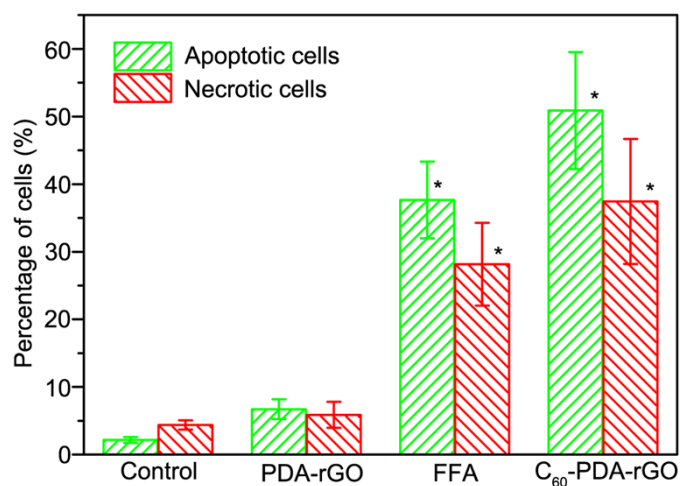


Fig. S7 C₆₀-PDA-rGO induced apoptosis in HeLa cells. HeLa cells were incubated with 50 $\mu\text{g}/\text{mL}$ PDA-rGO, FFA or C₆₀-PDA-rGO for 12 h prior to irradiation (Xe lamp, 2 W/cm², 9 min), respectively. DNA fragmentation was determined by flow cytometry. Data are presented as mean \pm S.D. (n=3). *p < 0.05 compared to the control group.