Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Photothermal and Photodynamic Therapy Reagents based on rGO-C_6H_4-COOH

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Supporting information contents

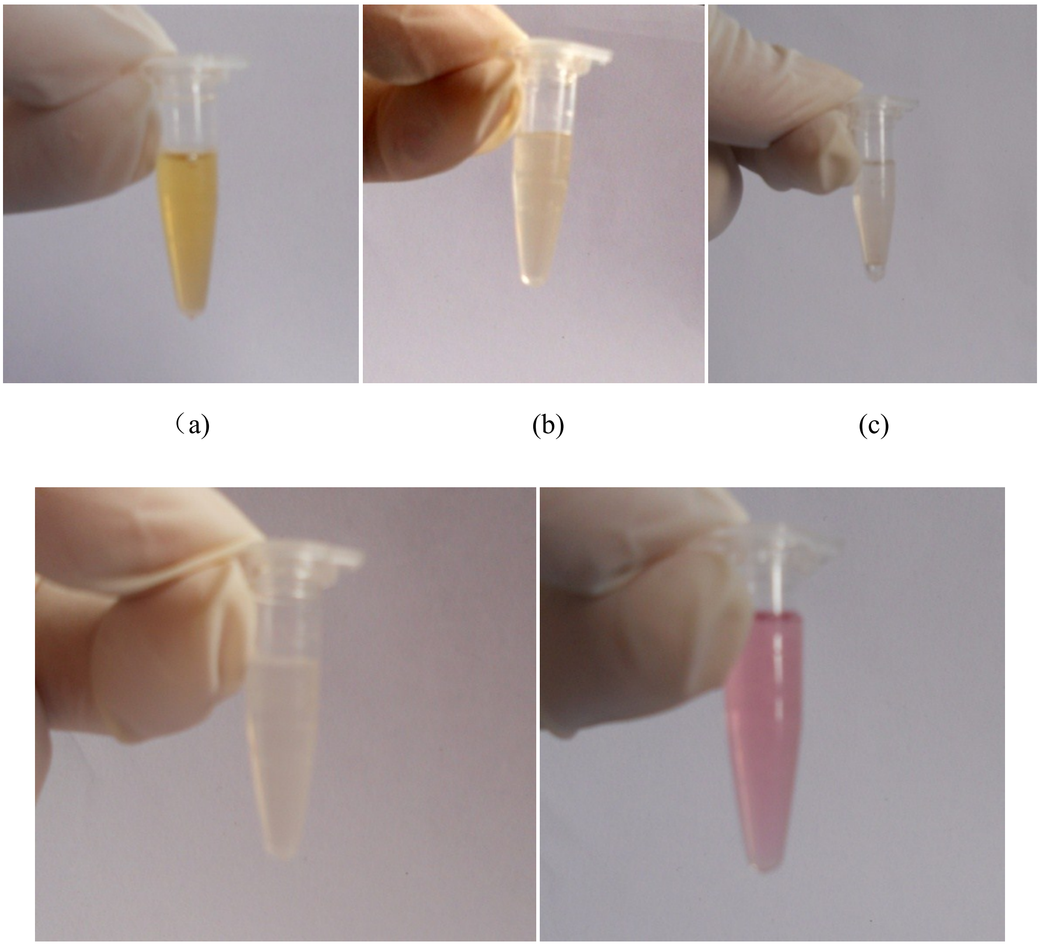
1. Synthesis of graphene oxide (GO), reduced graphene oxide (rGO) and rGO-C_6H_4-COOH.

1.1 GO was synthesized from natural graphites according to a modified Hummers method [S1-S4]. In general, natural graphite powder was first pre-oxidized through mixing natural graphite powder (20 g), concentrated sulfuric acid (H_2SO_4 , 30 mL), $K_2S_2O_8$ (10 g), and P_2O_5 (10 g). The mixture was incubated at 80℃ for 6 h to synthesize the product. The product was carefully washed with distilled water until the rinse water was pH 7, and then the product was dried in air at ambient temperature overnight. This pre-oxidized graphite powder (2 g) was put into gradually concentrated H_2SO_4 (0℃, 46 mL) and $KMnO_4$ (6 g) with stirring to keep the temperature of the mixture below 20℃. The mixture was then stirred at 35℃ for 2 h, and distilled water (92 mL) was added. Distilled water (280 mL) and 30% H_2O_2 (5 mL) were added, and the color of the mixture became bright brown over 20 min. The mixture was filtered and washed repeatedly with 10% HCl solution to remove residual metal ions, and then the mixture was washed with distilled water and filtered repeatedly until the solution became neutral. The product was dried to obtain a loose brown powder in air, and the product (0.25 g) was dispersed into distilled water (1 L) to make a bright yellow GO solution through sonication for 20 min. The GO solution was dialyzed with deionized water for 72 h to completely remove residual salts and acids. Any insoluble precipitation was removed by a centrifuge at 3000 rpm for 10 min. For experiments, the GO solution should be exfoliated by an ultrasonic probe at 500 W for 24 h, and then centrifuged at 10000 rpm for 6 min.

1.2 rGO was synthesized by a method originally presented by Li et al. [S5] Stable dispersion of highly conducting graphene sheets should generally be prepared by an R_{N2H4} /GO weight ratio of 7:10. More specifically, GO dispersion (0.025 wt%, $\approx$0.25 gL^{-1} , 150 mL) was mixed with water (150 mL), hydrazine solution (66.0 mL, 80 wt% in water), and ammonia solution (1050 mL, 28 wt% in water) in a 500 mL round-bottomed flask. The mixture was stirred for a few minutes and then incubated in a water bath (97–99℃) for 1 h. The resulting dispersions were permitted to cool at room temperature and dialyzed against 0.5% ammonia solution to remove the excess amount of hydrazine or any unnecessary compounds. The rGO ammonia solution could directly be used for the UV/Vis (U-4100) spectroscopy measurements. The rGO solid sample was obtained by freeze-drying for the FTIR (VERTEX-70, Bruker) spectra and the Raman spectra (Lab RAM HR800, Horiba JY). One drop of this ammonia solution was mounted on a thin film of amorphous carbon deposited on a copper grid for TEM (JEM-1400) imaging. The ionization of carboxylic acid groups of the rGO dispersion is strongly related to pH values. Once the rGO colloids are dried, they cannot be re-dispersed in water. The formation of stable rGO colloids should be mainly attributed to electrostatic repulsion [S5] and less hydrophilicity (i.e., the residual carboxylic acid groups in graphene sheets) than previously presumed [S6]. The Tyndall and salt effect of the colloidal nature and the diluted rGO dispersed sample solution gave rise to the Tyndall effect, like the GO dispersion solution, in which a laser beam passing through a colloidal solution leaves a discernible track as a result of light scattering. The Tyndall effect was also observed for graphene dispersed in ammonia water.

1.3 rGO-C_6H_4-COOH synthesis, structures, and properties: In our work, we used a new wet-chemical method [S7,S8] to demonstrate the chemical modification of graphene by covalently attaching organic groups onto the planar surface of rGO. The aryldiazonium ion solution was obtained according to the method below. Sodium nitrite (4.1 mmol) and 4-aminobenzoic acid (1.2 mmol) were dissolved in 0.25% NaOH solution (15 mL). The solution was then added dropwise to HCl solutions (0.1 $molL^{-1}$, 19.5 mL) in an ice bath with stirring. Before the aryldiazonium ion was introduced into the rGO sheets, the rGO colloidal solution (ammonia aqueous solution) had to be adjusted to the acidic conditions ($pH \leqslant 6.0$) by using the HCl solution in an ice bath. During the adjustment from the base solution to the acidic solution, the rGO colloids would precipitate and become a flocculent brown precipitate. Immediately, the aryldiazonium ion solution was added dropwise to the above rGO flocculent precipitation solution under stirring. The mixture was kept in the bath for 2 h without stirring to adsorb the aryldiazonium ion sufficiently to the rGO sheets. After 2 h, the mixture was sonicated for 2 h at 45℃ by using a 250 W sonicator. During sonication, the flocculent brown precipitates gradually disappeared and the solution changed from colorless to brown with a bubbled appearance. Then the mixture was sonicated continuously for 12 h to make multilayer graphene become the single-layer graphene. Dialyzing against distilled water was carried out for over 72 h to remove any ions, and then the solution was centrifuged at 10000 rpm for 3 min to remove the unreacted rGO sheets. The final solution, that is, rGO-C_6H_4-COOH solution was stored at 4℃. During the reaction, it was very important that the pH of the solution be strictly kept at acidic conditions, otherwise side reactions would occur and byproducts would appear. The rGO-C_6H_4-COOH solid sample was obtained through freeze-drying for FTIR spectroscopy. One drop of this solution was mounted on a thin film of amorphous carbon deposited on a copper grid for TEM imaging. One drop was placed on a silicon wafer for AFM (Nanoscope IIIA, Veeco) imaging.

2. The stability of rGO-PEI-TCPP in biological solutions

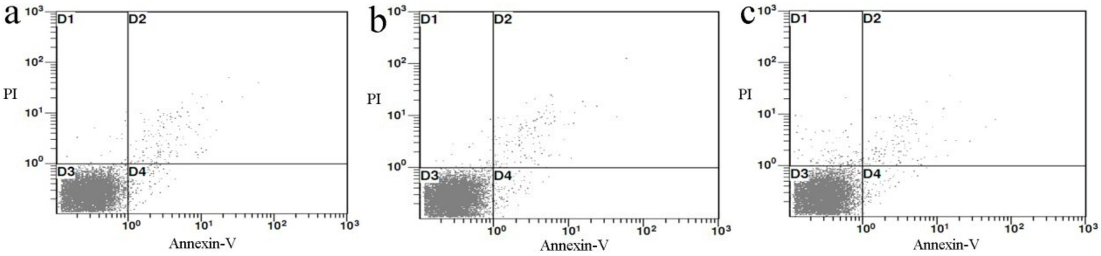


（a) (b) (c)

(d) (e)

Figure S1. Photos of rGO-PEI-TCPP in biological solutions. (a) in serum solution; (b) in 0.1 mol·L HAC media; (c) in 9% (wt%) NaCl solution; (d) in 1X PBS solution (pH = 7.4) (e) in cell culture medium solution. From above photos, one can see that rGO-PEI-TCPP exhibited excellent stability in biological solutions and no aggregation occurs.

3. Effect of rGO-PEI-TCPP concentration on apoptosis of CBRH7919 cell line



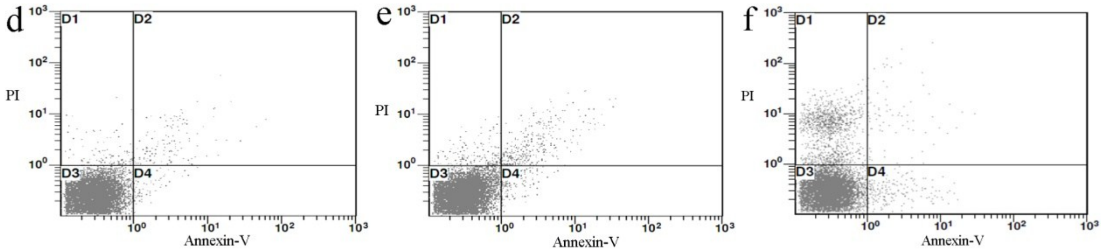
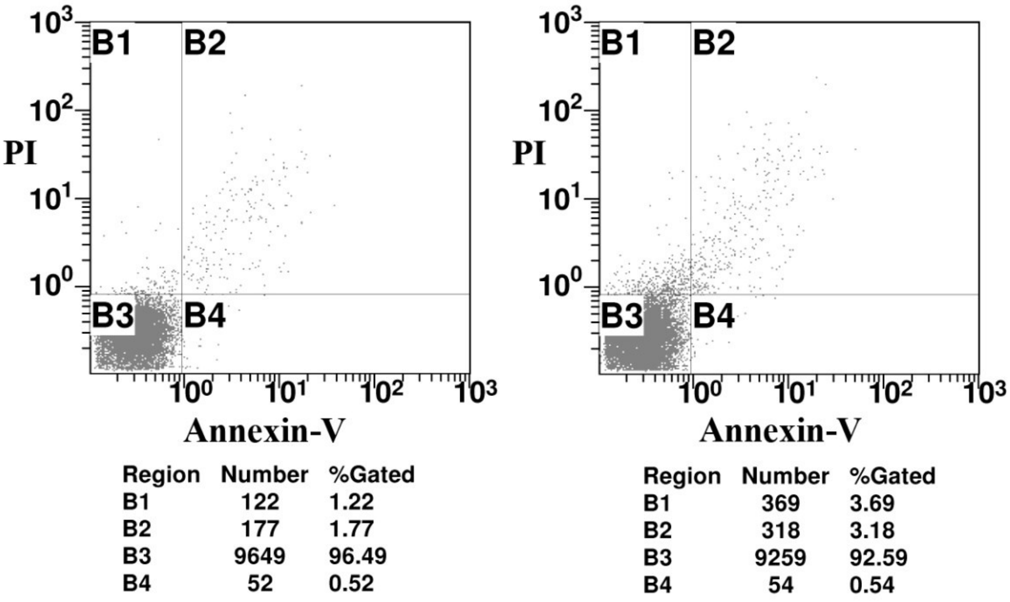


Figure S2. Effect of rGO-PEI-TCPP concentration on apoptosis of CBRH7919 cell line. a:Control group ; b:3 mg•L^{-1}; c:6 mg•L^{-1}; d:9 mg•L^{-1}; e:12 mg•L^{-1}; f: 15 mg•L^{-1}. In the FCM figures (a, b, c), D1, D2, D3 and D4 zone denote the cell necrosis period, the late apoptotic cells period, the normal cell death period and the early apoptotic cells period separately.

4. The FCM analysis of the toxicity of 410 nm laser irradiation alone to untreated CBRH-7919 cells by rGO-PEI-TCPP.



Region	Number	%Gated
B1	122	1.22
B2	177	1.77
B3	9649	96.49
B4	52	0.52

Region	Number	%Gated
B1	369	3.69
B2	318	3.18
B3	9259	92.59
B4	54	0.54

(a) (b)

Figure S3. The irradiation effect of 410 nm laser alone to untreated CBRH-7919 cells by rGO-PEI-TCPP. (a) Control group; (b) The irradiation group.

5. The confocal laser fluorescence microscopy imaging of the CBRH -7919 cancer cell which was induced to apoptosis by the rGO-PEI-TCPP under the laser irradiation.

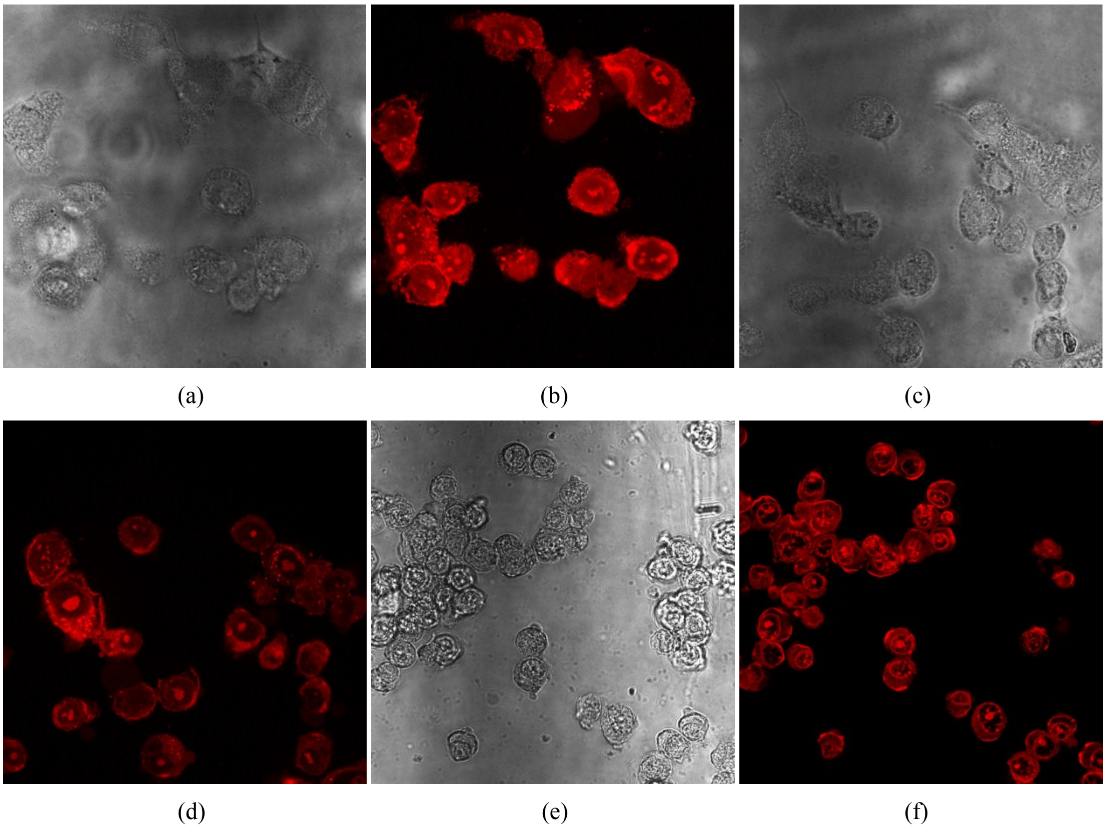
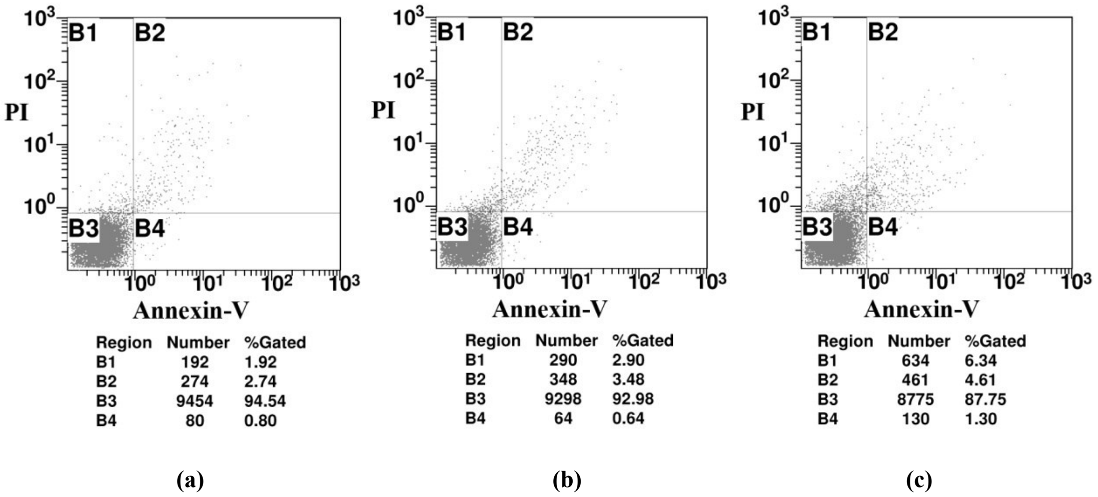


Figure S4. Confocal laser fluorescence microscopy imaging**.** (a) Control group (bright field); (b) Control group (Dark field); (c) 10 mg•L^{-1} rGO-PEI-TCPP group without irradiation (bright field); (d) 10 mg•L^{-1} rGO-PEI-TCPP group without irradiation (Dark field); (e) 10 mg•L^{-1} rGO-PEI-TCPP apoptosis group with irradiation (bright field); (f) 10 mg•L^{-1} rGO-PEI-TCPP apoptosis group with irradiation (Dark field).

6. The experiment of rGO-C_6H_4-CO-NH-PEI induces the CBRH7919 cancer cell apoptosis under the laser irradiation



(a)

Region	Number	%Gated
B1	192	1.92
B2	274	2.74
B3	9454	94.54
B4	80	0.80

(b)

Region	Number	%Gated
B1	290	2.90
B2	348	3.48
B3	9298	92.98
B4	64	0.64

(c)

Region	Number	%Gated
B1	634	6.34
B2	461	4.61
B3	8775	87.75
B4	130	1.30

Figure S5. The experiment of rGO-C_6H_4-CO-NH-PEI induces the CBRH7919 cancer cell apoptosis under the laser irradiation. (a) Control group; (b) 10 mg•L^{-1} rGO-C_6H_4-CO-NH-PEI group without irradiation; (c) 10 mg•L^{-1} rGO-C_6H_4-CO-NH-PEI group with irradiation.

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