Supplementary information

Fluorinated Graphene as An Anticancer Nanocarrier: An Experimental and DFT Study

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1.1. Synthesis of hydroxyl functionalized fluorinated graphite (FG-OH)

FGi (100 mg) was reacted with mixed alkali powder (KOH and NaOH) under 170 °C atmosphere condition for 2 h, according to our previous work with minor modifications.1, 2 For further exfoliation the obtained FG sheets, the product was dissolved in deionized (DI) water with low power ultrasonication for 2 h. Flows on the surface of the solution were removed and the products at the bottom of the solution were filtered and collected using the 0.45 μm microporous membrane. Then, the solid was rinsed by DI water until the pH value reached neutral and dried at 60 °C for 12 h under atmosphere.
Table S1 Chemical composition analyses of FGi and FG-OH samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Chemical composition obtained by XPS</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C (at %)</td>
<td>O (at %)</td>
<td>F (at %)</td>
<td>F/C</td>
<td>O/C</td>
</tr>
<tr>
<td>FGi</td>
<td>54.2</td>
<td>0.7</td>
<td>45.1</td>
<td>0.83</td>
<td>0.01</td>
</tr>
<tr>
<td>FG-OH-1</td>
<td>60.2</td>
<td>8.9</td>
<td>30.9</td>
<td>0.51</td>
<td>0.15</td>
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<tr>
<td>FG-OH-2</td>
<td>62.6</td>
<td>12.5</td>
<td>24.9</td>
<td>0.40</td>
<td>0.20</td>
</tr>
<tr>
<td>FG-OH-3</td>
<td>67.8</td>
<td>14.7</td>
<td>17.5</td>
<td>0.26</td>
<td>0.22</td>
</tr>
</tbody>
</table>

1.2. Synthesis of FGO material

FGO was synthesized from FG-OH by Hummers method with some modifications. FG-OH (6 g) was dispersed in 24 mL concentrated H$_2$SO$_4$ under mechanical stirring at 80 °C, then, K$_2$S$_2$O$_5$ (5 g) and P$_2$O$_5$ (5 g) were added and the solution was stirred for 4.5 h at 80 °C. After cooling down to room temperature, the
mixture was diluted by 1000 mL DI water and allowed to rest overnight. Then, the sample was filtered through a 0.45 μm microporous membrane and dried at 60 °C for 12 h under atmosphere after a rinse with DI water. The product was re-dispersed in 240 mL concentrated H₂SO₄, and then KMnO₄ (30 g) was added gradually under stirring keeping the temperature below 20 °C. 500 mL DI water was dropped into the mixture system and the solution was stirred for another 2 h with the temperature of 35 °C. Thus, a solution with brown color was obtained after the additions of 1.4 L DI water and 40 mL H₂O₂. The brown solution was orderly washed by 1 L of 5 wt % HCl and 1 L DI water. A dialysis bag (Mₘ = 8000-14000 Da) was used to dialyze the solution until the pH value reached neutral. Finally, FGO was obtained after vacuum freeze drying of the dialysate.

Fig. S2 FTIR data of FGO after being treated in phosphate buffered solution (PBS), cell culture medium and natural illumination for one week.
1.3. In vitro DOX loading and release from FGO

FGO (50 mg) was added into 50 mL phosphate-buffered saline (PBS) at pH value of 7.4, followed by the addition of 1 mL DOX (2 mg/mL) and stirring for 24 h at room temperature under darkness. The mixture was filtered, centrifuged and dried by vacuum freeze drying. The loading capacity of DOX on FGO was measured by the absorbance at 484 nm of UV–Vis spectrometer, after subtracting DOX dissolved in the filtrate.

The drug release from FGO was performed by adding FGO/DOX to PBS at pH = 5.4 and pH = 7.4. Briefly, 5 mg of FGO/DOX was dissolved in 2 mL of PBS, transferred into a dialysis bag (M<sub>w</sub>=3000Da), and bathing in 8 mL PBS with a shaking incubator at 37 °C. At set intervals, 3 mL of release medium was taken out and 3 mL fresh release medium was added to the system. The accumulative amount of released drug was determined by UV–vis spectrometer. The release experiments were repeated three times.

To determine the drug loading efficiency (DLE), FGO-DOX was dissolved in ultrapure water and analyzed by UV–visible spectrophotometer. The DLE of FGO-DOX was calculated according to eqn (1)

\[
\text{DLE} = \frac{\text{amount of drug on FGO}}{\text{total amount of drug added}} \times 100\% \quad (1)
\]

1.4. Cell Culture and in vitro cytotoxicity assay
HeLa cancer cells were obtained from Shanghai Institute of Material Medical. HeLa cells were cultured in Dulbecco’s modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in a 5% CO₂-containing atmosphere at 37 °C.

The cytotoxicities of FGO, FGO-DOX and free DOX were evaluated using the standard MTT assay. Briefly, HeLa cell were seed into a 96-well plate at a density of about 1 × 10⁴ cell per well. After incubation for 24 h, the culture media were replaced with 100 μL of fresh media containing FGO, FGO-DOX or free DOX at different concentrations for further 24 h. After the medium was removed and 100μL fresh medium added, 15 μL MTT (5 mg/mL in PBS) was added to the wells for 4 h. Then, culture medium was removed, and the formazan crystals were dissolved in DMSO (100 μL per well), followed by shaking for 20min. The option density (OD) was read using a microplate reader (DNM-9606, PERLONG) at a wavelength of 570 nm. The measured OD values of the blank, control, and experimental groups were coded as OD_{bla}, OD_{con}, and OD_{exp}, respectively. Finally, the cellular survival rate was calculated by the following equation:

\[
\text{Survival Rate} = \frac{\text{OD}_{\text{exp}} - \text{OD}_{\text{bla}}}{\text{OD}_{\text{con}} - \text{OD}_{\text{bla}}} \times 100\%
\]

The photothermal effect was assessed after irradiate cells (808 nm, 2.4 W/cm²) for 5min. The final values were the average measured in several parallels.

1.5. In vitro intracellular uptake

The cellular uptake of FGO-DOX and free DOX was examined by flow cytometry. HeLa cells were incubated into a 6-well plate at a density of about 1.5 × 10⁶ cell per well for 24 h and subsequently treatment with FGO-DOX and free DOX at the DOX concentration of 10 μg/mL. After incubation for 0.5 h, 2 h and 4 h, the
cells were rinsed three times with PBS and harvested using trypsin. Cells under
different treatment were washed and centrifuged by PBS, and then the HeLa cells
were suspended in PBS. The DOX fluorescence intensity of cells was analyzed with
the flow cytometry.

HeLa cells (1.5 × 10^6 cell per well) were incubated on glass cover slip in a 6-well
plate in fluorescence microscopy imaging. After 24 h for culture, the cells were
washed three times with PBS after treatment with FGO-DOX (DOX concentration of
10 μg/mL) for 4 h. Then fixed cells with 4% (w/v) paraformaldehyde for 20 minutes
and washed with PBS thrice to remove suspended cells and paraformaldehyde. The
microimages of the cells were observed by fluorescence microscopy.

1.6. Density functional theory (DFT) calculations

The structures studied in present work were designed and modeled by employing
the Gaussian09 software package. Global optimizations were performed at B3LYP/6-
31G** level of theory. Based on optimized structures, electrostatic potential (ESP)
method was carried out to predict the possible interaction sites between DOX and
FGO.\textsuperscript{41} Reduced density gradient (RDG) technique was used to study the nature of the
interactions and visualize non-covalent interactions in the real space by employing
Multiwfn software.

1.7. Characterization

The surface morphologies of the synthesized samples were analyzed by multiple
experimental apparatus, such as transmission electron microscopy (TEM) and high
resolution transmission electron microscopy (HRTEM, FEI Tecnai F30, operated at
300 kV) and X-ray diffraction (XRD, Rigaku D/MAX – 2400 X-ray diffractometer
with Ni-filtered Cu Ka radiation). The chemical composition of the samples was characterized by a Nicolet iS5 Fourier transformation infrared (FTIR) spectrometer. Additionally, a EscaLab 250Xi X-Ray photoelectron spectroscopy (XPS) was performed by using monochromated Al Kα irradiation with chamber pressure of $3 \times 10^{-8}$ Torr under testing conditions, in which all the binding energies were calibrated using 284.8 eV of C 1s. UV–Vis absorbance experiments were measured on a UV–Vis spectrophotometer (TU-1901). The photoluminescence (PL) spectra were performed on a FluoroMax-4 fluorescence spectrophotometer equipped with a 150 W Xe light source and double excitation monochromators. The photothermal property was tested by a NIR laser (Haoxuer, LDP-808-3000) with a temperature sensor. The Intracellular fluorescence intensity was analyzed with the flow cytometry (ACEA, Novocyte2040R). The fluorescence images were using a fluorescence microscopy (Olympus, PENIX73-DP80). Nanoscope IIIa multimode atomic force microscope (AFM, Veeco) in tapping mode was employed to study the morphology.

1.9. AFM data of FGO

![Fig. S3 AFM data of FGO.](image-url)
1.8. Photothermal comparison between FGO and GO

Fig. S4 Photothermal comparison between FGO and GO (0.1 mg/mL, 2.0 W/cm²)

References