Supplementary material for Fluorescence turn-off Ag/fluorinated graphene composite with high NIR absorption for effective killing cancer cells and bacteria

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1. Experimental details

1.1 Chemical reagents

Fluorinated graphite (FGi, Grade II) was purchased from Shanghai CarFluor Ltd. and used as provided. Folic acid (FA), 4', 6-Diamidino-2-phenylindole (DAPI), methyl thiazolyl tetrazolium (MTT), N-Hydroxysuccinimide (NHS), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC HCl), folic acid (FA) and doxorubicin hydrochloride were purchased from Sigma-Aldrich (Shanghai, PR China). All the other chemical reagents were purchased from Sinopharm Chemical Reagent Co., Ltd.

1.2 Exfoliation/activation of FGi to prepare fluorinated graphene (FG) and preparation of fluorinated graphene oxide (FGO)

To ensure as large contact area among the mixture as possible, the initial NaOH and KOH pellets were first milled into powder using a quartz mortar, and then blended uniformly with fluorinated graphite. To conduct the reaction, fluorinated graphite (100 mg) was blended with NaOH–KOH mixture powder (300 mg). Meanwhile, in order to obtain liquid eutectic alkali and mobile hydroxyl anion the weight ratio of NaOH were kept around 43.1%.

And to ensure more oxygen groups, the mixture was then heated at 170 ^{o}C for 4 h in air. After cooling down to room temperature, the product was dispersed in 100 mL Milli-Q water and sonicated at 40% power for 1 h dissolve alkali and further exfoliate the obtained graphene sheets. After the residual FGi that flows on the surface of the solution was carefully pipetted, the product was vacuum filtered onto a microporous membrane (0.45 μm), followed by being rinsed with Milli-Q water (until the pH value reaches neutral) and dried through vacuum freeze drying for 48 h.

To prepare fluorinated graphene oxide (FGO), Then 3 g FG was added in a mixed solution of concentrated H_2SO_4 (24 mL), P_2O_5 (5 g) and $K_2S_2O_8$ (5 g) and stirred for 6 h at 80 ^oC. Further oxidation was accomplished by adding 12 mL H_2SO_4 and 2 g $KMnO_4$ at 30 ^oC for 4 h. The formed FGO was obtained by filtration and purified by dialysis. FGO was dried by vacuum freeze drying for 48 h for following experiment.

1.3 Preparation of FGO-FA

FGO was functionalized with FA via amidation reaction between the COOH (from FGO) and NH_2 groups (from FA). N-hydroxysuccinimide (NHS, 200 mg) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, 125 mg) were mixed with 100 mg FGO, and ultrasonicated to make a homogeneous solution. 20 mL FA (0.5%, pH 8.0 in $NaHCO_3$ solution) was added and stirred overnight. The final product was first purified by dialysis against $NaHCO_3$ solution (pH 8.0) for two days and followed distilled water for one day.

1.4 Drug loading and release study

After introduction of oxygen and FA, DOX can be readily loaded on FGO-FA with different concentrations by stirring the mixture in dark overnight. The unloaded DOX was removed by filtered and collected after several density gradient centrifugations. The DOX loaded FGO-FA+Ag (FGO-FA+Ag+DOX) was dried using vacuum freeze drying. The drug loading capacity (DLC) was calculated according to eqn (1):

$$\mathrm{DLC\,(mg/mg)} = \frac{M_{DOX} - M'_{DOX}}{M_{FGO\text{-}FA+Ag}} \quad (1)$$

In the equations, M_{DOX} is the total amount of DOX added, M'_{DOX} is the amount of DOX without load and $M_{FGO\text{-}FA+Ag}$ is the total amount of FGO-FA+Ag added.

The acid condition triggered and NIR enhanced drug release was accomplished by dialysis of FGO-FA+DOX in phosphate-buffered saline (PBS) under different pH conditions. UV-vis spectrophotometer was employed to monitor the whole drug load and release process.

1.5 Cellular uptake and targeting specificity investigation

Briefly, about 1×10^6 HeLa cells and A549 cells per well were seeded in a six-well plate and pre-incubated in 2 mL DMEM medium for 24 h at 37 ^oC in a 5 % CO_2 humidified atmosphere. After the culture medium was replaced by fresh one, FGO-FA+DOX were added to wells with different dosages of DOX and cells untreated served as control. After incubation for fixed time, the cells were washed by PBS three times and digested with trypsin. 1.0 mL of PBS was added into each well and then centrifuged twice at 3500 rpm for 5 min. Subsequently, the supernatants were removed and 0.3 mL of PBS was added. For flow cytometry experiment, parameter of BL2 with an excitation light of 488 nm and detection channel of 572/28 was used.

1.6 In vitro fluorescence imaging

To obtain fluorescence microscope images, HeLa cells were seeded on pre-sterilized glass sheets in 6-well plates and then incubated at 37 ^oC for 24 h under a 5 % CO_2 humidified atmosphere. Subsequently, a fresh culture medium replaced the old one. FGO-FA+DOX were added to the wells and then the cells were further incubated for different times. The cells on glass sheets were washed with PBS more than three

times and immobilization by PBS buffered paraformaldehyde at room temperature for 20 min. The cells then were washed with PBS three times, and treated with 0.1% Triton 100-X in PBS for 12 min at room temperature. Finally, the cells were stained with DAPI to cell nuclei for 5 min at 37 ^{o}C, and then washed with PBS five times for further fluorescence microscope.

1.7 In vitro and in vivo combined chem-photothermal cancer therapy

The in vitro cytotoxicity assay of the samples was studied by MTT assay. The cells were cultured with a density of 6×10^3 cells per well, added into culture medium in a 96-well plate. Subsequently, samples with various concentrations of DOX were added into a 96-well plate, and then incubated for 24 h. 15 μL of MTT (5.0 mg mL^{-1}) was added to each well and incubated for another 4 h. In order to dissolve the MTT formazan crystals, 100 μL of DMSO was added to each well after the supernatant was removed carefully. The 96-well plate was shaken for 5 min and then the cell viability was detected. To calibrate the cellular survival rate, blank and control groups were set. In the blank group, only culture media was added; while in the control group, cells and culture media without samples were added. 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:

$$\text{Survival Rate} = \frac{OD_{exp} - OD_{bla}}{OD_{con} - OD_{bla}} \times 100\%$$

The results were expressed as the mean ± SD (standard deviation).

For photo-chemotherapy analyses, cells were first cultured for 24 h. Then samples were added into a 96-well plate with different concentrations and cells were incubated for 4 h. For the laser treatment groups, cells were irradiated by an 808 nm NIR laser for 5 min after incubation for 4 h. After another 4 h, the cells of the laser treatment groups were irradiated by an 808 nm NIR laser for another 5 min. After incubation 24 h, the cell cytotoxicity was tested using the same method as above.

Mice were anaesthetized and injected with 100 μL PBS, FGO-FA+Ag2 (50 μg/mL), and FGO-FA+Ag2+DOX (50 μg/mL) solution. The dose of drugs was about

5 mg/kg per injection. Injected mice with FGO-FA+Ag2 and FGO-FA+Ag2+DOX solution and 15 min later irradiated them with an NIR laser (808 nm, 2 W/cm^2) for 5 min. The tumor size was measured every other day, and calculated as the volume = (tumor length) × (tumor width)^2/2. Relative tumor volumes were calculated as V/V_0 (V_0 was the initial tumor volume).

1.8 Antibacterial experiments

Gram-negative E. coli and Gram-positive S. aureus were grown in the corresponding media for 12 h in a 37 °C shaker. When OD_{600} value reached 1 for E. coli and 0.5 for S. aureus (bacterial growth in the logarithmic phase), the bacterial suspensions were centrifuged and washed to completely remove the bacterial culture media. Finally, the bacterial pellets were re-suspended in physiological saline at a certain concentration. To evaluate the antibacterial activity of FGO-FA+Ag, bacteria suspensions with a bacterial concentration of 1.2 ×10^8 CFU mL^{-1} were used. sterilizat Sterilized FGO-FA+Ag of different Ag contents were added to the bacterial suspensions with a concentration of 0.1 mg mL^{-1} and incubated in a 37 °C shaker for 12 h. The nanocomposite was removed by centrifugation and diluted tenfold using 0.9% physiological saline. Then, 100 μL of the diluted suspensions were spread onto agar plates and incubated in a 37 °C shaker for 12 h. Finally, the number of bacterial colony in each group was counted.

1.9 Characterization and Measurement

The chemical composition of the prepared samples was investigated by Fourier transformation infrared (FTIR) spectrometer (Bruker, IFS 66 V/S, Germany), and X-ray photoelectron spectroscopy (XPS, EscaLab 250Xi). The morphology of samples was observed by transmission electron microscopy (TEM, FEI Tecnai F30). The

photothermal effect was evaluated by a NIR laser (Haoxuer, LDP-808-3000) equipped with a temperature sensor.. The cell uptake behavior was obtained by fluorescence microscope (Olympus, PENIX73-DP80) and the flow cytometry (ACEA, Novocyte2040R).

2. FTIR data of FGi

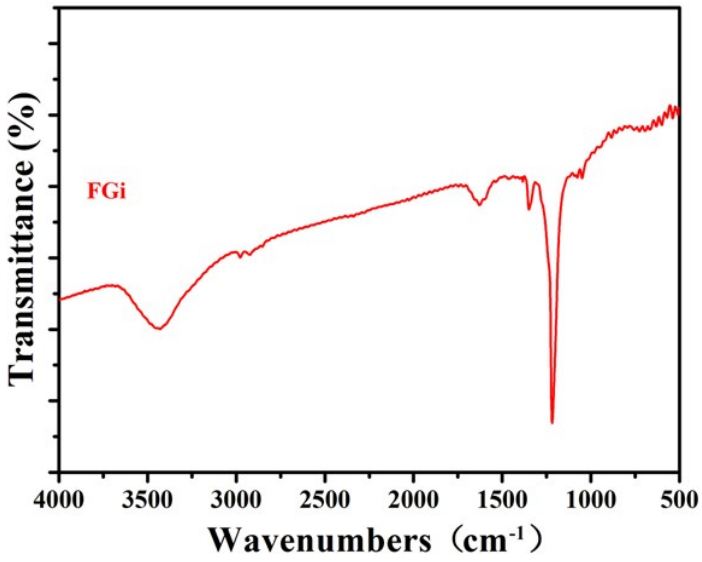

Figure S1 FTIR data of FGi

3. The photoluminescence performance of DOX under various excitation wavelengths

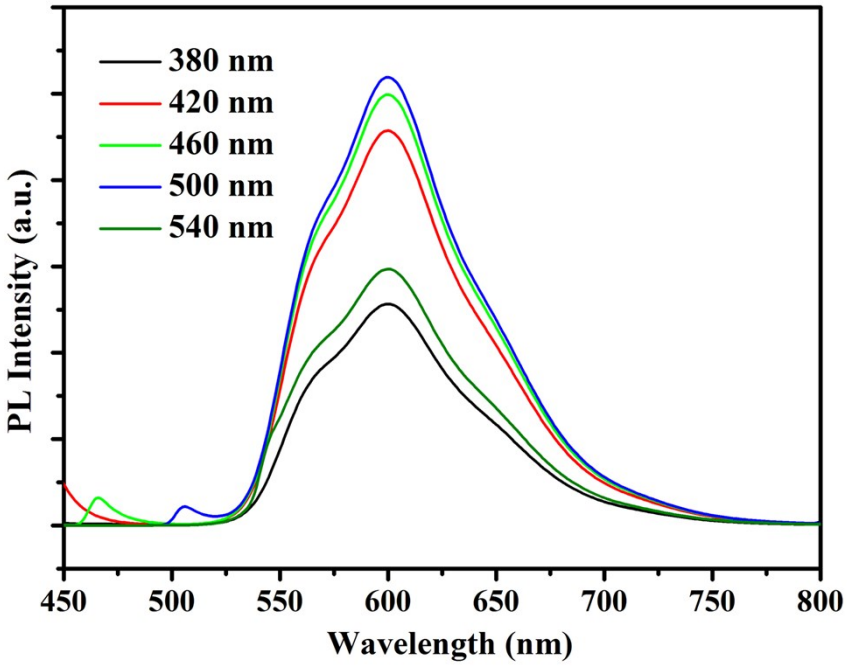

Figure S2 PL emission data of DOX

4. Comparison between HeLa cells with and without FA pretreatment

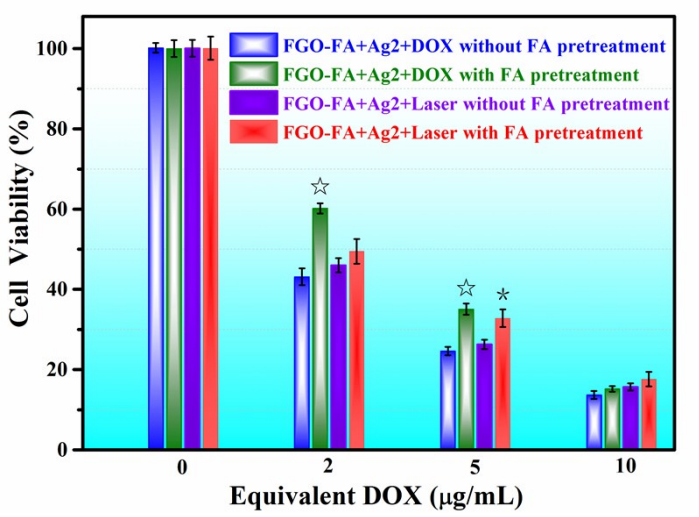

Figure S3 Comparison between HeLa cells with and without FA pretreatment incubated in FGO–FA+DOX, and FGO-FA+Ag2+Laser.

5. Comparison between HeLa cells with and without FA pretreatment

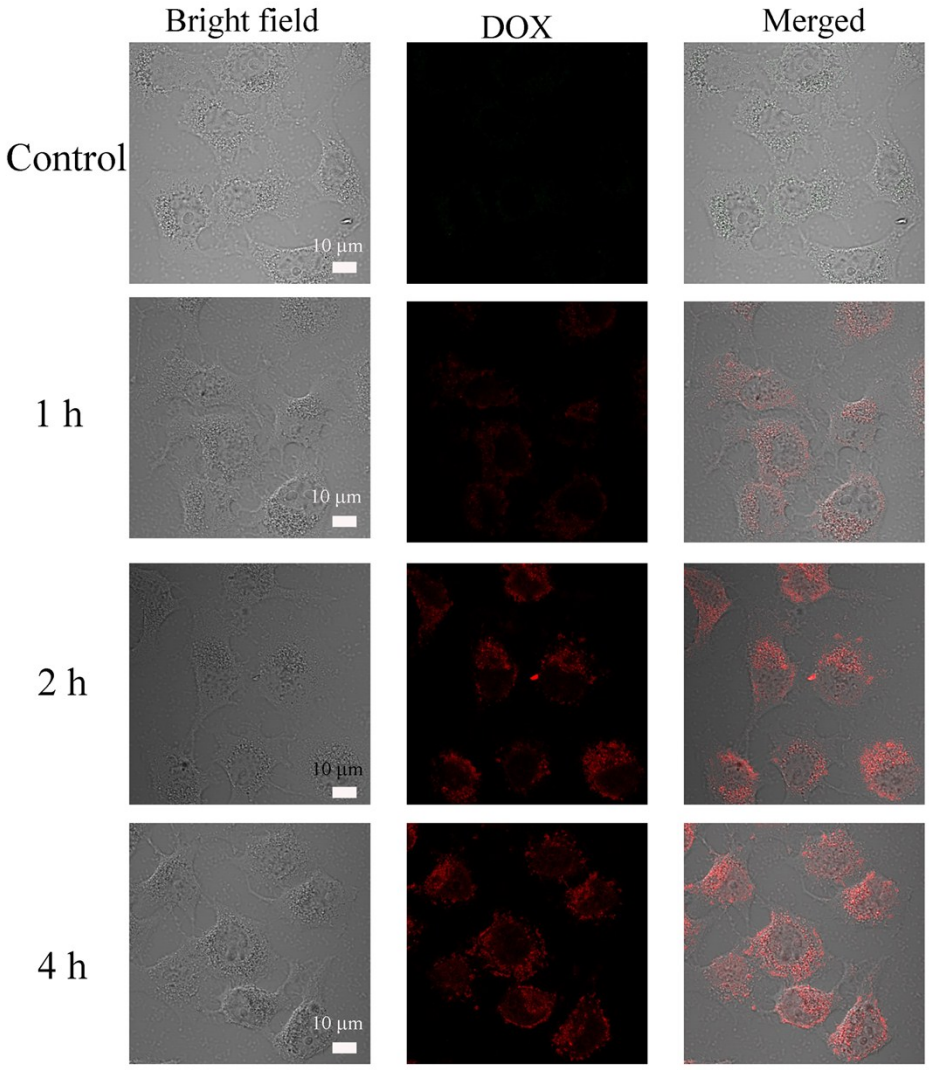

Figure S4 Fluorescence microscopes of cellular uptake for FGO–FA+Ag2+DOX in HeLa cells without drugs (control) and with drugs for different times.

6. Comparison between HeLa and A549 cell incubated with different samples

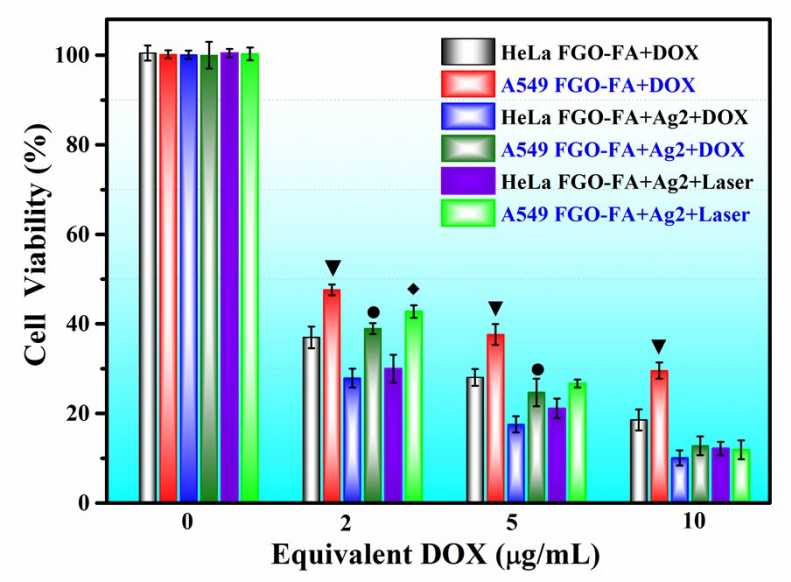

Figure S5 Comparison between HeLa and A549 cells incubated in FGO–FA+DOX, FGO-FA+Ag2+DOX and FGO-FA+Ag2+Laser for 48 h.