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Electronic supplementary information

Nano-sized Paramagnetic and Fluorescent Fluorinated Carbon Fiber with High NIR Absorbance for Cancer Chemo-photothermal Therapy

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1. Experimental details for synthesis and tests

1.1 Preparation of nano-sized FCO from FCE

Typically, 1 g FCE was first dispersed in 20 mL concentrated H_2SO_4, and then 1 g $K_2S_2O_8$ and 1 g P_2O_5 were added after heating up to 80 ^oC in the oil bath, incubation for 5 h under stirring. The oxidation process was further accomplished by adding 40 mL H_2SO_4 and 5 g $KMnO_4$ meanwhile keeping the temperature below 20 ^oC. After reaction at 35 ^oC for 3 h, 200 mL ultrapure water was added and the mixture was further sonicated for 3 h after adding 10 mL H_2O_2 and 150 mL 5% HCl. Then the mixture was filtered through a 0.22-μm membrane. The filtrate was collected, purified by dialysis and dried through vacuum freeze drying.

1.2 Drug loading, acid triggered and NIR enhanced drug release

1 mg DOX was added FCO-PVP (1mg/mL PBS), and then stirred for 24 h in the dark at room temperature. The solution was centrifuged and washed several times with PBS to completely remove any unloaded DOX. The solution was dried by freeze drying, and product of FCO-PVP+DOX was finally obtained. The DLE of FCO-PVP+DOX was calculated according to Eqn (1)

$$\mathrm{DLE} = \frac{\text{amount of drug on FCO - PVP + DOX}}{\text{total amount of drug added}} \times 100\% \quad (1)$$

The acid condition triggered and NIR enhanced release of drugs from FCO-PVP +DOX was performed by adding the products to PBS at 37 ^oC and at different pH values (5.0 and 7.4). The solutions were transferred into dialysis bags and constantly shaken at 37 ^oC in the dark. 4 mL dialysate was taken out and then replaced with 4 mL fresh PBS at regular time intervals. And for NIR enhanced release tests, the

solution was irradiated by an 808 nm laser for 5 min every 10 hours under the same conditions. The experiments were conducted in triplicate and related data were averaged. UV-vis spectrophotometer was employed to monitor the whole drug load and release process.

1.3 Intracellular uptake study of FCO-PVP+DOX and release

The cellular uptake of free DOX and FCO-PVP+DOX was first determined by Flow cytometry. About 2×10^6 Hela cells per well were seeded in a six-well plate, and pre-incubated under standard environment (310 K in 5 % CO_2), After the culture medium being replaced by a fresh one, free DOX and FCO-PVP+DOX were added (the dosages of DOX were 5 μg mL^{-1} and 10 μg mL^{-1}). As a control, cells untreated without free DOX or FCO-PVP+DOX under the same conditions were also cultured. After being incubated for 4 h, cells were washed by PBS and digested with trypsin. PBS was added and the mixture was centrifuged twice (3500 rpm for 5 min). Finally, the supernatant was removed and after being added 0.3 mL PBS, the cell uptake and drug release behavior was analyzed by flow cytometry[1,2].

For fluorescence microscopy, cells were cultured under the same conditons, and after 0.5 h and 4 h, cells were attached to the glass sheets and washed several times and then were immobilized by PBS paraformaldehyde (4% (w/v), 20 minutes). After being washed several times with PBS, the cells were treated with DAPI (5 min) and washed with PBS for three times before being investigated by fluorescence microscope[3].

1.4 Cytotoxicity assay and cancer treatment evaluation

Cells with a density of about 6×10^3 cells per well were added into a 96-well plate, and pre-incubated under standard environment (310 K in 5 % CO_2). Subsequently, FCO-PVP+DOX and free DOX (with various concentrations of 0.53, 1.59, 3.1, 5.2, 10.9, 20, 30 μg mL^{-1}) were added, and cells were then incubated for 24 h. After 15 μL of MTT (5.0 mg mL^{-1}) being added and incubated for another 4 h, 100 μL of dimethyl sulfoxide was added after the supernatant being removed carefully[4,5]. Cell viability was detected by MTT assay and calculated according to Eqn (2) :

$$\text{Cell Viability} = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}} \times 100\% \quad (2)$$

In Eqn (2), A_{blank}, $A_{control}$ and A_{sample} correspond to the absorbance of the blank, control and sample groups, respectively.

For photo-chemotherapy part, cells were cultured and samples were added under the same conditions. Subsequently, after being cultured by 4 h, cells were irradiated by 808 nm NIR laser for 5 min. Then cells were incubated for another 4 h, irradiated for another 5 min, and finally incubated for 24 h under the same conditions.[6] Cell viability was calculated by Eqn (2) using the same method under the same conditions.

1.5 Characterization

Morphology of the products was tested by scan electron microscopy (SEM, JE OL JSM-6701F), and transmission electron microscopy (TEM, JEOLJEM-2010), and the structure was measured by X-ray diffraction (XRD, Rigaku D/MAX-2400). Chemical composition was characterized by Fourier transformation infrared spectrometer (FTIR, IFS 66 V/S Bruker, Germany), and X-ray photoelectron

spectroscopy (XPS, EscaLab 250Xi). UV–Vis data were obtained by a UV–Vis spectrophotometer (UV-2600, Shimadzu). Photothermal performance of the samples was evaluated by a NIR laser (Haoxuer, LDP-808-3000) equipped with a temperature sensor. The cell uptake and drug release behavior in cells were obtained by flow cytometry (ACEA, Novocyte2040R), and fluorescence microscopy (Olympus, PENIX73-DP80).

2. FTIR spectra of FCE under different reaction conditions

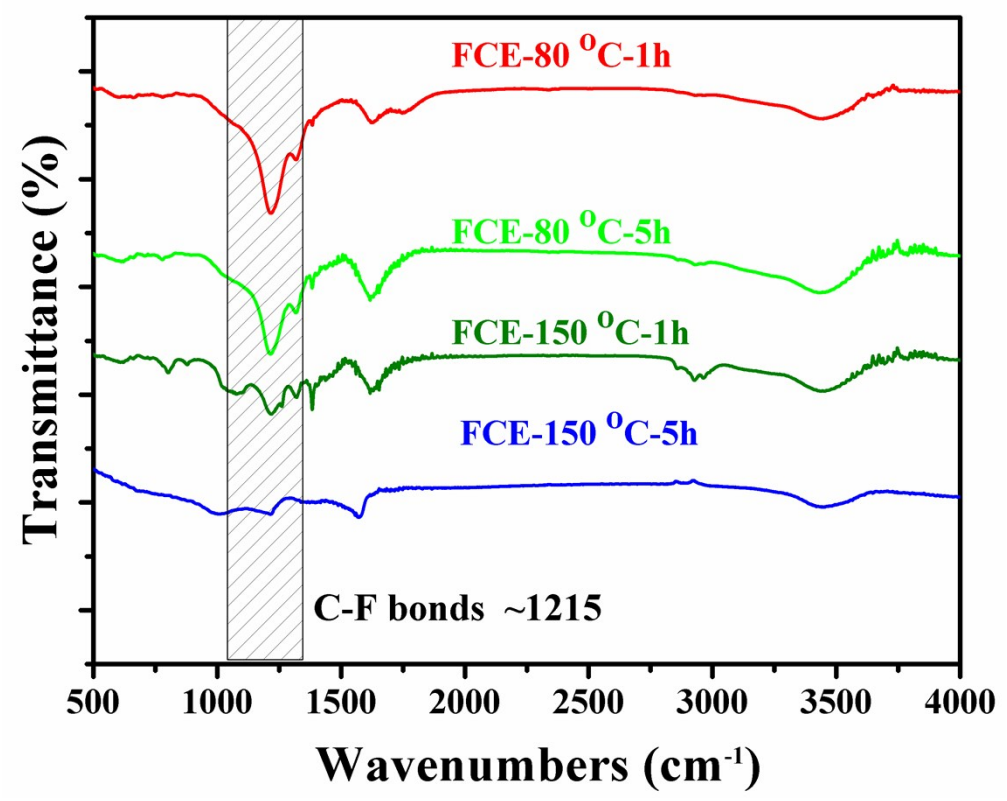

Fig. S1 FTIR spectra of FCE obtained at different reaction conditions.

2. Digital images of samples dispersed in water

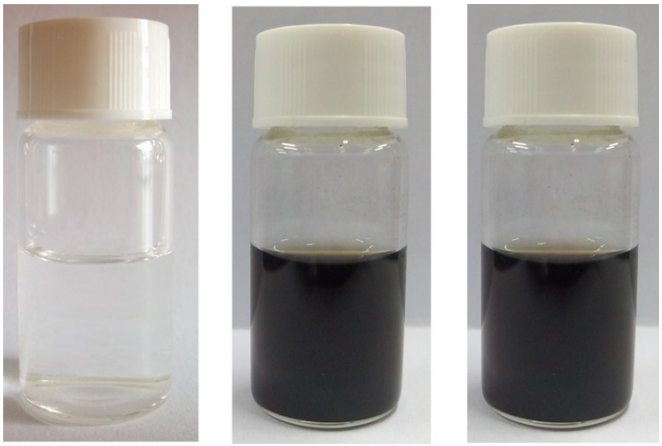

Fig. S2 Digital images of FC (The white sample floating on the water surface), FCE and FCO dispersed in water.

3. Zeta potential of FCE and FCO-PVP

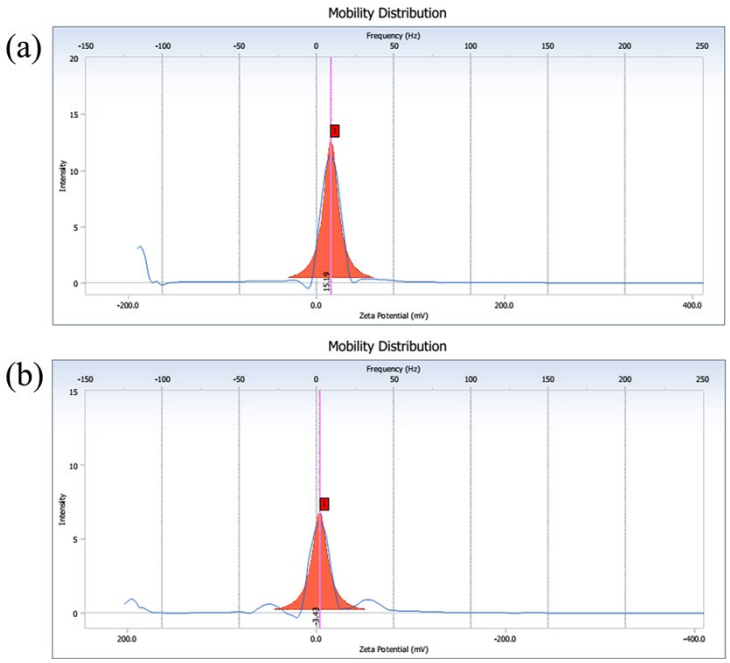

Fig. S3 Zeta potential of (a) FCE and (b) FCO-PVP.

4. Photothermal heating curves of FCO, GO and water solution.

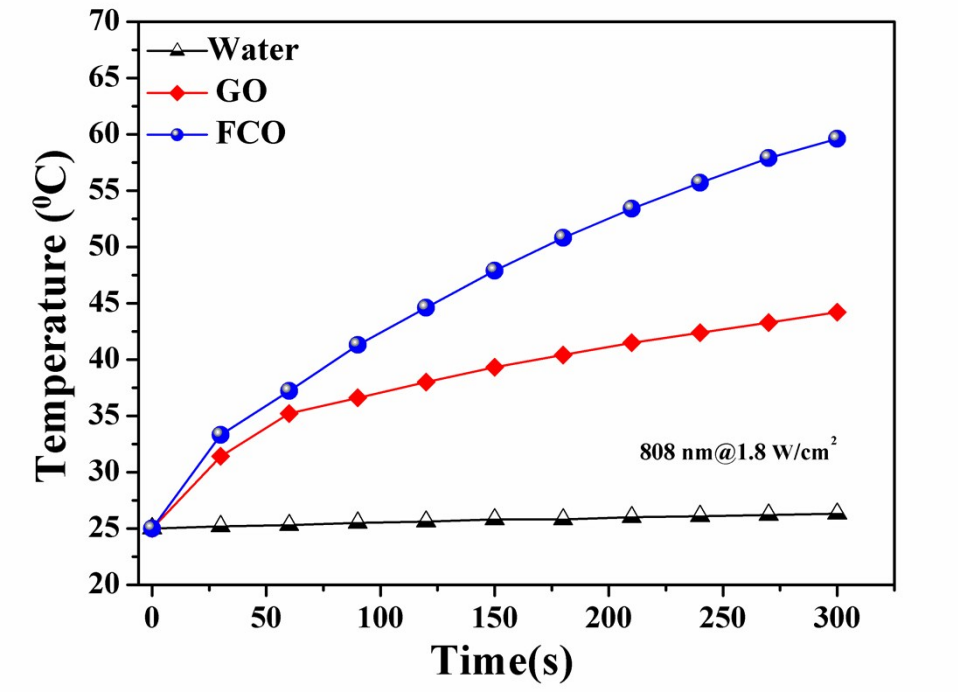

Fig. S4 Photothermal heating curves of FCO, GO and water under the same condition.

Fig. S3 showed photothermal heating curves of FCO, GO and water solution with a concentration of 100 μg mL^{-1} under a 1.8 W cm^{-2} NIR laser. FCO could be heated up to nearly 60 °C and in comparison the temperature of graphene oxide (GO) solution remained 43 °C within 300s,. And then FCO was synthesized by a facile and effective method without modified by photothermal materials and FCO had better photothermal conversion performance with a low concentration and power density in a shorter time compared to GO.

5. Drug loading ratio of FCO without being modified by PVP

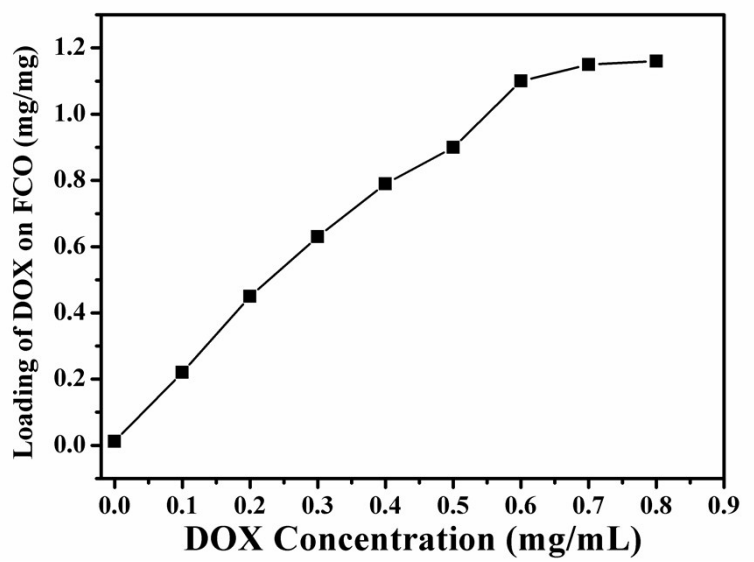

Fig. S5 The loading of DOX on FCO at different concentrations of DOX.

6. pH-triggerred and NIR enhanced drug release of FCO-PVP+DOX

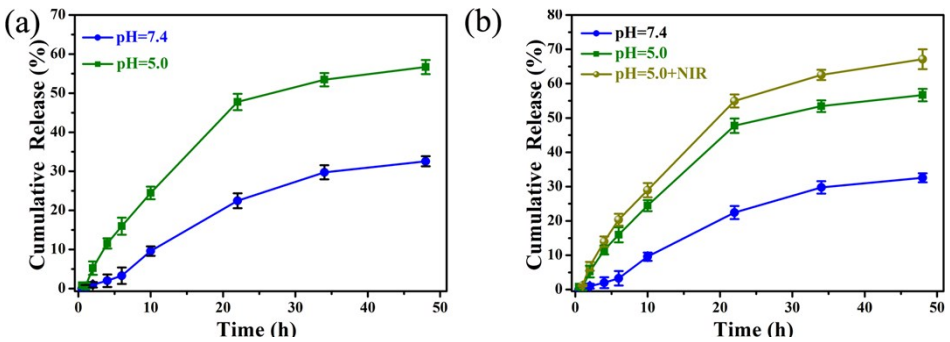

Fig. S6 (a) pH-responsive drug release of FCO-PVP+DOX. (b) NIR enhanced drug release.

7. Photoluminescence (PL) performance study of DOX and fluorescence microscopy images for cells incubated with FCO-PVP

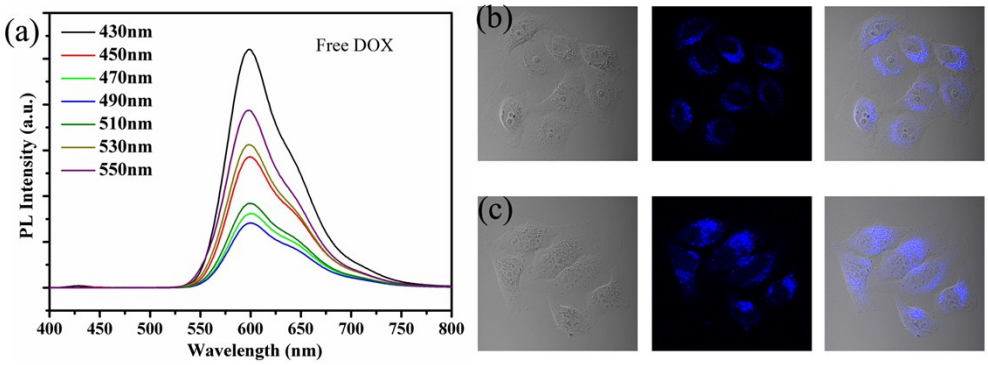

Fig. S7 (a) PL performance of DOX under different excitation wavelengths; cells incubated with FCO-PVP for (b) 0.5 h and (c) 4 h.

8. Cell viabilities of Hela cells under higher drug doses

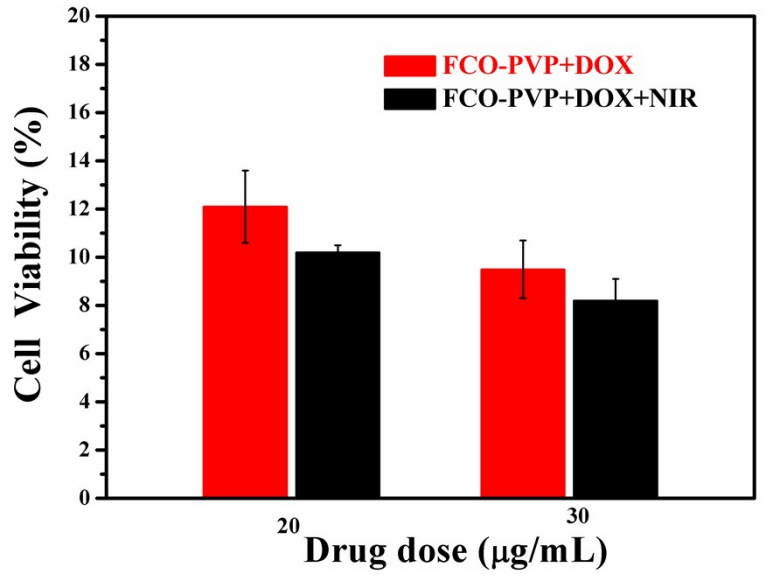

Fig. S8 Cell viabilities of Hela cells under higher drug doses for FCO-PVP+DOX and FCO-PVP+DOX+Laser.

Notes and references

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