Supporting Information

Chiral graphene-based supramolecular hydrogels toward tumor therapy

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1. Experimental section

1.1. Materials

Natural graphite powder was obtained from Qingdao Huarun Graphite Co., LTD, China (325 meshes). H_2SO_4 (purity≥98%), $KMnO_4$, $NaNO_3$, H_2O_2 (30%), NaOH, HCl (36–38%) were purchased from Aladdin Chemistry. Terephthaloyl Chloride, D/L-Phenylalanine Methyl Ester Hydrochloride, were purchased from Sigma-Aldrich Company. Ethanol (C_2H_5OH), Methanol (CH_3OH), Diethylene Glycol ($C_4H_{10}O_3$), Dichloromethane (CH_2Cl_2), Triethylamine ($C_6H_{15}N$) were purchased from Shanghai TITAN Technology Co., Ltd. All aqueous solutions were prepared using ultrapure water (18 MU) from a Milli-Q system (Millipore). Calcein-AM/propidium iodide (PI), Hoechst 33258 and Trypsin were purchased from Shanghai Shaoxin Biotechnology Co., Ltd. Fetal bovine serum (FBS) and DMEM cell culture medium were purchased from Thermo Fisher Scientific (China) Co., Ltd. Human breast cancer cells (T47D cells) were obtained from Shanghai FUHENG Biotechnology Co., Ltd. Human skin

fibroblasts (Hs27 cells) were obtained from Tongpai (Shanghai) Biotechnology Co., Ltd. All of the chemicals were used as received without further purification.

1.2. Preparation of D/LPFEG gelator

Terephthaloyl Chloride (2.6 g, 13.0 mmol) dissolved in DCM (20 mL) was slowly added to a 100 mL mixture of D-Phenylalanine Methyl Ester Hydrochloride (6.0 g, 26.2 mmol) and triethylamine (8.0 mL, 58.4 mmol) under magnetic stirring. After 24 h of stirring, the mixture was spinned to eliminate the excess solvent. The residual substance in the flask is dissolved in ethanol, then the insoluble substance p-Ph(D-Phe-OMe)$_2$ was obtained by filtering and drying. The intermediate product (5.1 g, 10.5mmol, 85%) is dissolved in methanol to form a suspension, and 15 mL of NaOH aqueous solution is added under stirring overnight to obtain a clear solution. Then, 3.0 M HCl was used to adjust the pH of the solution to obtain gel precipitation. The gel was filtered and washed multiple times with deionized water, and the p-Ph(D-Phe-OH)$_2$ (4.6 g, 9.9 mmol, 90%) was obtained after vacuum drying. Overall yield of DPF: 76.5%. ^{1}H NMR (500 MHz, DMSO-d6, δ, ppm): δ = 12.81 (s, 2H), 8.82 (d, J = 8.2 Hz, 2H), 7.83 (s, 4H), 7.34-7.29 (m, 4H), 7.26 (t, J = 7.6 Hz, 4H), 7.21-7.14 (m, 2H), 4.62 (ddd, J = 10.7, 8.1, 4.5 Hz, 2H), 3.20 (dd, J = 13.8, 4.4 Hz, 2H), 3.06 (dd, J = 13.8, 10.6 Hz, 2H). EI-MS for $C_{26}H_{24}O_6N_2$ calcd. 460.1634; found 461.1701 $[M+H]^+$, 921.3318 $[2M+H]^+$. Similarly, LPF was obtained as a white solid after freeze-drying (4.2 g, 82%). ^{1}H NMR (500 MHz, DMSO-d6, δ, ppm): δ = 12.84 (s, 2H), 8.85 (d, J = 8.1 Hz, 2H), 7.86 (d, J = 3.2 Hz, 4H), 7.37-7.31 (m, 4H), 7.29 (t, J = 7.6 Hz, 4H), 7.24-7.17 (m, 2H), 4.65 (ddd, J = 10.7, 8.1, 4.5 Hz, 2H), 3.23 (dd, J = 13.8, 4.5 Hz, 2H), 3.09 (dd, J = 13.8, 10.7 Hz, 2H). EI-

MS for C26H24O6N2 calcd. 460.1634; found 461.1704 $[M+H]^+$, 921.3323 $[2M+H]^+$.

p-Ph(D-Phe-OH)$_2$ (4.6 g, 9.9 mmol) was dissolved in diethylene glycol and 0.5 mL of concentrated HCl was added. After reacting at 130 ℃ for 3.5 h, the clarified solution was poured into the ice water and gel precipitation was produced. Finally, the sediment was filtered and washed with deionized water for several times, and DPFEG gelator (4.2 g, 6.6 mmol, 91%) was obtained in an oven. ^{1}H NMR (500 MHz, DMSO-d6, δ, ppm): δ = 8.97 (d, J = 7.8 Hz, 2H), 7.84 (s, 4H), 7.34-7.24 (m, 8H), 7.23-7.16 (m, 2H), 4.67 (ddd, J = 10.1, 7.8, 5.3 Hz, 2H), 4.57 (dt, J = 14.2, 5.5 Hz, 3H), 4.18 (qdd, J = 11.9, 6.0, 3.7 Hz, 4H), 3.63-3.49 (m, 4H), 3.52 – 3.38 (m, 12H), 3.18 (dd, J = 13.8, 5.3 Hz, 2H), 3.11 (dd, J = 13.8, 10.0 Hz, 2H). EI-MS for $C_{34}H_{40}O_{10}N_2$ calcd. 636.2720; found 659.2570 $[M+Na]^+$. LPFEG gelator (3.6 g, 5.6 mmol, 85%) was synthesized in the same way. ^{1}H NMR (500 MHz, DMSO-d6, δ, ppm): δ = 8.98 (d, J = 7.8 Hz, 2H), 7.85 (s, 4H), 7.34-7.25 (m, 8H), 7.23-7.16 (m, 2H), 4.68 (ddd, J = 10.1, 7.8, 5.3 Hz, 2H), 4.59 (t, J = 5.4 Hz, 2H), 4.19 (qdd, J = 11.9, 5.9, 3.7 Hz, 4H), 3.63-3.56 (m, 2H), 3.55 (ddd, J = 11.4, 6.0, 4.0 Hz, 2H), 3.47 (q, J = 5.3 Hz, 4H), 3.41 (dd, J = 5.5, 3.9 Hz, 4H), 3.19 (dd, J = 13.8, 5.3 Hz, 2H), 3.11 (dd, J = 13.8, 10.0 Hz, 2H). EI-MS for $C_{34}H_{40}O_{10}N_2$ calcd. 636.2720; found 659.2566 $[M+Na]^+$.

1.3. Preparation of GO

Briefly, 2 g of graphite powder was first combined with 1 g of $NaNO_3$, and was then added into the appropriate amount of concentrated H_2SO_4 while stirring in an ice bath. Subsequently, 6 g of $KMnO_4$ was added in several times and the temperature was maintained under 20 ℃. After the mixture was stirred overnight, 150 mL of deionized

water (DI) was slowly poured, and then 15 mL of 30 wt.% H_2O_2 was added to reduce the residual oxidant. Finally, the mixture was filtered and washed with 5% HCl solution several times to remove the overabundance of residual ions. The product was kept in a vacuum drying oven at 80 ℃ for storage.

1.4. In vitro cytotoxicity study.

Human skin fibroblasts are used to evaluate the biotoxicity of the materials, while human breast cancer cells are used to assess the antitumor effect of the composite hydrogels. The cytotoxicity of the materials to HS27 cells and T47D cells was detected by CCK-8 method after culture within the specified time. The relative cell viability was calculated as following Equation (1):

Cell viability (%) = [(As-Ab) / (Ac-Ab)] × 100% (1)

Where As is the absorbance of experimental well, containing cell culture medium, CCK-8 and the substance to be tested. Ab is a blank well, without cell culture medium, CCK-8 and substance. Ac is the control group, containing cell culture medium and CCK-8, without substance to be tested.

2. Additional Experimental Data and Figures

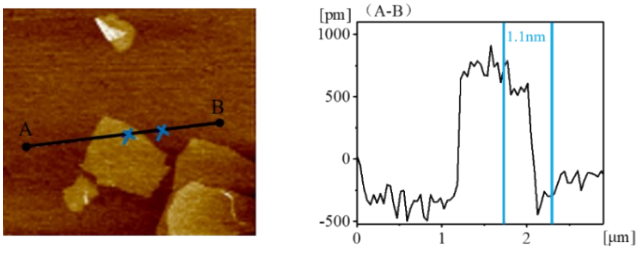


Figure S1. AFM image of the GO sheets.

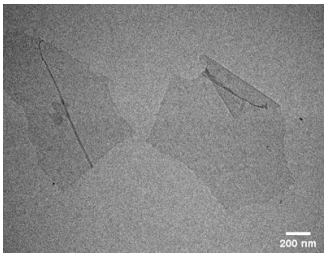


Figure S2. TEM image of the GO sheets.

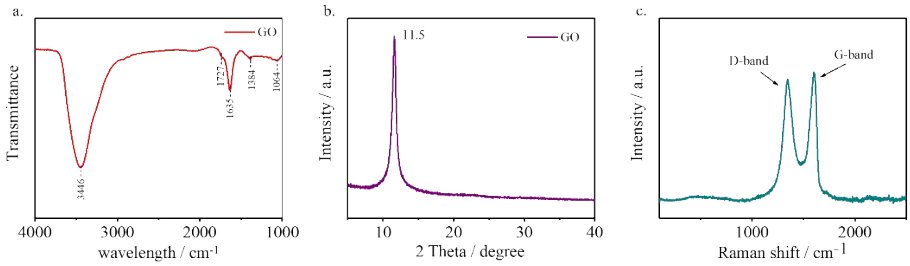


Figure S3. GO characterizations. (a) Fourier Transform infrared spectroscopy, (b) X-ray diffraction and (c) Raman spectra of GO.

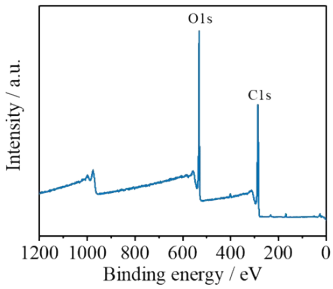


Figure S4. X-ray photoelectron spectroscopy (XPS) survey spectrum of GO.

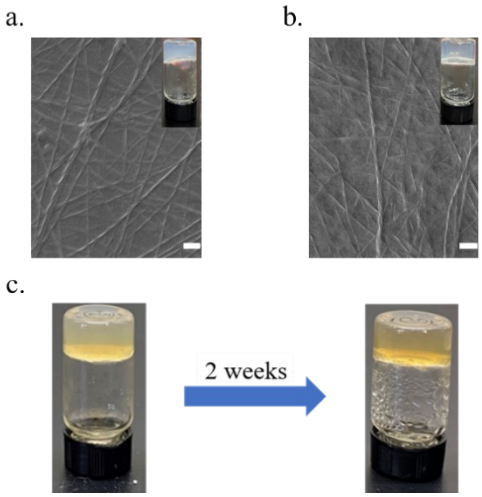


Figure S5. SEM images of (a) LPFEG and (b) DPFEG hydrogels. (c) The stability of

DPFEG-GO hydrogel after 2 weeks. (Scale bar: 0.5 μm).

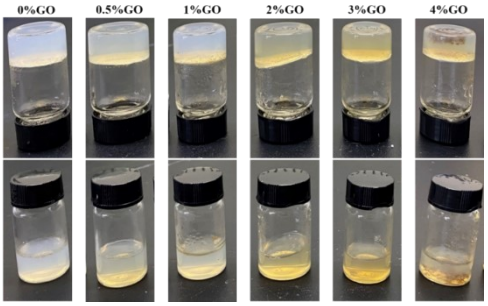


Figure S6. Photographs of GO with different proportions.

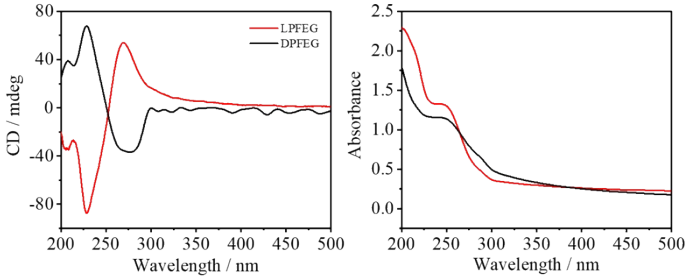


Figure S7. CD spectra and corresponding UV absorption of LPFEG and DPFEG hydrogels.

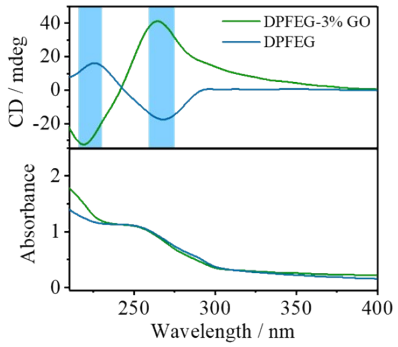


Figure S8. CD and corresponding UV-vis spectra of DPFEG and DPFEG-3% GO.

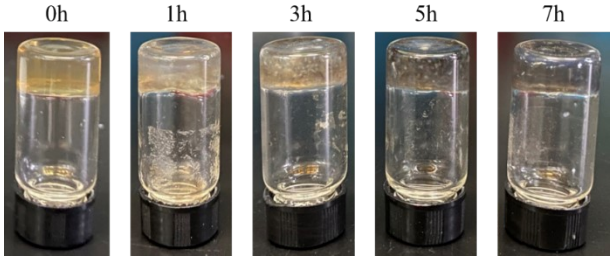


Figure S9. Photographs of DPFEG-GO hydrogel under NIR light irradiation with different time intervals.

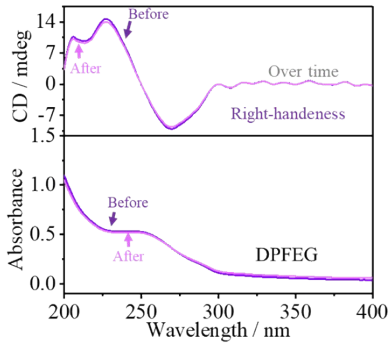


Figure S10. CD spectra and corresponding UV absorption of DPFEG hydrogels after NIR.

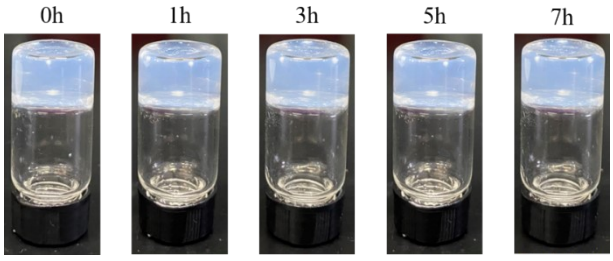


Figure S11. Photographs of DPFEG hydrogel under NIR light irradiation with different time intervals.

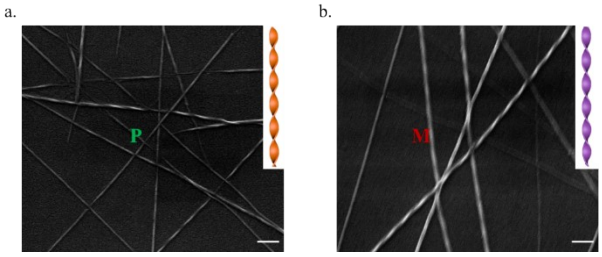


Figure S12. SEM images of (a) DPFEG hydrogel (b) DPFEG-GO hydrogel. (Scale bar: 0.5 μm).

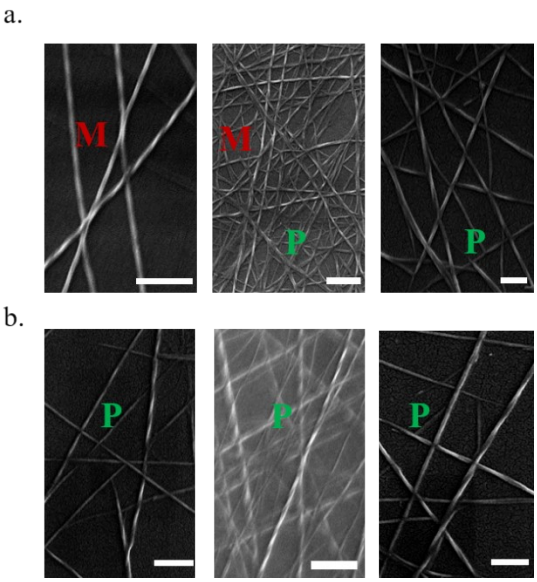


Figure S13. SEM images of (a) DPFEG-GO hydrogel (b) DPFEG hydrogel with extension of NIR light irradiation. (Scale bar: 1 μm).

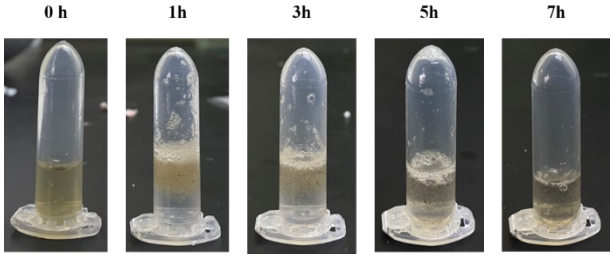


Figure S14. Images of time-dependent change in DPFEG-GO hydrogel during drug release process.

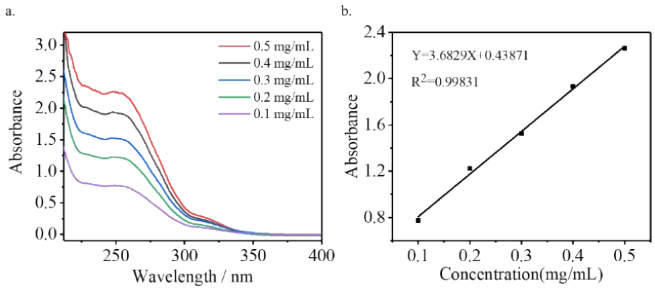


Figure S15. (a) UV-vis absorption of oxaliplatin at different concentrations. (b) Standard calibration curve of oxaliplatin.

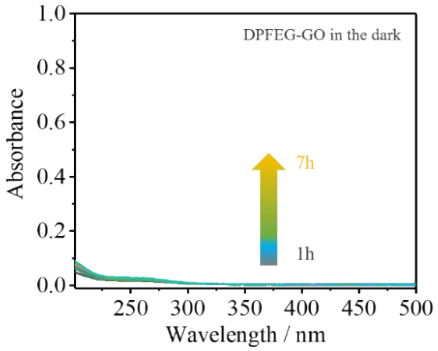


Figure S16. UV-vis detection of released R-OXA from DPFEG-GO hydrogel under NIR light irradiation with different time.

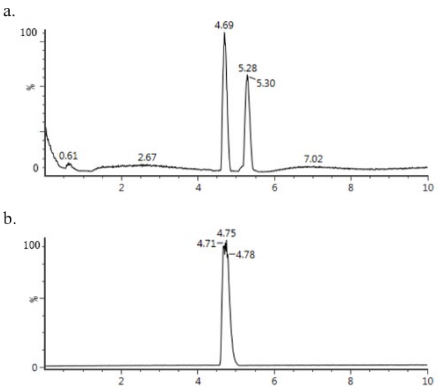


Figure S17. UPLC-TOF-MSe chromatograms of (a) OXA racemates and (b) corresponding solution desorbed from DPFEG-GO hydrogel.

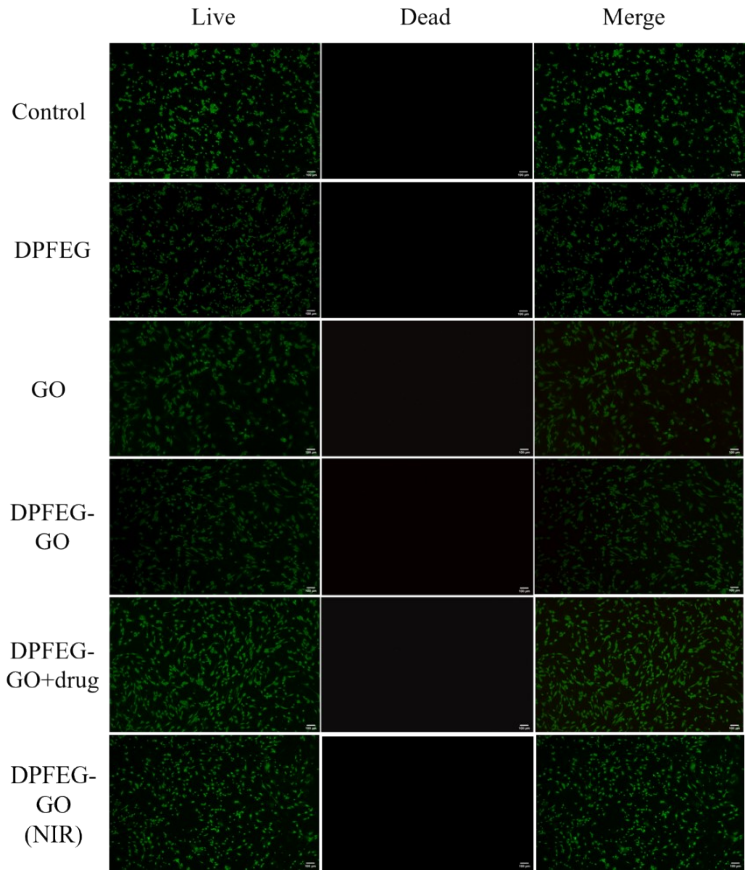
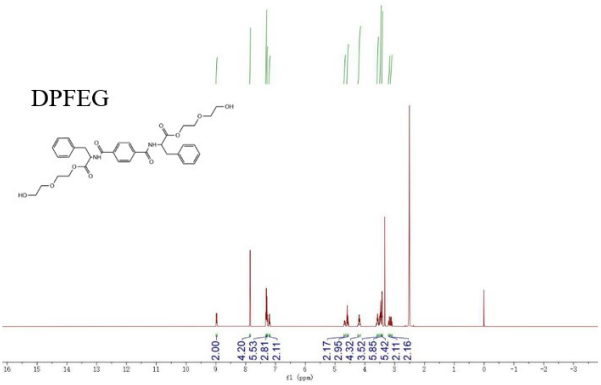


Figure S18. Fluorescence images of calcein co-stained Hs27 cells with various hydrogels (cells without any treatment is used as control). Scale bar: 100 μm.



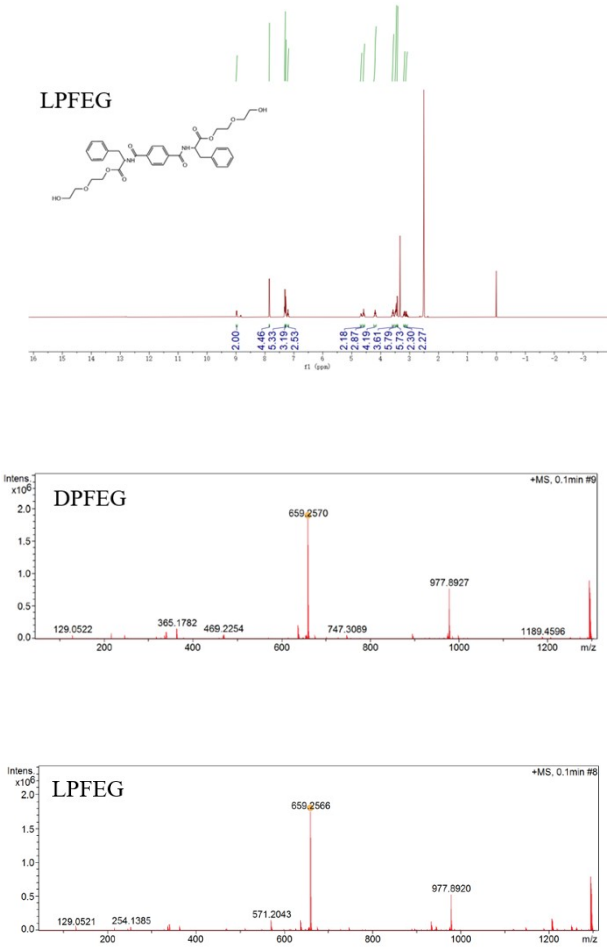


Figure S19. ^{1}H NMR and EI-MS measurement of D/LPFEG.

References

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