Supporting Information

Dual-prodrug cascade activation for chemo/chemodynamic mutually beneficial combination cancer therapy

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Supplementary Experimental Section

Materials. Cinnamaldehyde (CA), 2-hydroxyethyl methacrylate (HEMA), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl), 4-(dimethylamino) pyridine (DMAP), methacryloyl chloride, 4-nitrophenyl chloroformate, 4-(hydroxymethyl) phenylboronic acid pinacol ester and doxorubicin hydrochloride (DOX·HCl) were purchased from Energy Chemical. 1, 1, 1-tris (hydroxymethyl)ethane, ferrocene-carboxylate (Fc) and 4-Cyano-4-(phenylcarbonothioylthio)pentanoic acid were purchased from Aladdin. 2,2'-

azobisisobutyronitrile (AIBN), 2-(diisopropylamino) ethyl methacrylate (DPA) and $mPEG_{2000}$ were obtained from Sigma-Aldrich. 2',7'-dichlorofluorescin diacetate (DCFH-DA), calcein-AM/pyridine iodide (PI) live/dead cell staining kit, 4′,6-diamidino-2-phenylindole (DAPI), JC-1 probe and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were obtained from Solarbio.

Synthesis of CAMA monomer and amphiphilic polymer PEG-PCA. The PEG-RAFT agent was synthesized via esterification reaction between $mPEG_{2000}$ and 4-Cyano-4-(phenylcarbonothioylthio) pentanoic acid in the presence of EDC·HCl and DMAP (Scheme S1). After reaction, an excess of diethyl ether was added to the mixture for precipitation. The ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of PEG-RAFT agent was shown in Figure S1. The appearance of characteristic peaks in ^{1}H NMR indicated successful synthesis. ^{1}H NMR (400 MHz, Chloroform-d): δ 7.95-7.88 (m, 2H), 7.60-7.53 (m, 1H), 7.44-7.38 (m, 2H), 4.31-4.23 (m, 2H), 3.65 (s, 176H), 3.38 (s, 3H), 1.94 (s, 3H).

The (5-methyl-2-styryl-1,3-dioxan-5-yl) methyl methacrylate (CAMA) monomer was synthesized according previous report via a two-step procedure (Scheme S2).[1] First, 1,1,1-tris (hydroxymethyl) ethane (16 mmol) and CA (8 mmol) were dissolved in anhydrous tetrahydrofuran (60 mL) containing 5 Å molecular sieves. Then added p-toluenesulfonic acid (0.8 mmol) was added into the solution under stirring. After 12 h of reaction at room temperature, triethylamine was added to terminate the reaction. The product was purified by silica gel chromatography (hexanes/ethyl acetate = 3:1),

obtaining white precursor CA-1. Secondly, CA-1 (2 mmol) and triethylamine (4 mmol) were dissolved in anhydrous dichloromethane (40 mL) and cooled in an ice-water bath. Methacryloyl chloride (4 mmol) was dropwise added into the mixture, then the mixture was stirred overnight at room temperature. The product was purified by silica gel chromatography (hexanes/ethyl acetate = 4:1). The ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of CAMA was shown in Figure S2.

Then the amphiphilic polymer PEG-PCA was synthesized using Reversible Addition-Fragmentation Chain Transfer (RAFT) polymerization method (Scheme S2). PEG-RAFT agent (0.02 mmol), CAMA (0.4 mmol) and AIBN (0.005 mmol) were dissolved into *N,N*-dimethylformamide (DMF) (3 mL) and added into a flask. The flask was sealed under argon and the reaction was carried out at 70 ℃ for 24 h. The PEG-PCA was obtained by dialysis against pure water and lyophilization. The ^{1}H-NMR (400 MHz, DMSO-d6) spectrum of PEG-PCA was shown in Figure S3.

Synthesis of FcMA monomer and amphiphilic block copolymer PEG-*b*-P(DPA-*co*-Fc). The 2-(methacryloyloxy) ethyl ferrocene-carboxylate (FcMA) monomer was synthesized according previous method (Scheme S3).[2] Fc (8 mmol), HEMA (16 mmol) and DMAP (1 mmol) were dissolved in anhydrous dichloromethane (DCM) (100 mL). The mixture was cooled to 0 °C under argon flow and stirred for 30 min. EDC·HCl (16 mmol) in anhydrous DCM was dropwise added into the mixture. The mixture was stirred at 0 °C for 1 h and then at room temperature for 24 h. The product was purified by silica gel chromatography (hexanes/ethyl acetate = 12:1),

obtaining orange solid FcMA. The ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of FcMA was shown in Figure S4.

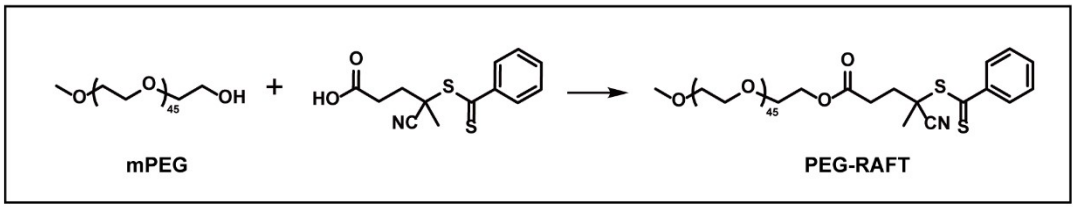
The amphiphilic block copolymer PEG-*b*-P(DPA-*co*-Fc) was also synthesized via RAFT polymerization (Scheme S3). PEG-RAFT agent (0.02 mmol), FcMA (0.2 mmol), DPA (0.8 mmol), and AIBN (0.005 mmol) were dissolved into 1,4-dioxane (3 mL). The flask was sealed under argon and the reaction was carried out at 80 ℃ for 24 h. The PEG-*b*-P(DPA-*co*-Fc) was obtained by dialysis against pure water and lyophilization. The ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of PEG-*b*-P(DPA-*co*-Fc) was shown in Figure S5.

Synthesis of BDOX. The BDOX was synthesized according the previous literature (Scheme S4).[3] Firstly, 4-(hydroxymethyl) phenylboronic acid pinacol ester (2 mmol) and DMAP (3 mmol) were dissolved in anhydrous DCM (15 mL) and cooled to 0 °C. 4-Nitrophenyl chloroformate (3 mmol) in anhydrous DCM was dropwise added into the mixture. After 12 h of reaction at room temperature, the product was purified by silica gel chromatography (hexanes/ethyl acetate = 10:1), obtaining white precursor NCPB. The ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of NCPB was shown in Figure S6. Secondly, DOX·HCl (0.34 mmol), NCPB (0.52 mmol) and triethylamine (1.03 mmol) were dissolved in of anhydrous DMF (5 mL) and the reaction was carried out overnight at room temperature in the dark. The product was purified by silica gel chromatography (DCM: methanol = 25:1) to obtain BDOX as a red powder. The ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of BDOX was shown in Figure S7.

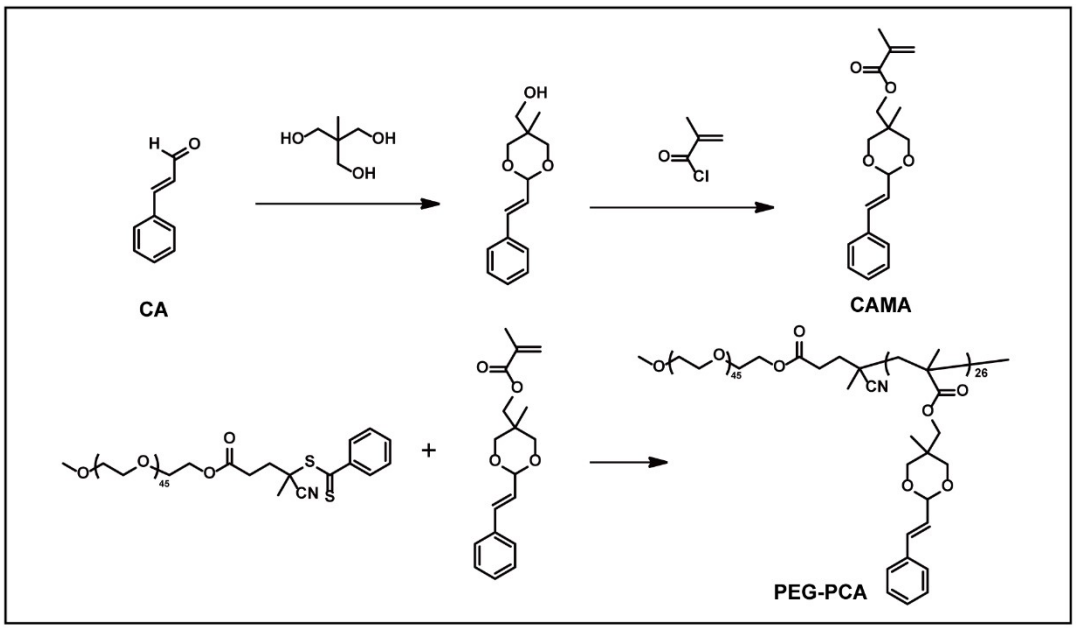
Preparation and characterization of BDOX@PFc-PCA NPs. PEG-*b*-P(DPA-*co*-Fc) (2 mg), PEG-PCA (1 mg) and BDOX (0.2 mg) were dissolved in THF (1 mL). Then the mixture was dropwise added into deionized water (4 mL) under stirring. The solution was dialyzed (MWCO: 3.5 kDa) against deionized water to remove organic solvent. Other control groups, including PFc NPs, PCA NPs and PFc-PCA NPs, were prepared by the similar experimental steps.

Characterizations. ^{1}H-NMR spectra were recorded on 400 MHz Bruker®. The morphology was characterized by transmission electron microscope (FEI, Tecnai G2 F20). The size and zeta potential were measured by laser particle size analyzer (Malvern, Zetasizer nano ZS90). UV-vis absorption spectra were measured by UV-vis absorption spectrometer (Hitachi, UH5300). Laser confocal scanning microscope images were determined by Zeiss laser scanning confocal microscope (UltraView Vox).

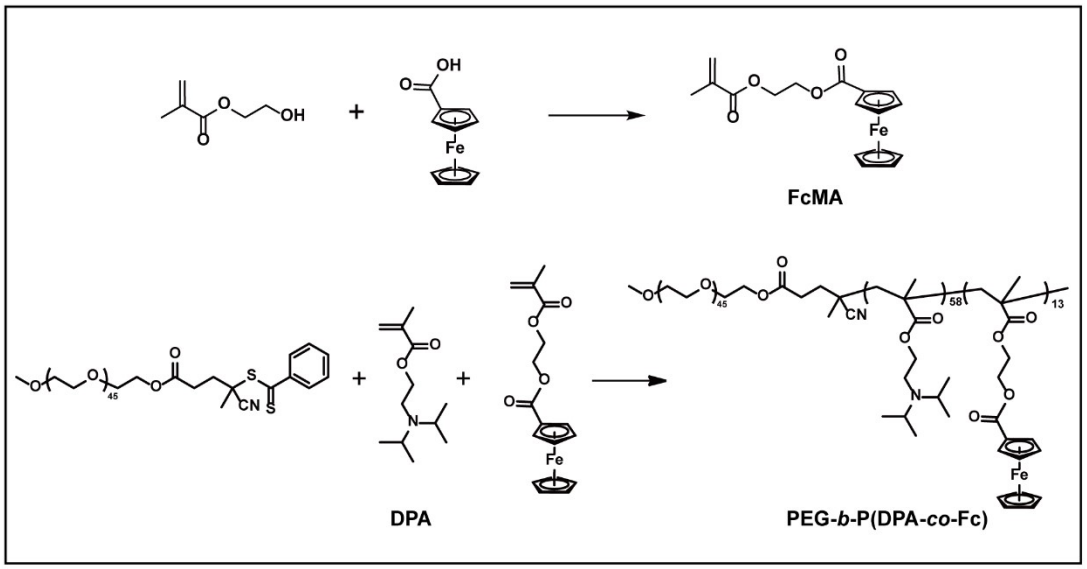
Supplementary Figures



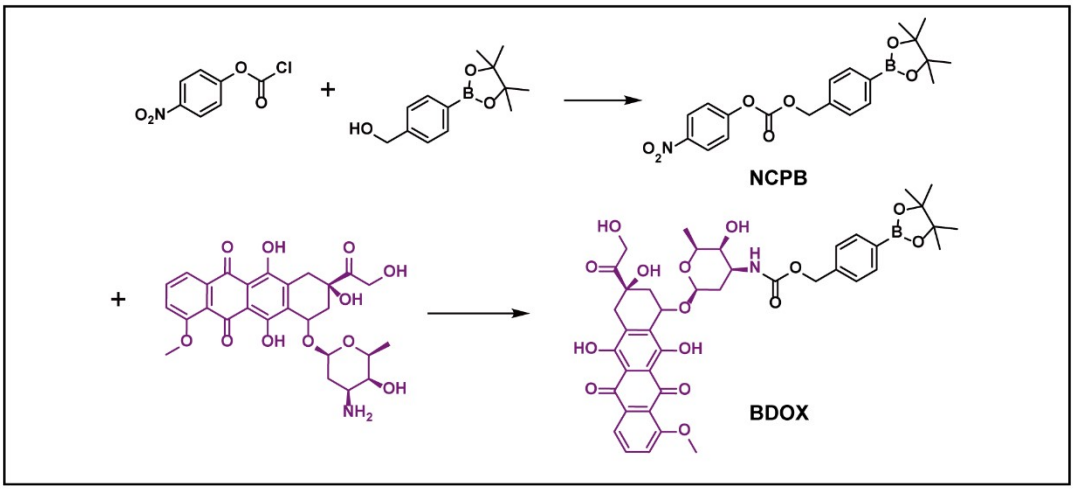
Scheme S1. Synthesis process of PEG-RAFT.



Scheme S2. Synthesis process of PEG-PCA.



Scheme S3. Synthesis process of PEG-*b*-P(DPA-*co*-Fc).



Scheme S4. Synthesis process of BDOX.

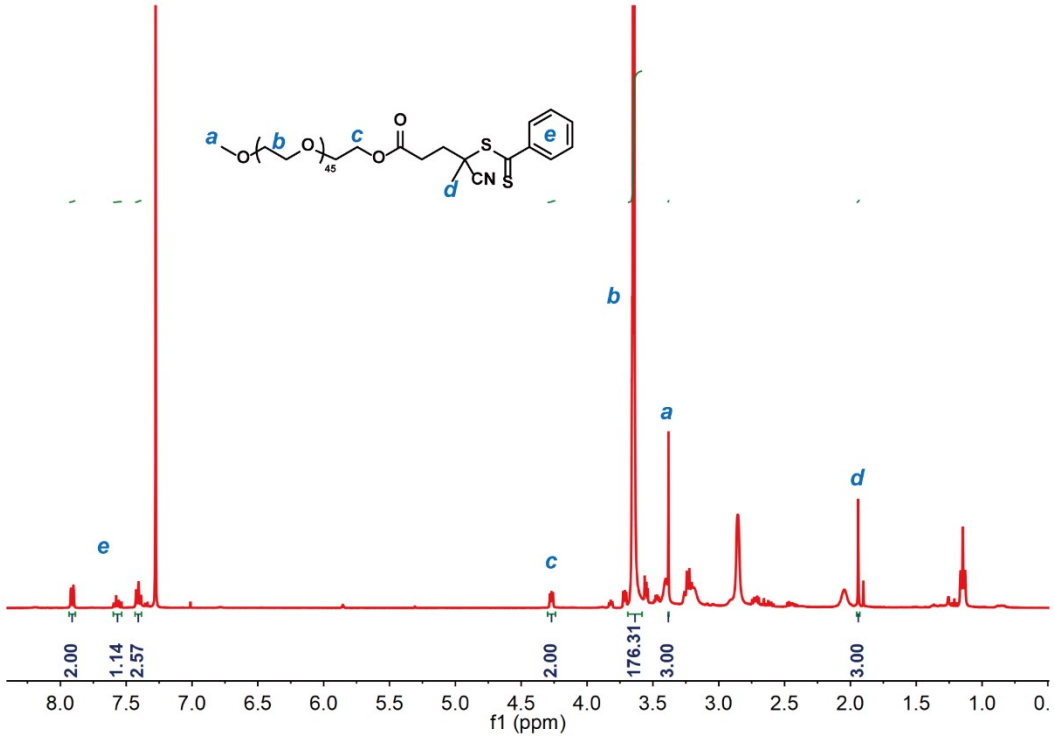


Fig. S1 ^{1}H NMR spectrum of PEG-RAFT.

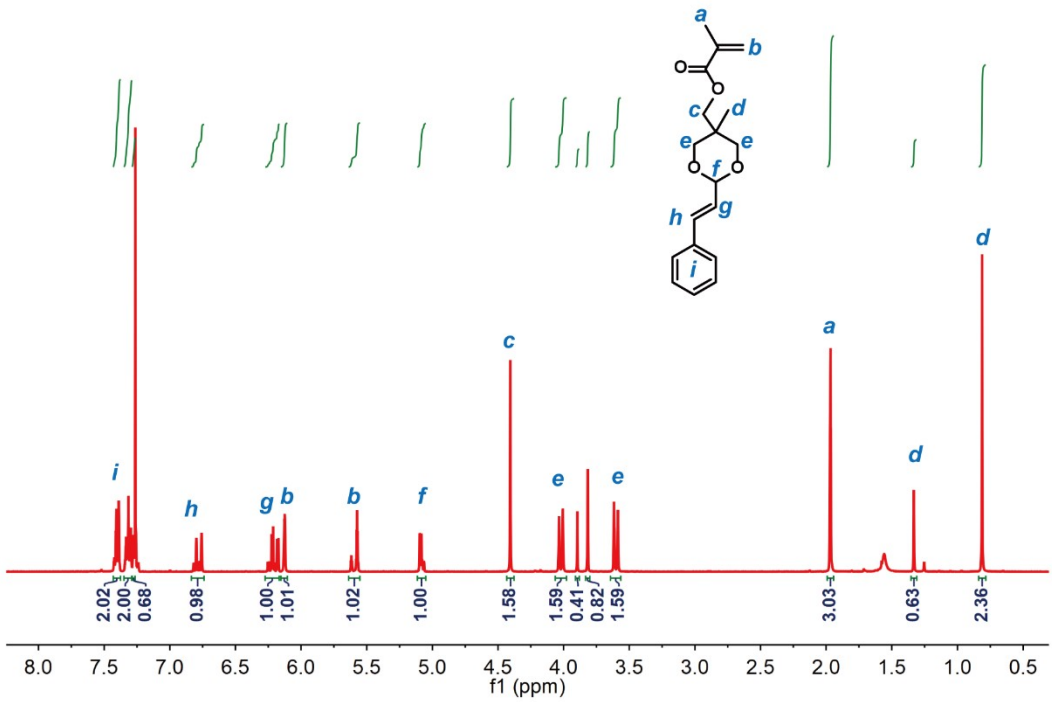


Fig. S2 ^{1}H NMR spectrum of CAMA.

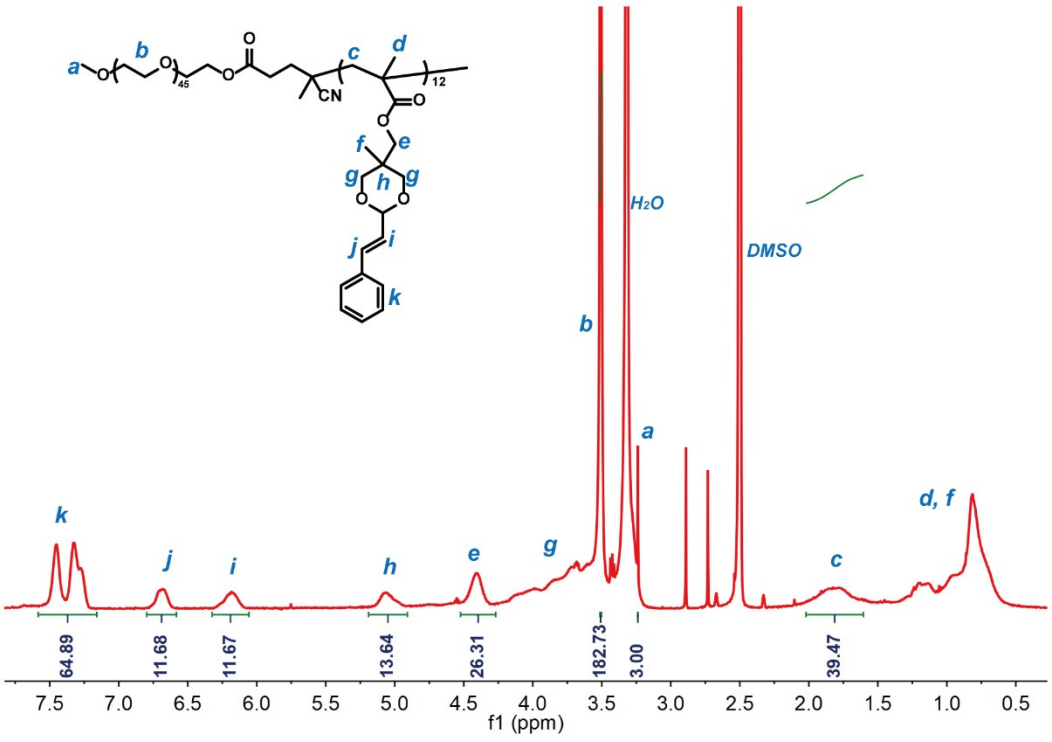


Fig. S3 ^{1}H NMR spectrum of PEG-PCA.

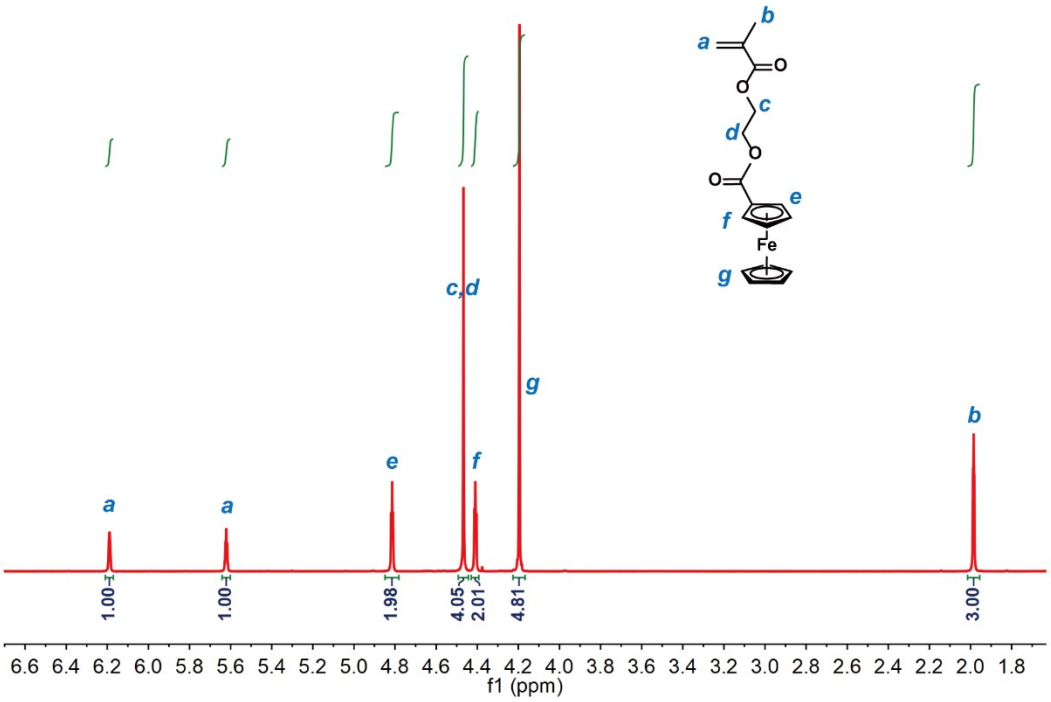


Fig. S4 ^{1}H NMR spectrum of FcMA.

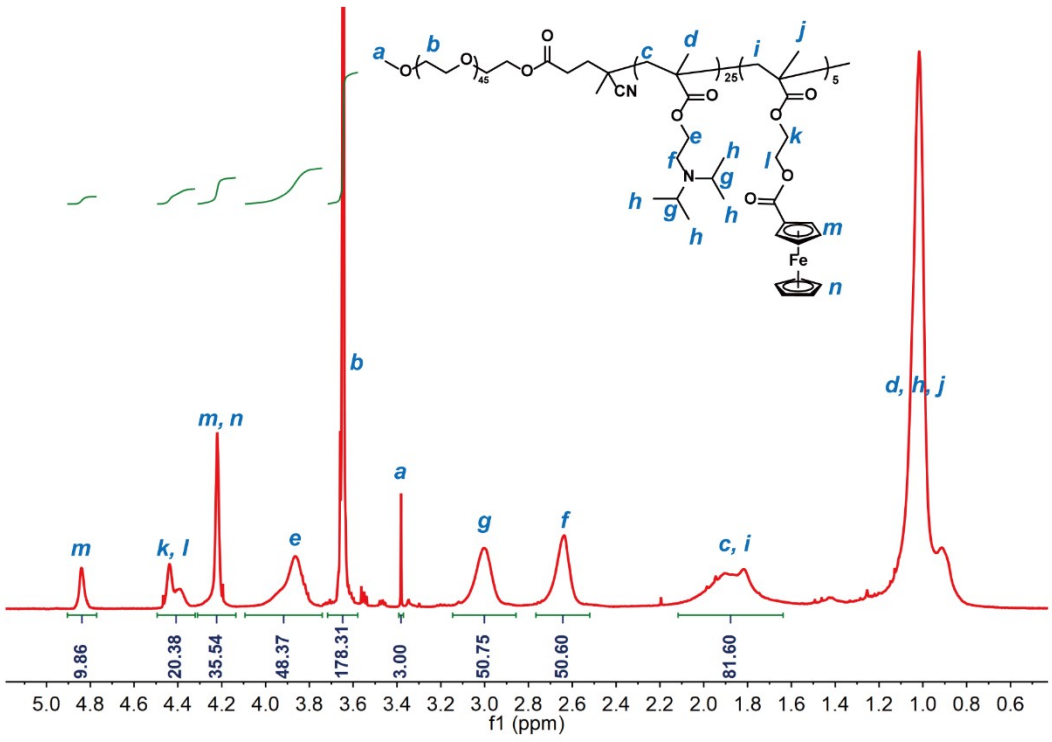


Fig. S5 ^{1}H NMR spectrum of PEG-*b*-P(DPA-*co*-Fc).

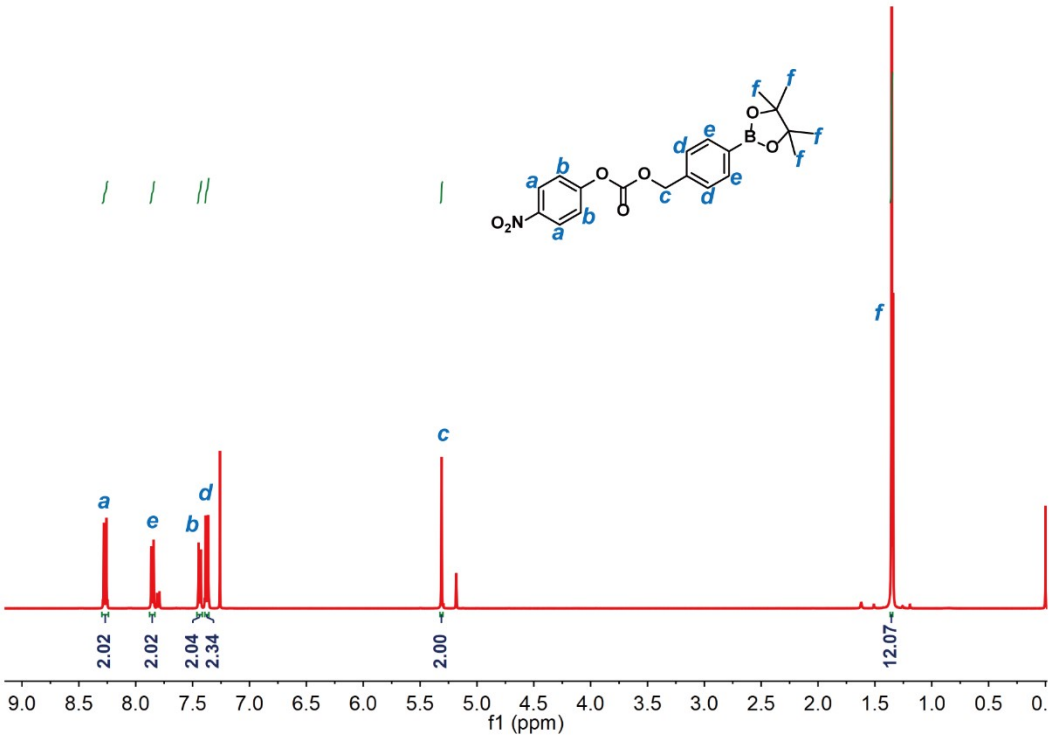


Fig. S6 ^{1}H NMR spectrum of NCPB.

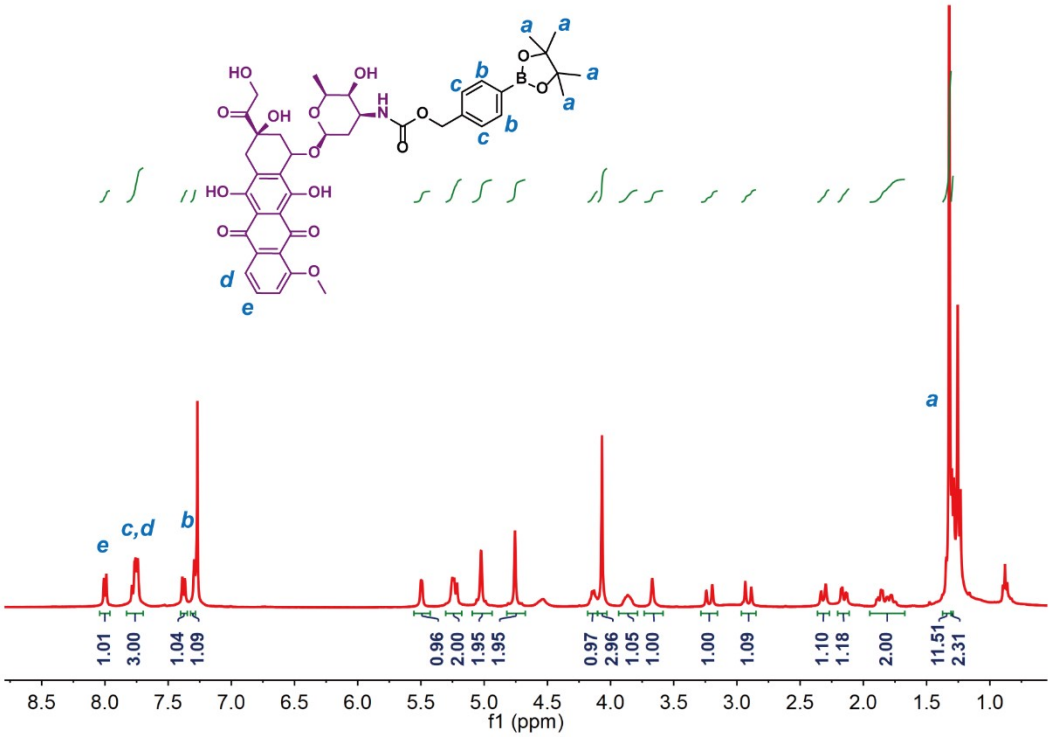


Fig. S7 ^{1}H NMR spectrum of BDOX.

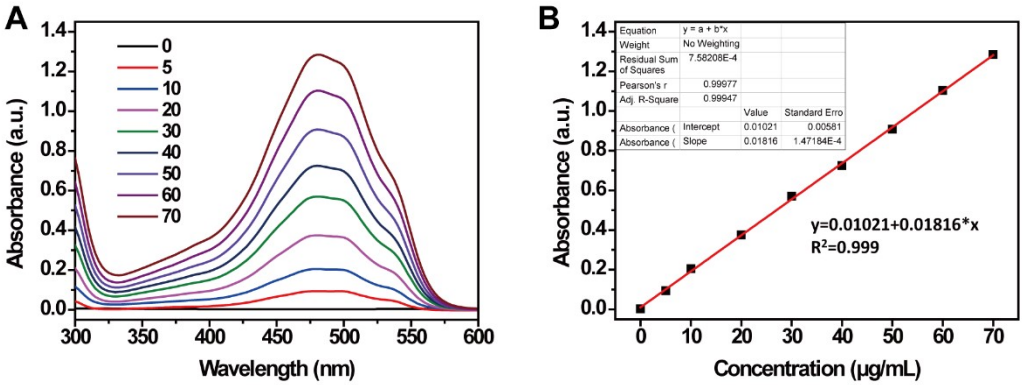


Fig. S8 The UV–vis absorption spectra (A) and standard curve (B) of DOX.

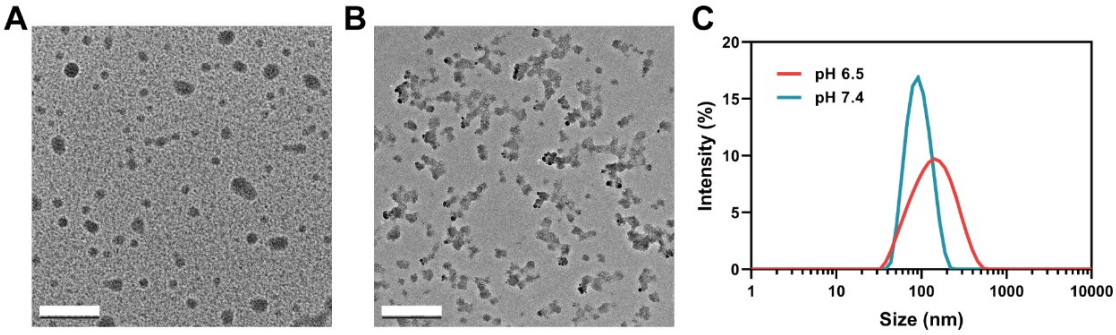


Fig. S9 TEM images of BDOX@PFc-PCA NPs at pH 7.4 (A) and pH 6.5 (B). Scale bars: 200 nm. (C) Relevant size distribution analyzed by DLS.

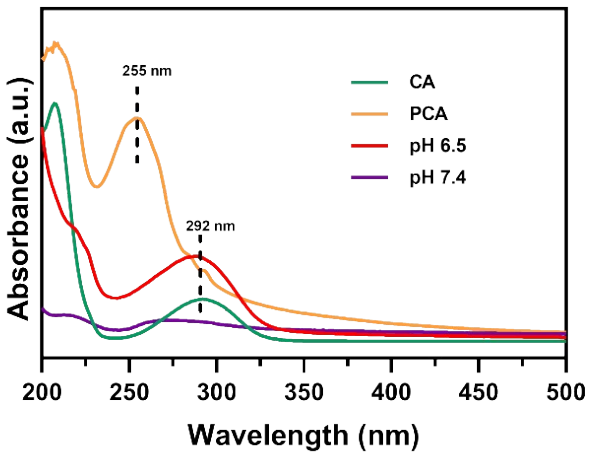


Fig. S10 CA release analysis by UV–vis spectroscopy.

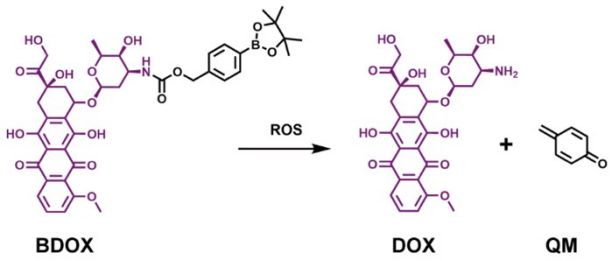


Fig. S11 Structure change of BDOX under the ROS stimulus.

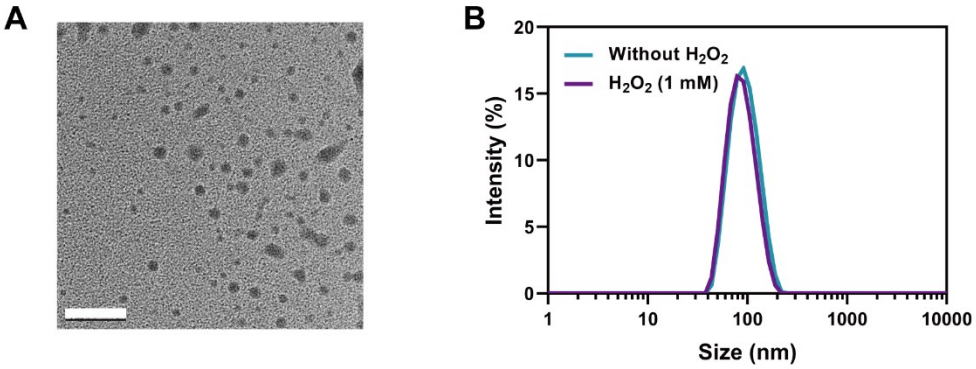


Fig. S12 (A) TEM image of BDOX@PFc-PCA NPs treated with H_2O_2 (1 mM). Scale bar: 200 nm. (B) Relevant size distribution analyzed by DLS.

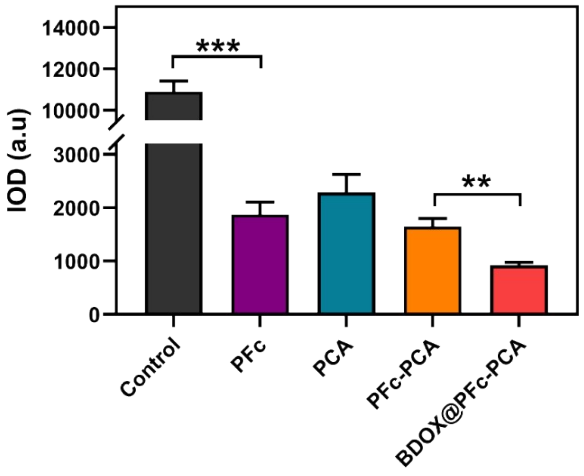


Fig. S13 Integrated optical density (IOD) of GSH analyzed by Image J. *p < 0.05, **p < 0.01, ***p < 0.001 (t-test).

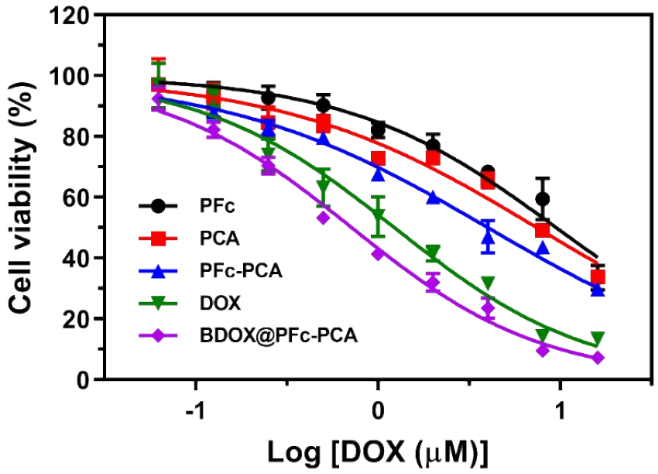


Fig. S14 Viability of 4T1 cells incubated with different samples for 48 h.

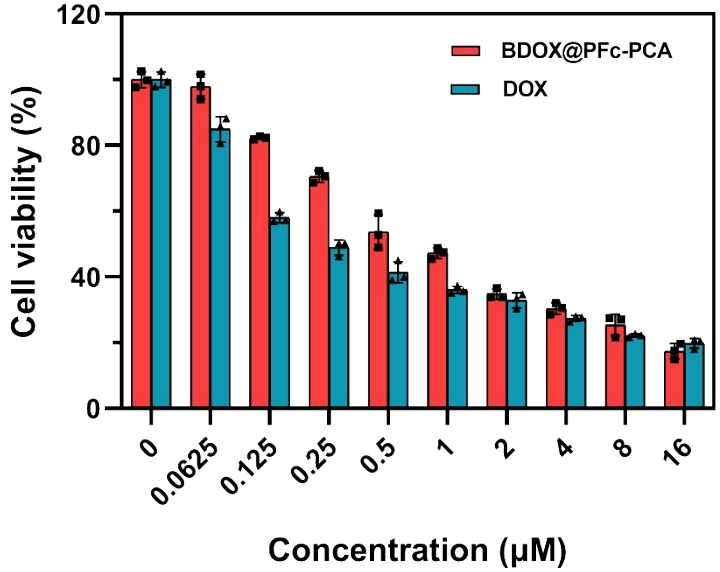


Fig. S15 Viability of 3T3 cells incubated with different samples for 48 h.

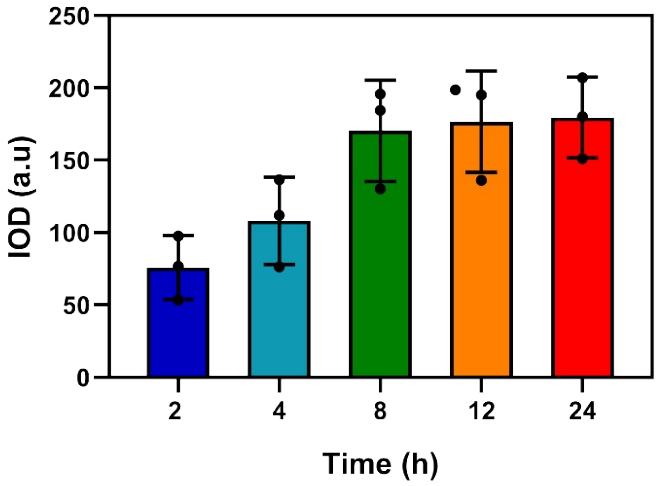


Fig. S16 The fluorescence intensities of tumor site at different time points (analyzed by Image J).

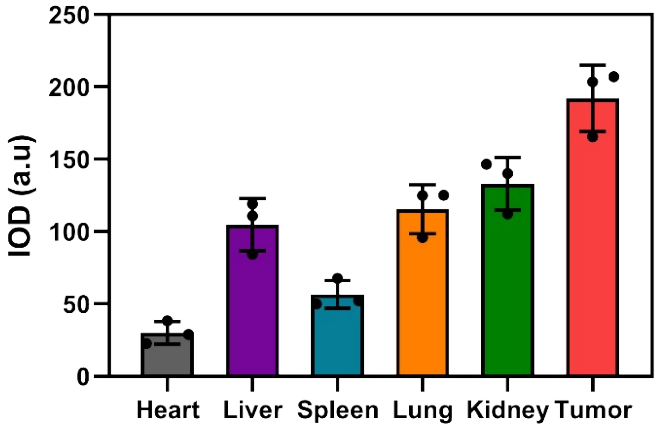


Fig. S17 The ex vivo fluorescence intensities of major organs and tumor at 24 h post-injection (analyzed by Image J).

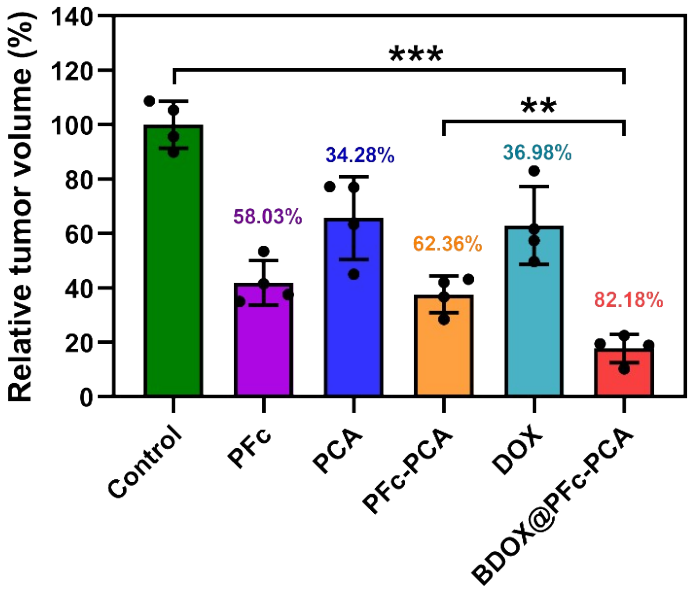


Fig. S18 The inhibition rate of tumor growth (IRG) at the end of treatment (**p < 0.01, ***p < 0.001).

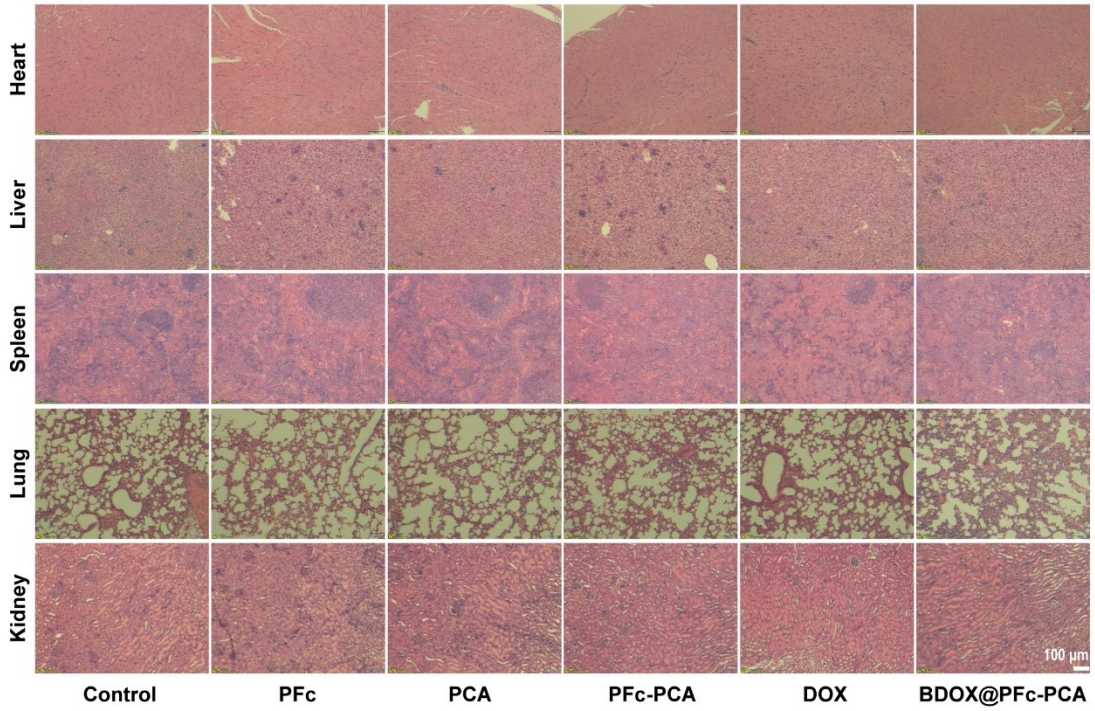


Fig. S19 H&E staining images of major organs (heart, liver, spleen, lung and kidney) after different treatments.

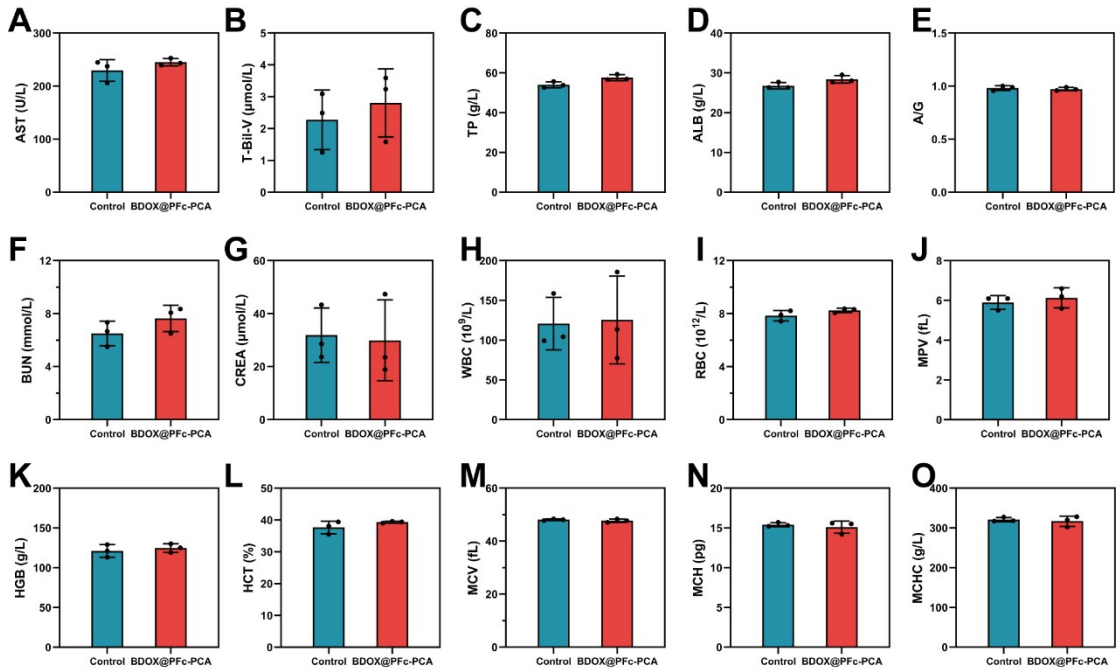


Fig. S20 (A-G) Biochemical indexes: (A) AST; (B) T-Bil-V; (C) TP; (D) ALB; (E) A/G; (F) BUN; (G) CREA. (H-O) Routine blood analysis: (H) WBC; (I) RBC; (J) MPV; (K) HGB; (L) HCT; (M) MCV; (N) MCH; (O) MCHC.

REFERENCES

1. Z. Feng, J. Guo, X. Liu, H. Song, C. Zhang, P. Huang, A. Dong, D. Kong and W. Wang, *Biomaterials*, 2020, **255**, 120210
2. L. Liu, L. Rui, Y. Gao and W. Zhang, *Polymer Chemistry*, 2015, **6**, 1817-1829.
3. M. Ye, Y. Han, J. Tang, Y. Piao, X. Liu, Z. Zhou, J. Gao, J. Rao and Y. Shen, *Adv. Mater.*, 2017, **29**, 1702342.