Supporting Information

Erythrocyte Membrane-camouflaged Fluorescence Covalent Organic Framework for Starving/Nitric Oxide/Immunotherapy of Triple-Negative Breast Cancer

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Materials and methods

Materials.

4,4',4"-triformyltrphenylamine (TFTPA), 1,3,5-tris(4-aminophenyl)triazine (TAPT), glucose, Hu, *N*-(1-naphthyl) ethylenediamine dihydrochloride, sulfanilic acid, titanium oxysulfate - sulfuric acid hydrate were purchased from Aladdin Industrial Co. Ltd. (Shanghai, China). 1,4-dioxane, acetic acid (HAc), phosphoric acid, mesitylene, methanol (MeOH), tetrahydrofuran (THF), $NaNO_2$, and H_2O_2 were purchased from Sinopharm Chemical Reagent Co. Ltd. GOx and horseradish peroxidase (HRP) were purchased from Shanghai Yuanye Bio-Technology Co. Ltd. (Shanghai, China). DSPE-PEG2000-FA and DSPE-PEG2000-Cy5 were purchased from Chongqing Yusi Pharmaceutical Technology Co. Ltd. (Chongqing, China). 2, 2'-azino-di(3-ethylbenzthiazoline) sulfonate (ABTS) was purchased from Aladdin Industrial Co. Ltd. (Shanghai, China). Dulbecco's modified Eagle medium (DMEM) was obtained from Sangon Biotech Co. Ltd. (Shanghai, China). Fetal bovine serum (FBS) was purchased from Dingguo Biotech Co. Ltd. (Shanghai, China). CPG (1826) oligodeoxynucleotides with the sequence of 5'-TCCATGACGTTCCTGACGTT-3' were synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China). CCK-8 kit, 3-amino, 4-aminomethyl-2',7'-difluorescein, diacetate (DAF-FM diacetate), 3,3'-dioctadecyloxacarbocyanine perchlorate (DIO), DAPI, BCA Protein Assay Kit were purchased from Beyotime Institute of Biotechnology (Shanghai, China). Mouse TNF-α and TGF-β1 ELISA kit were purchased from Dakewe Biotech Co. Ltd. (Beijing, China). Deionized water was prepared by the Millipore system (18.25 MΩ cm).

Apparatus.

X-ray diffraction (XRD) profile was collected via Smartlab SE X-ray powder diffractometer (Rigaku, Japan). Fourier transform infrared (FT-IR) spectra were measured on a Nicolet iS50 FTIR spectrometer (Thermo Fisher, USA). UV-vis absorption spectra were tested on a UV-2550 UV-vis spectrophotometer (Shimadzu, Japan). Transmission electron microscope (TEM) photograph was obtained on a HT-7700 TEM (Hitachi, Japan). N_2 adsorption-desorption isotherms were examined on ASAP-2460 gas-sorption analyzer at 77 K (Micromeritics, USA). Hydrated size and zeta potential (ζ) were tested on a Zetasizer Nano ZS90 (Malvern, UK). pH value was measured by a PB-10 pH-meters (Sartorius, Germany). Fluorescence spectra were acquired on a F-7000 spectrometer (Hitachi, Japan). The flow cytometric analysis was performed on flow cytometer (BD LSR Fortessa). Cell imaging were collected by a Nikon C2 confocal laser scanning microscope (CLSM) (Nikon, Japan).

Preparation of COF nanosheets.

The synthesis of COF was conducted through a solvothermal method based on our previous work.[1] In typically, TAPT (28.1 mg, 0.08 mmol) and TFTPA (26.3 mg, 0.08 mmol) were dissolved in 3.15 mL mixed solvent of mesitylene/1, 4-dioxane (1:4, v/v) in a 10 mL glass ampoule. Then, 0.6 mL of H_2O and 0.9 mL HAc were added. The suspension was sonicated for 2 min and degassed for freeze-pump-thaw cycles for three times under a liquid N_2 bath. After that, the tube was flame-sealed under vacuum and then heated to 120 °C for 72 h in an oven for reaction. After cooling to room temperature, the yellow solid was collected by filter and washed by H_2O and THF for several times. The COF nanosheets were obtained by ultrasonic exfoliation for 6 h in water and then collected through centrifugation (13000 rpm, 10 min) for further use.

Preparation of EM and FEM.

The EM fragments were prepared by hypotonic lytic method according to the published protocols.[2] In brief, fresh whole blood (2 mL) was first obtained from female BALB/c mouse (6 - 8 w) and 1.5 mg EDTA per mL blood was added for anticoagulation. The blood suspended in water (5 mL, 40% v/v) was centrifuged at 2,000 rpm for 10 min at 4 °C, then the EM was washed with PBS three times. Haemolysis was performed by treating EM in 0.25-diluted PBS and incubated in ice bath for 1 h. The lysed EM was collected through centrifugation at 4,000 rpm for 20 min at 4 °C, and then washed with PBS three times and freeze-dried. EM fragments were obtained through sonication of 0.5 mg mL^{-1} EM in PBS for 5 min. For the preparation of FEM, the EM (1.0 mL, 0.5 mg mL^{-1}) was incubated with 50 μg of DSPE-PEG-FA at 37 °C for 30 min. Then, the solution was centrifuged at 1200 rpm for 8 min and washed with PBS three times. The successful modification of FA on EM was also identified via CLSM through instead of DSPE-PEG-FA with DSPE-PEG-Cy5 during the preparation procedure.

Preparation of COF@HGC.

COF@HGC nanocomposites were obtained via a facile one-pot method. Generally, a solution of Hu (2 mM, 0.5 mL), CPG ODN (100 nM, 100 μL) and GOx (1 mg mL^{-1}, 0.5 mL) were added into COF suspension (1 mg mL^{-1}, 1 mL) and stirred at room temperature for 24 h. After that, the suspension was centrifuged at 10000 rpm for 10 min and washed with deionized water for 3 times to remove the unloaded substances. The as-obtained COF@HGC was freeze-dried and stored at 4 °C before use. For quantitative analysis, all of the supernatant was collected.

Preparation of COF@HGC@FEM.

COF@HGC@FEM was prepared by mixing COF@HGC suspension (0.5 mg mL^{-1}, 1 mL) with FEM (0.5 mg mL^{-1}, 1 mL) and ultrasonicated in ice water for 5 min for membrane coating.

The excessive FEM fragments were removed by centrifugation. Then, the obtained COF@HGC@FEM was freeze-dried and stored at 4 °C before use.

Assessment of enzyme activities of GOx and Hb.

The activity of GOx was tested by using a GOx/HRP cascade/ABTS colorimetric assay. Typically, 5.0 mM glucose, 100 nM HRP and 2.0 mM ABTS were added in 1 mL buffer (PBS, 10.0 mM). Then, free GOx (0.528 μg), COF (30 μg), COF@HGC (30 μg), and COF@HGC@FEM (30 μg) were added into mixture, respectively. After 30 min, the amount of H_2O_2 generated through enzyme reaction was monitored by the absorption of ABTS-diradical at 418 nm. The peroxidase activity of FEM or free bHb was investigated using the H_2O_2/ABTS colorimetric assay. H_2O_2 (1 mM, 2μL), ABTS (2 mM, 20 μL) were added into 1 mL buffer (PBS, 10.0 mM). Then, 0.654 μg bHb, or 30 μg of COF, COF@HGC or COF@HGC@FEM were added into these mixture suspension, respectively. The mixture was allowed to react at 25 ^oC for 30 min and the absorption of the ABTS-diradical at 418 nm was recorded to assess their peroxidase activity.

Cascade reaction activity assays.

The cascade reaction for NO production in the presence of glucose was measured by Griess reagent based on its maximum UV absorption at 540 nm. The standard curve for NO measurement was established by using a $NaNO_2$ solution with different concentrations (6.25, 12.5, 25, 50, 100 μM). For the cascade catalysis experiments, COF (COF@HGC or COF@HGC@FEM) (1 mg mL^{-1}, 300 μL) and glucose (50 mmol L^{-1}, 500 μL) were added in 4.2 mL PBS (10.0 mM), and then reacted at 25^oC for 3 h. After that, the supernatant was collected for NO detection. The effects of pH and concertation on this reaction were also investigated. The cascade enzymatic reaction was conducted under different pH conditions (6.8 and 7.4) or different concentrations of COF@HGC@FEM (10, 20, 30, 40 and 50 μg mL^{-1}), respectively. To monitor the pH variation in the catalytic process, the COF (COF@HGC or COF@HGC@FEM) (1 mg mL^{-1}, 300 μL) and glucose (50 mmol L^{-1}, 500 μL) were added in 4.2 mL H_2O. The pH of the suspension was detected by pH meter at different time. The glucose consumption was monitored by a DNS method. Briefly, at different time points, the reaction mixture (100 μL) was centrifuged and the supernatant was added into DNS (0.3 mL) and then bathed in boiling water for 5 min. The concentration of glucose was determined by monitoring the absorbance at 540 nm by using a standard curve. In addition, the cascade reaction kinetics of COF@HGC@FEM (30 μg mL^{-1}) was also investigated in the presence of different amounts of glucose (0 - 20 mM). Michaelis-Menten equation and Lineweaver-Burk fitting were adopted to calculate the kinetic parameter.

SDS-PAGE assay.

Polyacrylamide gel electrophoresis (PAGE) assay was adopted to characterize the proteins on COF@HGC, COF@HGC@FEM. FEM, free GOx and COF were used as controls, respectively. The as-prepared samples were individually lysed in SDS buffer, and the total protein amount was determined by a BCA protein assay kit. The samples were subsequently heated at 95 °C for 5 min, then, 20 μg of each sample was loaded into each well and separated in a 10 % SDS-PAGE at 120 V for 2 h. The resulted polyacrylamide gel was subjected to stain with Coomassie brilliant blue for 30 min and rinsed with the decolorizing solution several times before taking pictures.

Stability test.

To evaluate the long-term stability, 30 μg mL^{-1} of COF or COF@HGC@FEM were dispersed in H_2O, PBS, DMEM, 10% FBS solution to stand for 0, 3, 5, 7, and 14 d, respectively. Accordingly, the size distribution of materials was monitored.

The drug release behavior.

To study the release kinetics, 1 mL of COF@HGC@FEM solution (1 mg mL^{-1}) was added into a dialysis bag (MWCO = 200 kDa) and immersed in 9 mL PBS with different pHs (pH = 7.4 or 5.5) with continuous stirring for 200 rpm. At different time points, 1 mL of releasing media was collected and replaced with an equivalent amount of fresh medium. The cumulative amount of Hu released was determined by HPLC. To quantify GOx and CPG, the releasing media was separated using a 30 kDa millipore. Then, the content of GOx and CPG were quantified by UV absorbance at 260 and 280 nm through their standard calibration curve.

Cell culture.

In this experiment, human normal breast cell line (MCF-10A), human TNBC cell line (MDA-MB-231 cells), murine TNBC cell line (4T1 cells), murine macrophages (RAW264.7 cells) and human carcinoma lung cell line (A549 cells) were selected. These cell lines were incubated in a DMEM culture medium containing 10% FBS and 1% penicillin-streptomycin. All the cells were cultured in a CO_2 incubator with 5% CO_2 humidified atmosphere at 37 °C.

Cellular targeted uptake.

The 4T1, MDA-MB-231 or A549 cells were seeded into 24-well plate with the density of 1.0×10^5 each well and incubated for 12 h. Then, the culture medium was renewed with 500 μL fresh DMEM containing 30 μg mL^{-1} COF@HGC@EM or COF@HGC@FEM, and then incubated for another 3 h. Then, the cells were washed with PBS for 3 times, fixed with 4% paraformaldehyde for 5 min and stained with DAPI for 5 min. Finally, the cells were subject for fluorescence imaging with 405 nm laser excitation and collected emission at 460 ± 25 nm

for blue channel. For the green channel, COF@HGC@FEM was excited with a 488 nm laser and the emission wavelength at 525 ± 25 nm was collected.

In vitro cytotoxicity test.

MCF-10A, 4T1 or MDA-MB-231 cells (100 μL in DMEM) were seeded in 96-well plates with the density of 6.0×10^3 each well and incubated for 12 h. After that, the tumor cells were incubated with fresh neutral (100 μL, pH 7.4) or acidified DMEM (100 μL, pH 6.8) containing different concentrations (0, 1, 5, 10, 25, 50, 100, and 200 μg mL^{-1}) of COF, COF@HGC or COF@HGC@FEM for 24 h, respectively. Subsequently, the incubation solution was discarded and rinsed with PBS for 3 times. Finally, the cell of each well was incubated with 100 μL DMEM solution containing 10 μL CCK-8 solution for another 4 h, followed by measurement the optical density (OD) per well at 450 nm.

In vitro permeability assay in MTSs.

Multicellular tumor spheroids (MTSs) were fabricated using previous method.[3] In brief, the 96-well plate was pre-coated with 100 μL of 2 % low melting point agarose to avoid cell adhesion. Then, 100 μL of cell suspension (about 5.0×10^3 cells) was added into each well and incubated for 3 d to prepare MTSs. The regular tumor spheroids (400 ~ 500 nm in diameter) were selected and incubated with 30 μg mL^{-1} COF or COF@HGC@FEM for 12 h. Then the MTSs were washed and imaged in different penetration depths by CLSM with a 488 nm laser excitation and emission at 525 ± 25 nm.

Intracellular NO staining.

The intracellular NO was detected by a commercial NO specific probe of 3-amino, 4-aminomethyl-2', 7'-difluorescein, diacetate (DAF-FM DA). The 4T1 or MDA-MB-231 cells were seeded in a 24-well plate with a density of 1.0×10^5 cells per well and adherent growth for 12 h. Then, the cell was pretreated with DAF-FM DA probe (5.0 μM) for 30 min. After that, fresh DMEM solution (500 μL) containing 30 μg mL^{-1} of COF (COF@HGC or COF@HGC@FEM) was added into each well for another 3 h co-incubation, respectively. Finally, the cells were washed with PBS for three times, fixed with 4% paraformaldehyde for 5 min, and stained with DAPI for 5 min. Then, it was subjected for CLSM imaging with a 488 nm laser excitation and collected emission wavelength range at 470 ± 50 nm.

Live/dead cell staining assays.

4T1 or MDA-MB-231 cells were seeded into the 24-well plate with a density of 5.0×10^5 and adherent growth for 12 h. The culture medium was renewed with 500 μL fresh DMEM containing 100 μg mL^{-1} of COF, COF@HGC or COF@HGC@FEM. After incubation for 24 h, cells were washed with PBS three times and stained with calcein-AM/PI according to the

protocol, followed by imaging with CLSM. The green channel for live cell imaging was excited by 488 nm laser and collected wavelength range at 470 ± 50 nm. The red channel for dead cell imaging was excited by 561 nm laser and collected wavelength range at 590 ± 20 nm.

Cell apoptosis assay

4T1 or MDA-MB-231 cells were seeded into six-well plates with a density of 10^5 cells/well and incubated for 12 h. Then, the culture medium was renewed with 1.5 mL fresh DMEM containing 100 μg mL^{-1} COF, COF@HGC or COF@HGC@FEM, and then incubated for another 24 h. Then, the cells were washed, collected and stained with the Annexin V-FITC/PI cell assay kit (Beyotime Biotechnology Company, China). Lastly, the samples were detected by flow cytometry.

Wound healing assay.

4T1 or MDA-MB-231 cells (1.5 mL, 1.0×10^6 cells per mL) were seeded into a 6-well plate and adherent growth for 12 h. The tumor cells were scratched with a sterile 200 μL pipette tips to create wound. Then, the cells were washed with PBS for 3 times and the scratched width at 0 h were taken by a fluorescence microscope. Subsequently, cells were incubated with culture media (1.5 mL) containing 100 μg mL^{-1} COF (COF@HGC or COF@HGC@FEM) for 24 h, respectively. The treated cells were rinsed and taken photo again. The width of wound healing was analysis with ImageJ software.

Transwell assay.

4T1 or MDA-MB-231 cells (200 μL, 6.0×10^4/well) in DMEM without FBS were seeded in the upper chamber (8 μm filters) and then treated with 100 μg mL^{-1} COF (COF@HGC or COF@HGC@FEM) for 12 h, respectively. The lower chamber was supplemented with 800 μL DMEM medium containing 10% FBS. After that, the migrated cells on the lower surface of the filter were rinsed with PBS and fixed with 4% paraformaldehyde for 20 min. Finally, the chamber was stained with 0.1% crystal violet and imaged via a fluorescent microscope.

Macrophage polarization in vitro.

In order to evaluate the CPG polarization of M2 to M1 macrophages, a transwell co-incubation system with 0.4 μm polycarbonate porous membranes was adopted. At first, M2 macrophages were obtained by inducing RAW246.7 cells with IL-4 (20 ng mL^{-1}) for 24 h. At same time, 1.0×10^4 4T1 cells were seeded on the upper chamber of transwell plate for 12 h, then treated with 100 μg mL^{-1} COF, COF@HGC or COF@HGC@FEM for 3 h, respectively. After that, the upper chambers were transferred to co-incubate with M2 macrophages which were seeded on the lower chamber of 24-well plate for 24 h. After that, the M2 macrophages were stained with cell membrane dye (DIO) and cell nuclei dye (DAPI), then observed by

CLSM. In addition, the cell supernatant was collected for cytokine (TNF-α, TGF-β1) assay by ELISA kits. For analysis of changes in macrophage phenotype, the co-incubation macrophages in the lower chamber were collected and stained with PE-F4/80, FITC-CD80 and APC-CD206 for flow cytometry. PE-F4/80 and FITC-CD80 were used to label M1 macrophages, PE-F4/80 and FITC-CD206 were used to label M2 macrophages.

Animals and tumor model.

All experimental procedures on mice were performed in compliance with the relevant laws and the Guidelines of Animal Experimental Ethics Committee of East China Normal University. All mice experiments have been approved by the committee (No. m20210224). The TNBC tumor model was established by subcutaneous injection of 4T1 cells (100 μL, 1.0×10^6) into the right armpit of female BALB/c mice (8 weeks old, ~20 g). When the tumor size reached ~ 100 mm^3, fluorescence imaging and therapeutic experiments were performed.

In vivo fluorescence imaging.

In vivo fluorescence imaging was conducted by intravenous injection of 100 μL COF or COF@HGC@FEM (1.0 mg mL^{-1}) into 4T1 tumor-bearing mice. The fluorescence imaging was recorded at 2, 6, 12, 24, and 48 h post-injection using an in vivo IVIS fluorescence imaging system. For another group, after 24 h post-injection, the mice were sacrificed, and then the major organs and tumors were collected for fluorescence imaging with an excitation wavelength and emission wavelength at 488 nm and 525 ± 25 nm, respectively.

The pharmacokinetics of COF@HGC@FEM.

COF@HGC@FEM nanoparticles (1 mg mL^{-1}, 100 μL) were intravenously injected to healthy BALB/c mice (n=3). Then, 50 μL of blood samples were collected via eye puncture at different time points (0.08, 0.5, 1, 2, 4, 6, 8 and 24 h). The blood samples were centrifugated at 3,000 rpm for 5 min to obtain plasma and the concentration of COF@HGC@FEM were detected by a fluorescence spectrophotometer using the corresponding standard calibration curve.

Hemolysis assay.

To obtain red blood cells (RBCs), fresh mouse blood (2.0 mL) was centrifuged at 3000 rpm at 4°C for 5 min and washed with PBS for 5 times. Afterward, the RBCs were re-suspended in 50 mL PBS to achieve 4% RBC suspension (v/v). The suspension of RBCs (50 μL) was mixed with 950 μL of COF or COF@HGC@FEM with different concentrations (25, 50, 75, 100 and 200 μg mL^{-1}), and then incubated at 37 °C for 8 h. For comparison, the RBCs suspended in PBS or water were used as the negative and positive controls, respectively. After that, all samples

were centrifuged and recorded the absorbance of the supernatant at 540 nm by a UV-Vis spectrophotometer. The ratio of hemolysis was calculated by the following equation,

$$Hemolysis(\%) = \frac{A_{sample} - A_{negative}}{A_{positive} - A_{negative}} \times 100\%$$

Antitumor effect and safety evaluation.

The tumor-bearing mice were randomly divided into 6 groups (n = 3 per group). Each group was accepted the following curation, (1) PBS, (2) COF, (3) free CPG, (4) COF@GOx, (5) COF@HGC, and (6) COF@HGC@FEM. PBS (100 μL), COF (100 μL, 1.0 mg mL^{-1}), CPG (100 μL, 23 μg mL^{-1}), COF@GOx (100 μL, 1.0 mg mL^{-1}), COF@HGC (100 μL, 1.0 mg mL^{-1}), and COF@HGC@FEM (100 μL, 1.0 mg mL^{-1}). The CPG was subcutaneously injected into the right axilla of mice. The other materials were intravenous injection via tail vein every 2 d for 7 times with a curation period of 14 d. The tumor size and body weight were measured before every treatment. The tumor volume (V) could be calculated by the following equation, Tumor volume (mm^3) = (Length × $Width^2$)/2. At the end of the administration, the mice were anesthetized, and the blood was collected for plasma biochemical assay. The vital organs as well as tumors were collected and fixed for further use. The main organs were sectioned into slices for H&E staining. The tumor slices were subjected to H&E and Ki-67 immunochemical staining. For macrophage polarization analysis, the tumors under different treatment were isolated, and digested by 0.25% trypsin and filtered through a cell strainer (100 μm) to form single cell suspension. After stained with corresponding antibodies of PE-F4/80, FITC-CD11b, APC-CD80, and APC-CD206 according to manufacturer's instructions, the samples were analyzed by using flow cytometry. The CD11b+ positive macrophage were initially identified and then further gated by the expression of CD80+F4/80+(M1 macrophage) or CD206+ F4/80+(M2 macrophage). The tumor slices were subjected to FTIC-CD80 and APC-CD206 immunofluorescence staining. For the detection of cytokine level in tumors, the supernatant of tumor cells was used to measure TNF-α and TGF-β1 level by using ELISA kits (Dakewe, China).

Statistical analysis.

All results are acquired in triplicate and expressed as mean ± SD. One-way analysis of variance (ANOVA) was adopted for multiple comparisons. Tukey's post-test was used for all data analysis. P value of *P < 0.05, **P < 0.01, ***P < 0.001 represents statistically significant.

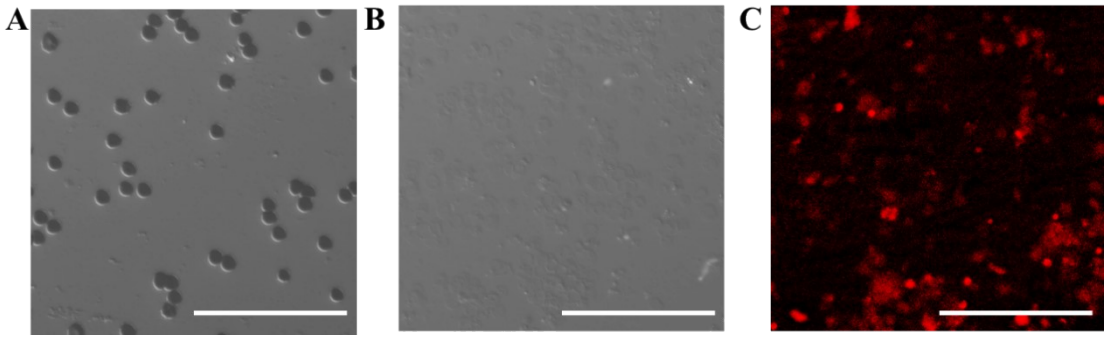

Fig. S1 Optical microscopy images of (A) erythrocyte and (B) erythrocyte treated with 0.25-diluted PBS solution. (C) CLSM of EM stained with DSPE-PEG-Cy5. Scar bar = 50 μm.

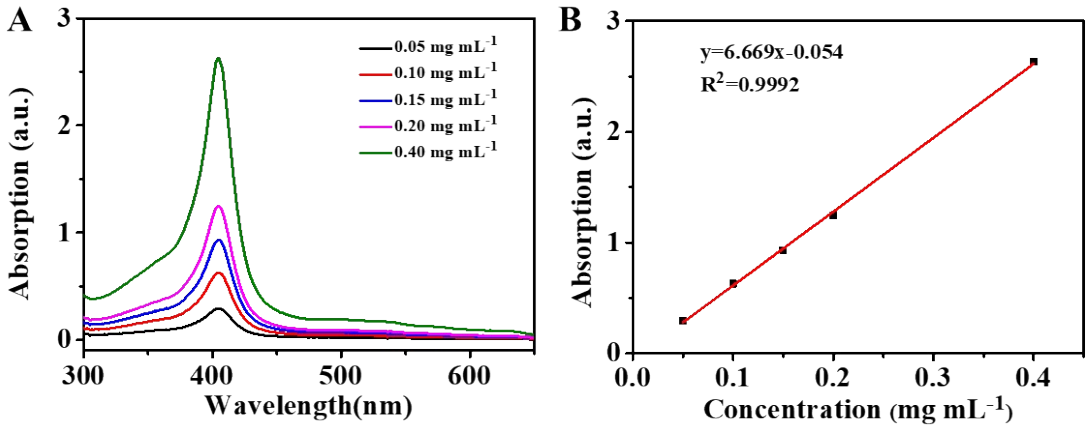

Fig. S2 (A) UV-Vis absorption spectra of different concentrations of bHb, and (B) Linear relationship between the UV-Vis absorption at 405 nm and the concentration of bHb.

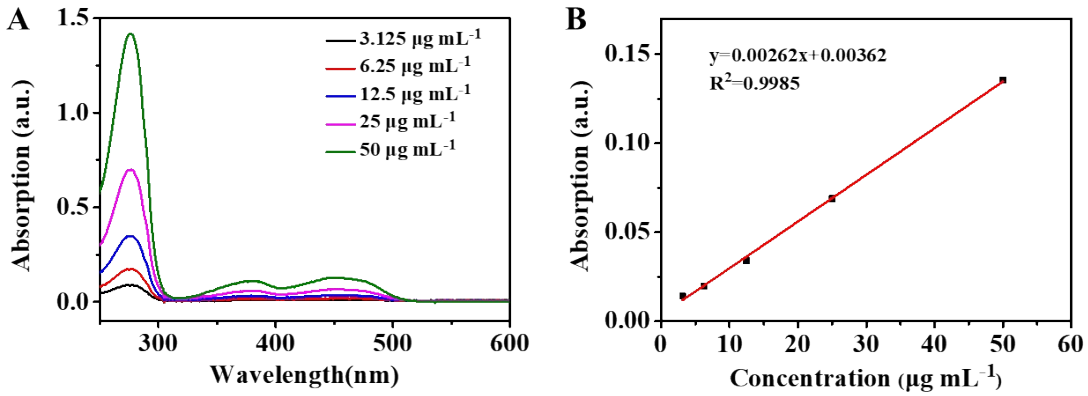

Fig. S3 (A) UV-Vis absorption spectra of different concentrations of free GOx, and (B) Linear relationship between the UV-Vis absorption at 425 nm and the concentration of GOx.

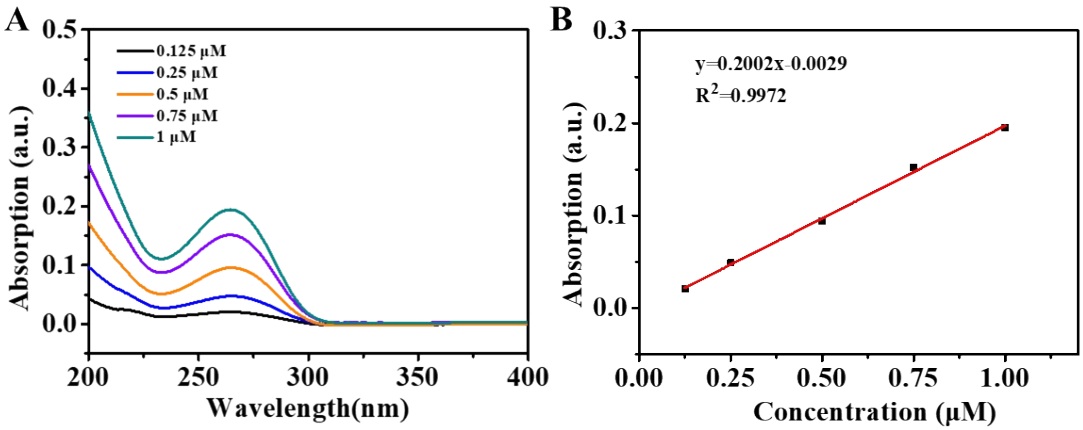

Fig. S4 (A) UV-Vis absorption spectra of different concentrations of CPG, and (B) Linear relationship between the UV-Vis absorption at 260 nm and the concentration of CPG.

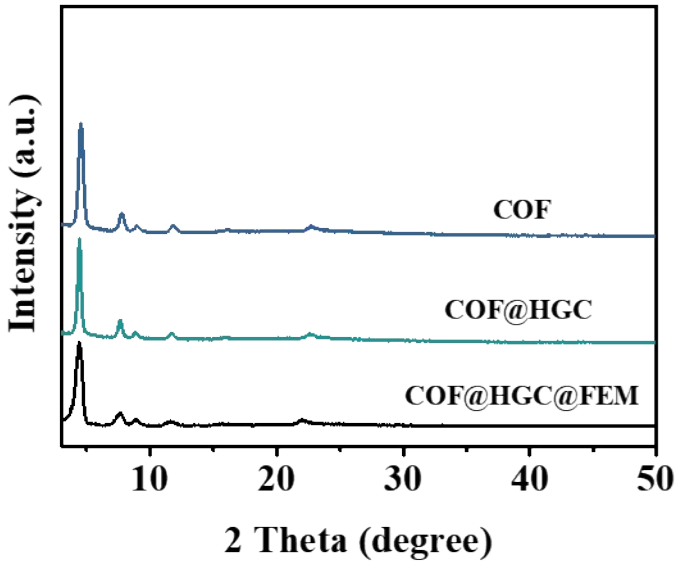

Fig. S5 The XRD pattern of COF, COF@HGC, COF@HGC@FEM.

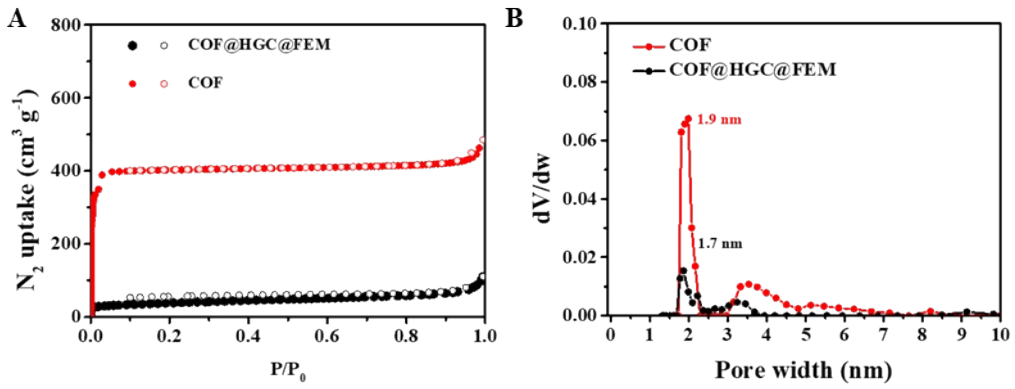

Fig. S6 N_2 adsorption (filled symbols) and desorption (empty symbols) isotherms of (A) COF and COF@HGC@FEM. Pore size distribution curves of (B) COF and COF@HGC@FEM.

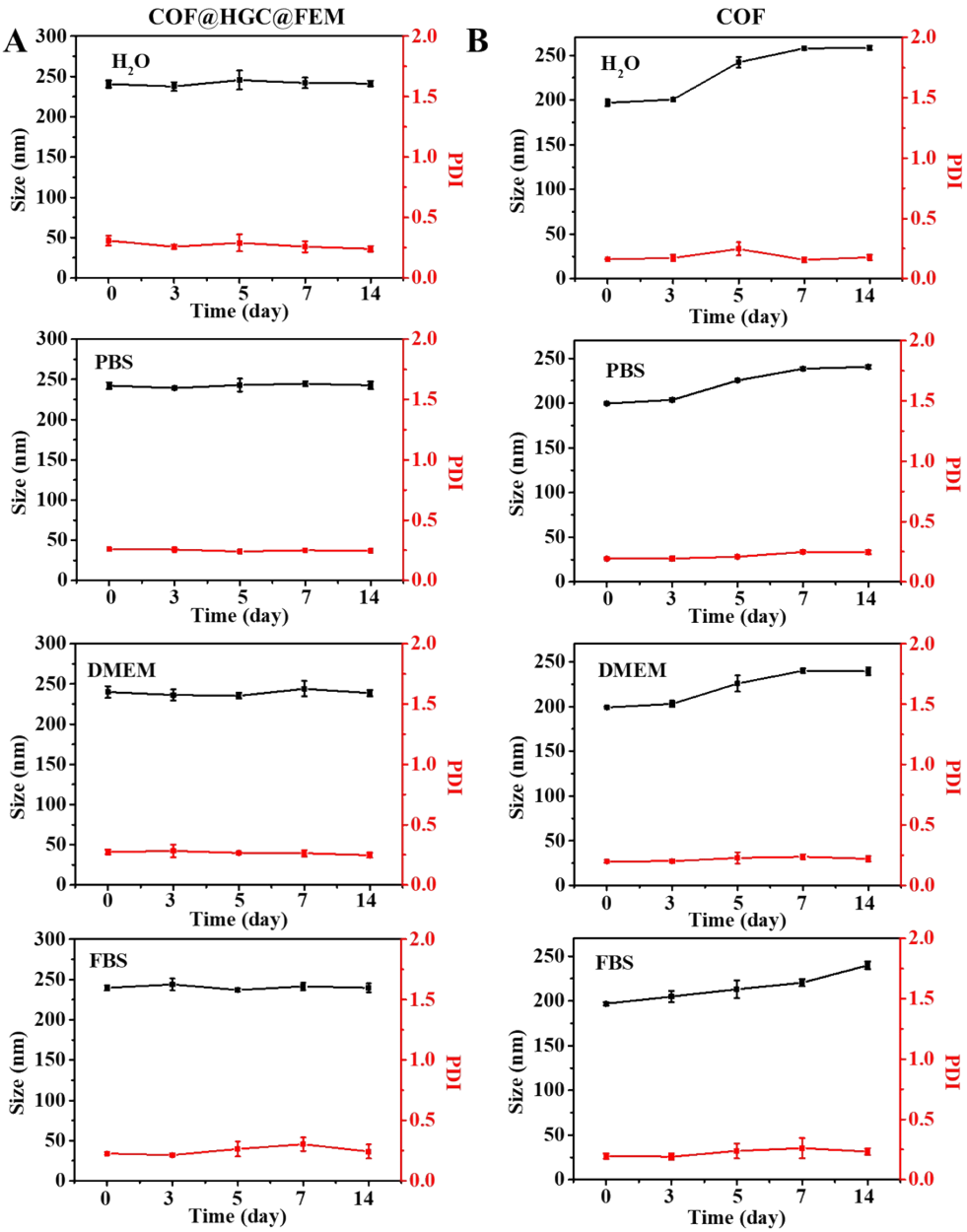

Fig. S7 Variation of size and polydispersity index (PDI) of (A) COF@HGC@FEM and (B) COF in H_2O, PBS, DMEM, 10% FBS solution at different times based on dynamic light scattering (DLS).

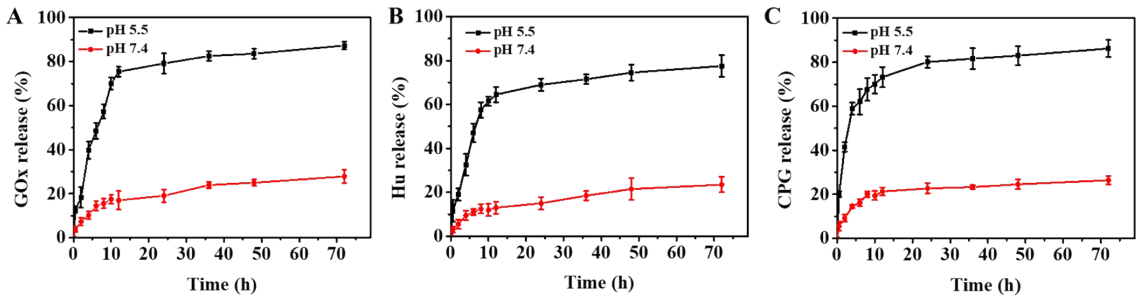

Fig. S8 The release profiles of (A) GOx; (B) Hu; (C) CPG from COF@HGC@FEM in PBS at pH 5.5 and 7.4.

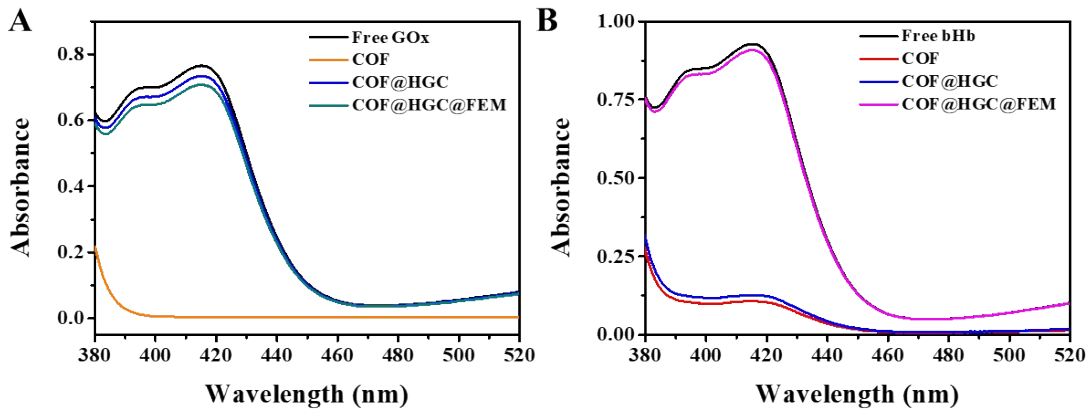

Fig. S9 UV-Vis absorption spectra of ABTS in presence of (A) free GOx, COF, COF@HGC or COF@HGC@FEM in the presence of 5.0 mM glucose, 100 nM HRP and 2.0 mM ABTS, respectively, and (B) free bHb, COF, COF@HGC or COF@HGC@FEM and 1.0 mM H_2O_2, respectively.

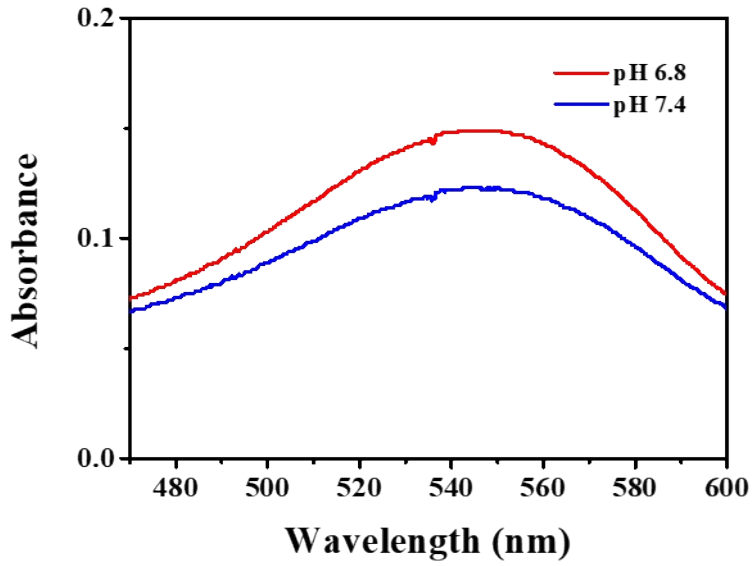

Fig. S10 Absorption spectra of Griess reagent triggered by COF@HGC@FEM catalytic reaction in the presence of 5.0 mM glucose at different pH (6.8 and 7.4).

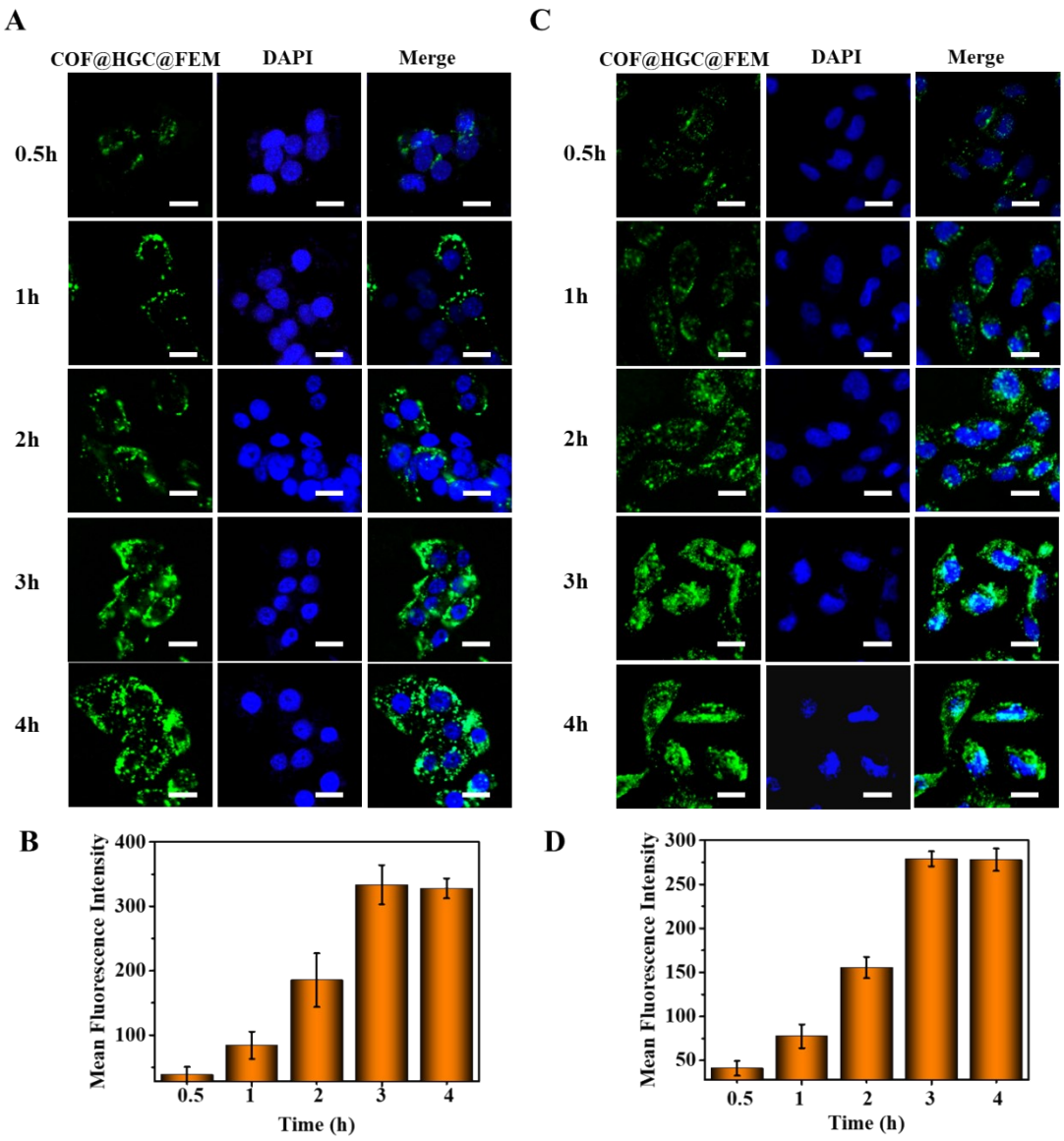

Fig. S11 (A) CLSM of 4T1 cells treated with COF@HGC@FEM for different time. Scale bar = 25 μm. (B) Corresponding mean fluorescence intensity statistic of 4T1 cells after COF@HGC@FEM treatment for different time. (C) CLSM of MDA-MB-231 cells treated with COF@HGC@FEM for different time. Scale bar = 25 μm. (D) Corresponding mean fluorescence intensity statistic of MDA-MB-231 after COF@HGC@FEM treatment for different time.

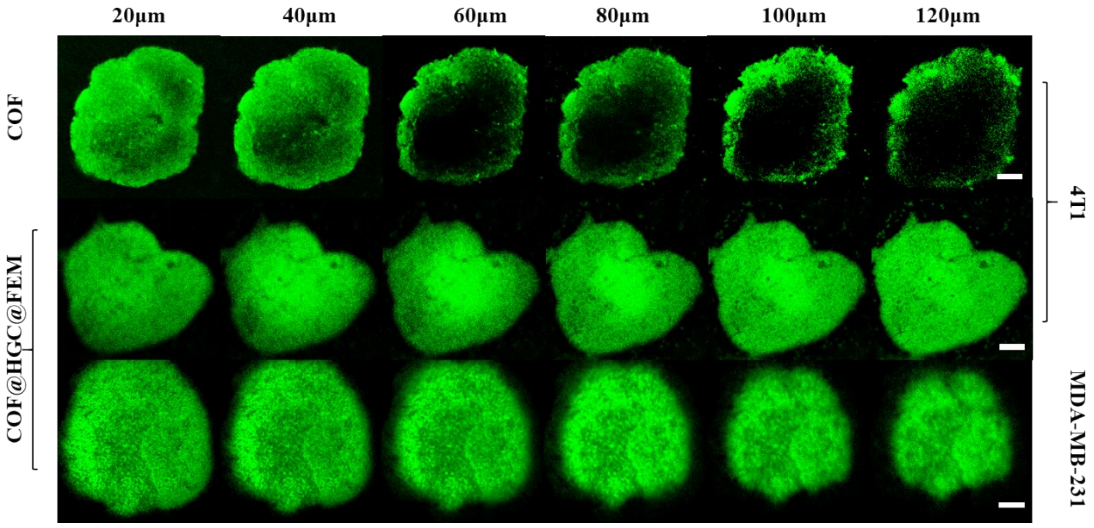

Fig. S12 CLSM of 4T1 or MDA-MB-231 MTSs at different depths after 12 h incubation with COF or COF@HGC@FEM. Scale bar = 100 μm.

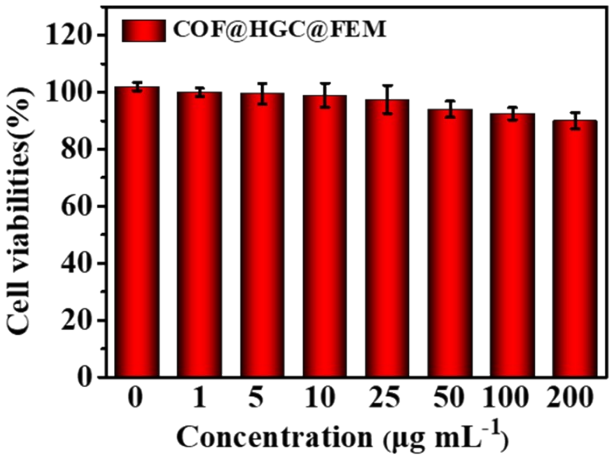

Fig. S13 Cell viability of MCF-10A cells treated with different concentrations of COF@HGC@FEM for 24 h.

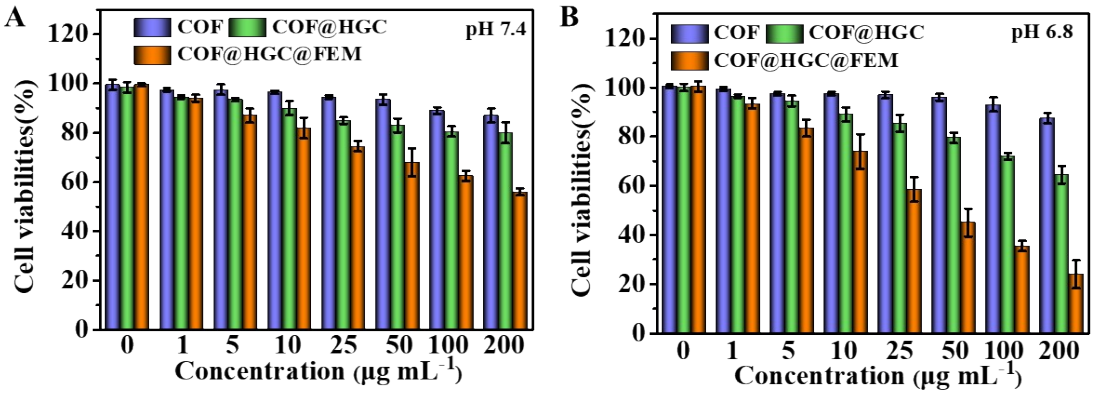

Fig. S14 Cell viability of MDA-MB-231 cells treated with different concentrations of COF, COF@HGC or COF@HGC@FEM at pH 7.4 (A) and 6.8 (B) for 24 h, respectively.

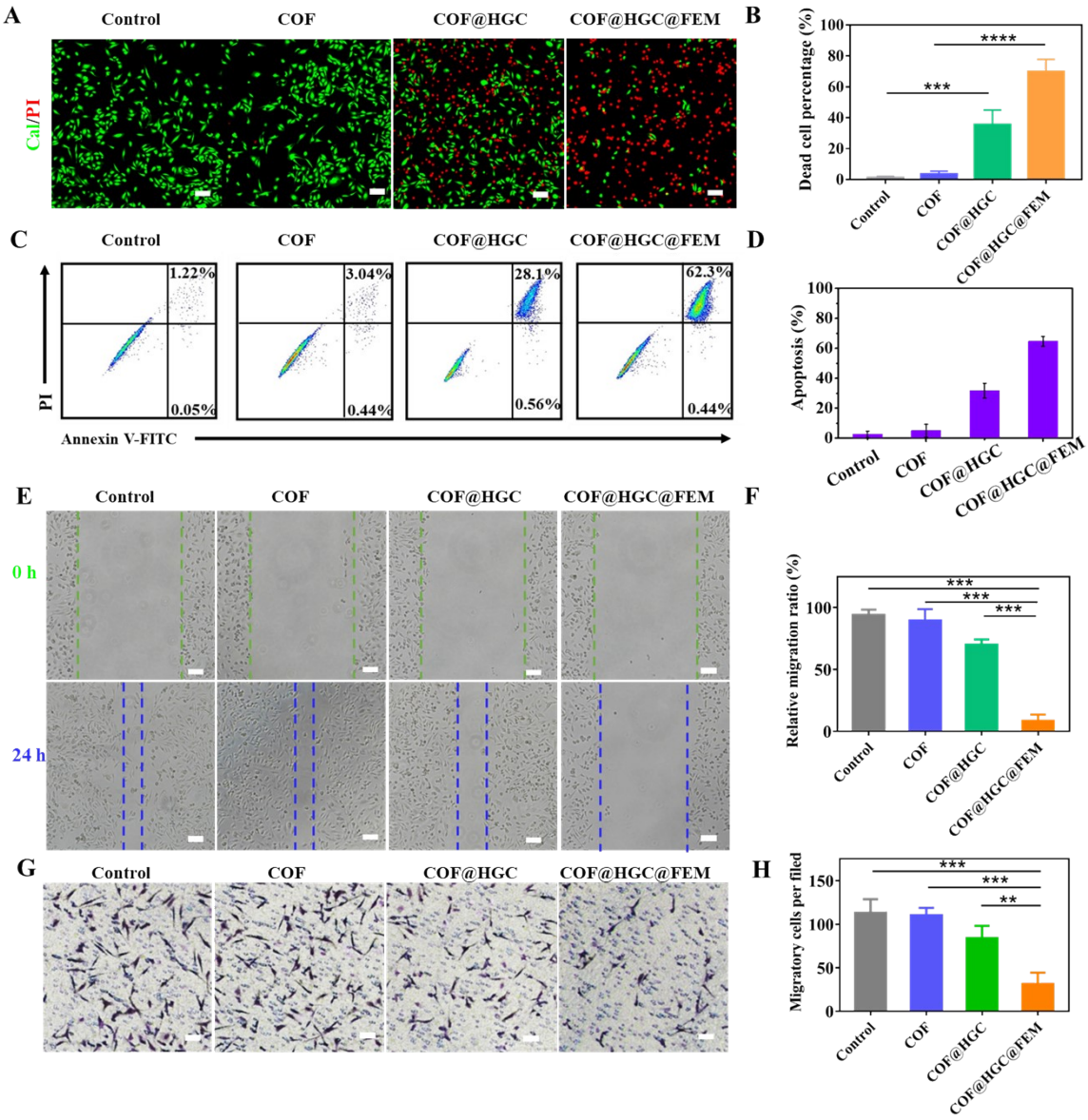

Fig. S15 (A) CLSM images of MDA-MB-231 cells co-stained with calcein-AM and PI after incubated with COF, COF@HGC or COF@HGC@FEM for 24 h, respectively. Scale bar = 100 μm. (B) Percentage of dead cells according to calcein-AM and PI staining. (C) Flow cytometric plots of MDA-MB-231 cell apoptosis after different treatments. (D) quantitative analysis of MDA-MB-231 cell apoptosis from flow cytometry analysis. (E) Wound healing images and (F) corresponding wound migration ratio of MDA-MB-231 cells after treated with COF, COF@HGC or COF@HGC@FEM for 24 h. Scale bar = 100 μm. (G) Microscopy images and (H) quantification of transwell migration number of MDA-MB-231 cells treated with COF, COF@HGC or COF@HGC@FEM for 24 h. Scale bar = 100 μm. Data were presented as the mean ± SD (n = 3).

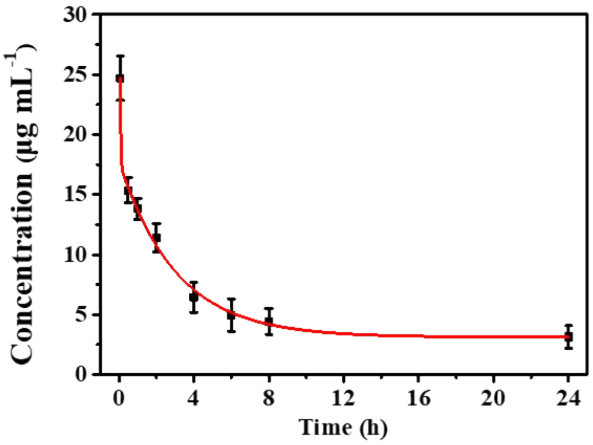

Fig. S16 The pharmacokinetic profiles of COF@HGC@FEM after intravenously injection at different time points. The data are presented as mean ± SD (n = 3).

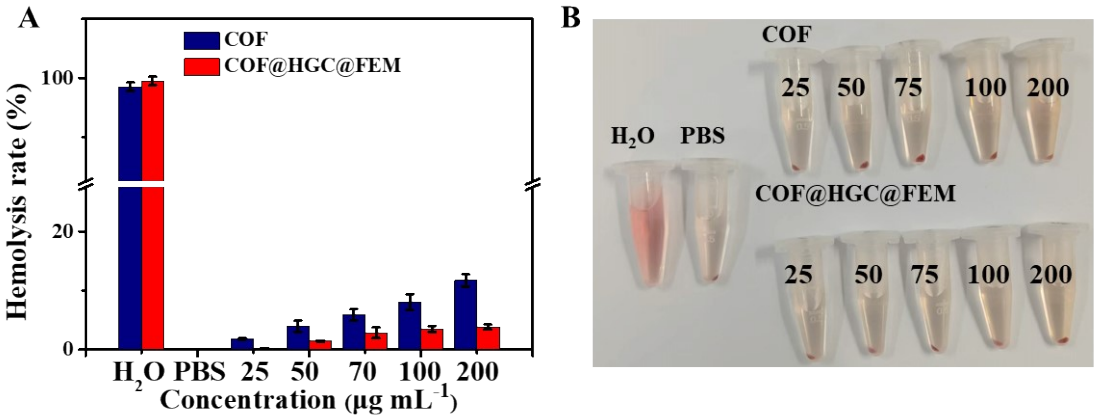

Fig. S17 (A) The hemolysis rate of the RBC supernatant in H_2O, PBS and corresponding system with different concentrations of COF or COF@HGC@FEM. (B) The photographs of hemolysis in corresponding system. PBS and H_2O were used as negative and positive controls.

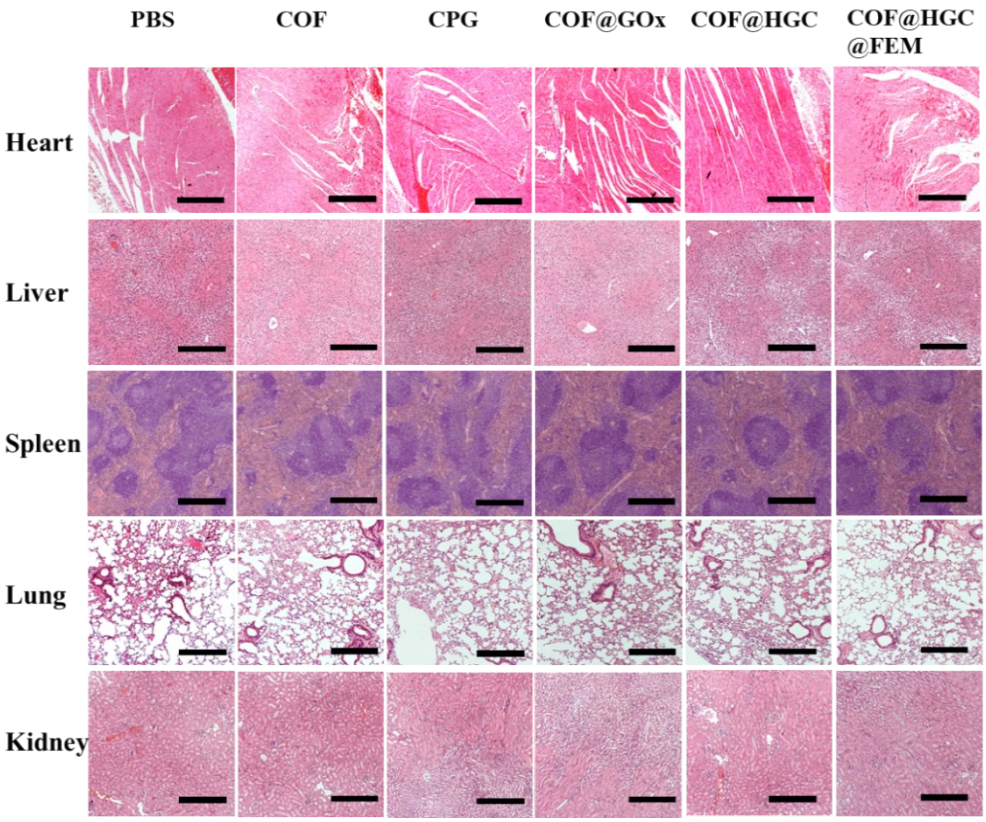

Fig. S18 H&E staining of heart, liver, spleen, lung and kidney of mice on the 14th day after different treatments. Scale bar = 200 μm

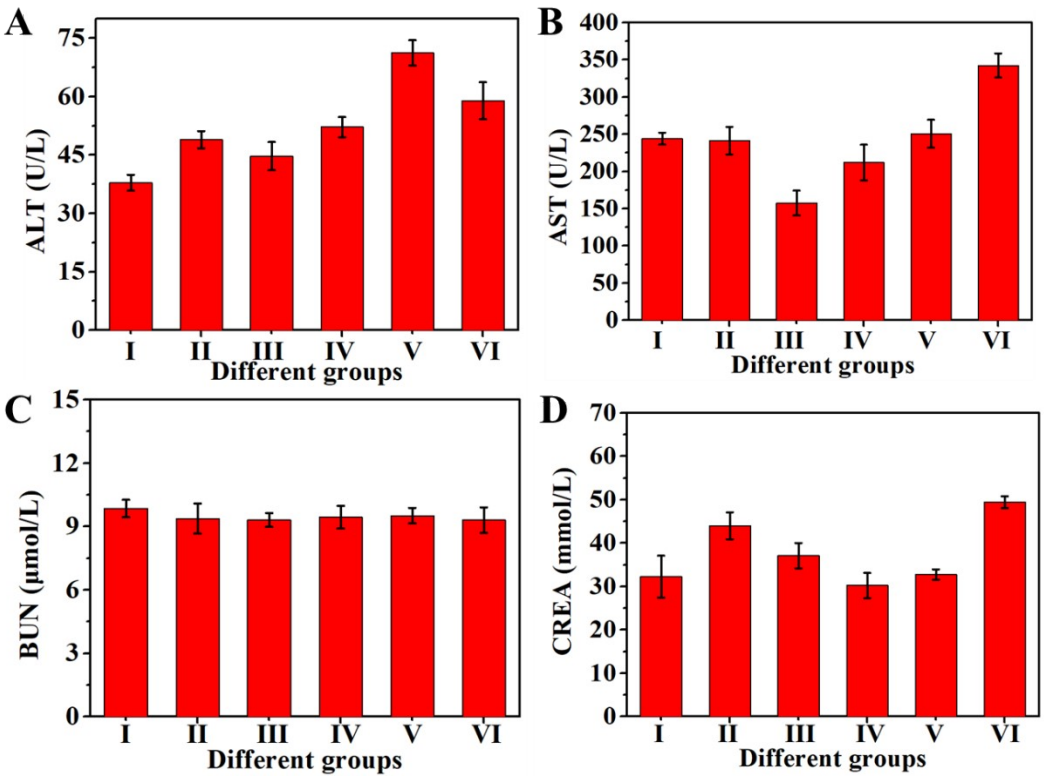

Fig. S19 Blood biochemical levels of ALT, AST, and BUN, CREA on the 14th day after treatments.

References

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