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Electronic Supplementary Information

# Engineering Lauric Acid-based Nanodrug Delivery Systems for Restoring Chemosensitivity and Improving Biocompatibility of 5-FU & OxPt against *Fn*-Associated Colorectal Tumor

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## Supplemental experimental procedures

**Materials and Instruments.** All chemicals used for synthesis were purchased from Sigma-Aldrich and directly used as received. $^{1}$H NMR spectra of the polymers were recorded using a Bruker Avance III 400 spectrometer. Dynamic light scattering (DLS) experiments were carried out with a Zetasizer Nano ZS90 instrument (Malvern Instruments, Southborough, MA). The stability of the nanoparticles was tested in 10% of the serum at different time. The morphology of nanoparticles was perceived via transmission electron microscope (TEM, JEM-2100F, JEOL Ltd., Japan). The morphologies of bacteria were investigated by scanning electron microscope (SEM) performed on a JSM-6700F instrument (JEOL Ltd., Japan) at an acceleration voltage of 10 kV.

**Synthesis of 5-FU-LA.** 5-FU-LA was synthesized according to the literature.[1] 5-FU (500 mg, 3.8 mmol) reacted with formaldehyde (37% wt.) in aqueous solution (2.5 mL formaldehyde and 2.5 mL water) at 60 °C. The reaction was stirred for 1 h and gave a mixture of N-1-hydroxymethyl-5-fluorouracil, N-3-hydroxymethyl-5fluorouracil and N,N'-1,3-bis(hydroxymethyl-5-fluorouracil). Then, the mixture was cooled in an ice-bath (0-5 °C) and 15 mL acetonitrile were added. Then, DCC (1.27 g, 6.08 mmol), DMAP (34 mg, 0.266 mmol) and lauric acid (1.23 g, 6.14 mmol) were added to achieve the esterification. The reaction mixture was stirred for 1 h at 0 °C, then 24 h at room temperature and monitored by thin-layer chromatography. The secondary product dicyclohexyl urea (DCU) was separated by filtration and eliminated after several washings with acetonitrile. The product was recovered by solvent evaporation under reduced pressure and purification on a silica gel chromatographic column (dichloromethane/ethyl acetate 95:5 v/v) (yield: 28%).

**Synthesis of PG.** PG was synthesized according to the literature.[2] Citric acid (500 mg) and glycidol (10 mL) were added into a round bottom flask and stired at 20 °C for 24 h. When the reaction was terminated, a compound was in the form of transparent viscous colloid. The crude product was dissolved in distilled water, and dialyzed against distilled water for 24 h using dialysis membrane tubing (MWCO = 3,000 Da) for 48 h. After lyophilizing, hyperbranched PG was obtained as a colorless product with a high viscosity (yield: 48%).

**Synthesis of Pt-COOH.** The carboxylated oxaliplatin (Pt-COOH) was synthesized as previously described.[3] Oxaliplatin (500 mg, 1.25 mmol) was suspended in acetic acid (10 mL). $H_2O_2$ (30% 0.5 mL) was added every 30 minutes and the mixture stirred for another 12 h. Then acetic acid was removed by vacuum distillation and the residue was purified by a silica gel chromatography with ethyl acetate/methanol (3:1, v/v) as the eluent. The white powder (OxPt-OH) was obtained (yield: 51%). Then the OxPt-OH (300 mg, 0.64 mmol) and succinic anhydride (152 mg, 1.52 mmol) were suspended in N,N-Dimethylformamide (DMF, 10 mL), heated to 50 °C and stirred overnight. The solvent was concentrated under a vacuum, and the crude product was suspended in acetone. The solution was filtered, and the product was

precipitated by the addition of diethyl ether. The white product (OxPt-COOH) was collected by filtration, washed with diethyl ether, and dried in a vacuum oven at 50 °C (yield: 47%).

**Cell and Bacterial Culture.** All cell lines were obtained from the cell bank of the Chinese Academy of Sciences (Shanghai, China). Mouse fibroblast cells (L929 cells), murine colorectal cancer cells (CT26 cells) and human colorectal cancer cells (HCT116 cells) were cultured in RPMI-1640 supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics in a humidified incubator at 37 ℃ (95% room air, 5% $CO_2$). Human HT29 colorectal cancer cells were maintained in McCoy's 5A Medium (Invitrogen) containing 10% of FBS. *Fn* (ATCC 25586) and *Clostridium butyricum* (*C. butyricum*, ATCC 19398) were purchased from Shanghai Huzheng Biological Technology Co., Ltd (China) and used for bacterial experiments cultured with brain heart infusion (BHI) broth (Qingdao Hope Biotechnology, China). And *Escherichia coli* (*E. coli*, gram-negative bacterium) were cultured with Luria-Bertani (LB) culture medium.

**Cell Viability Assay.** For viability assay, the cells were cultured and grown in 96-well plates for 24 h, with 5,000 cells seeded into each well. When cells reached 70% confluences, they were incubated with the fresh complete medium containing test samples with appropriate concentrations for another 24 h. Cell proliferation was measured using the MTT according to the manufacturer’s instructions. And the absorbance intensity was measured at 560 nm using a Versamax microplate reader (Germany). Results were quantified by determining cell viability relative to that of untreated cells.

**Hemolysis assay.** The specific experimental process was as follows. 6% Sheep red blood cell suspension (20 μL) was added to the materials (980 μL) of different concentrations (25, 50, 75, 100 μg/mL) at 37 ℃. After 3 h, the supernatant of erythrocyte suspension were collected by centrifuged, and measured by the Varioskan LUX microplate reader at 541 nm. PBS and $H_2O$ were used as negative control and positive control, respectively. The hemolysis ratio was calculated using the following formula:

$$Hemolysis\ ratio\ (\%) = \frac{A - A_N}{A_P - A_N} \times 100$$

Among them, A, $A_N$ and $A_P$ were the absorbance of the samples, negative control and positive control respectively. The experiment was repeated three times independently.

## Supplementary figures

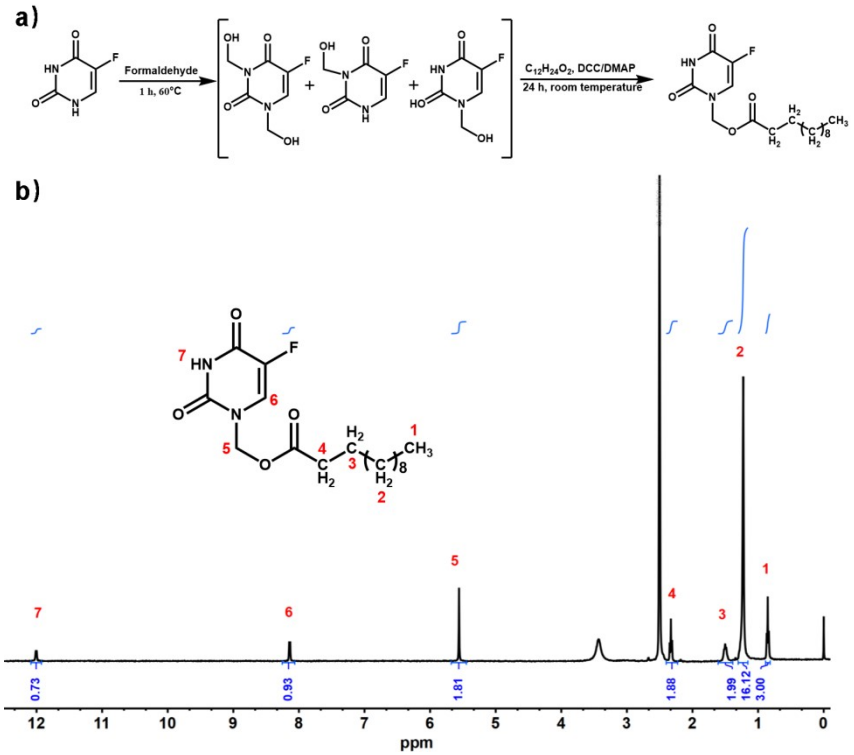


**Figure S1.** a) Synthetic scheme of 5-FU-LA. b) $^1$H NMR spectrum of 5-FU-LA in DMSO-$d_6$.

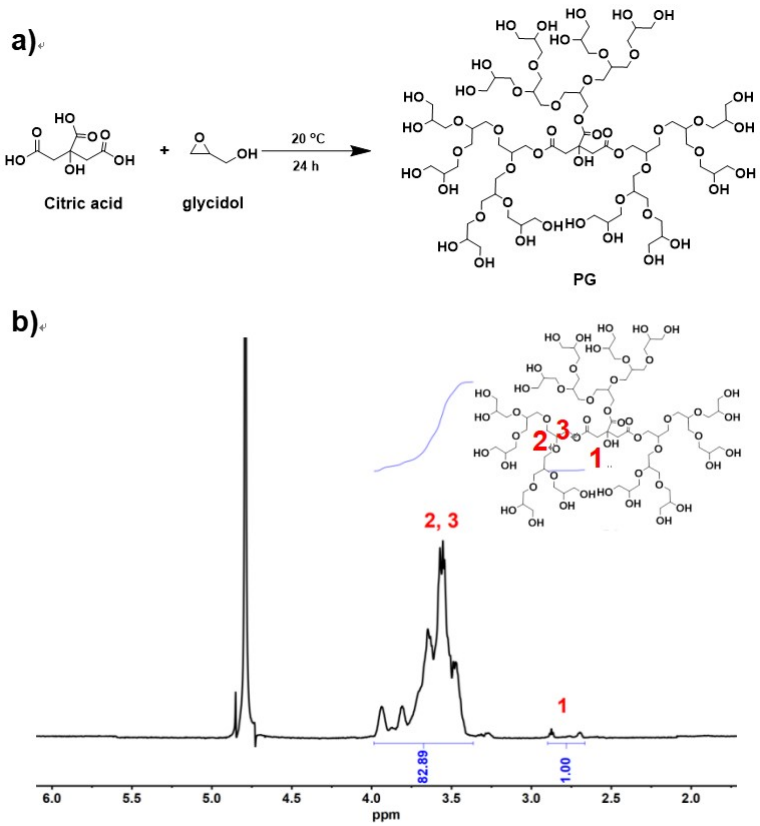


**Figure S2.** a) Synthetic scheme of PG. b) $^1$H NMR spectrum of PG in $D_2O$.

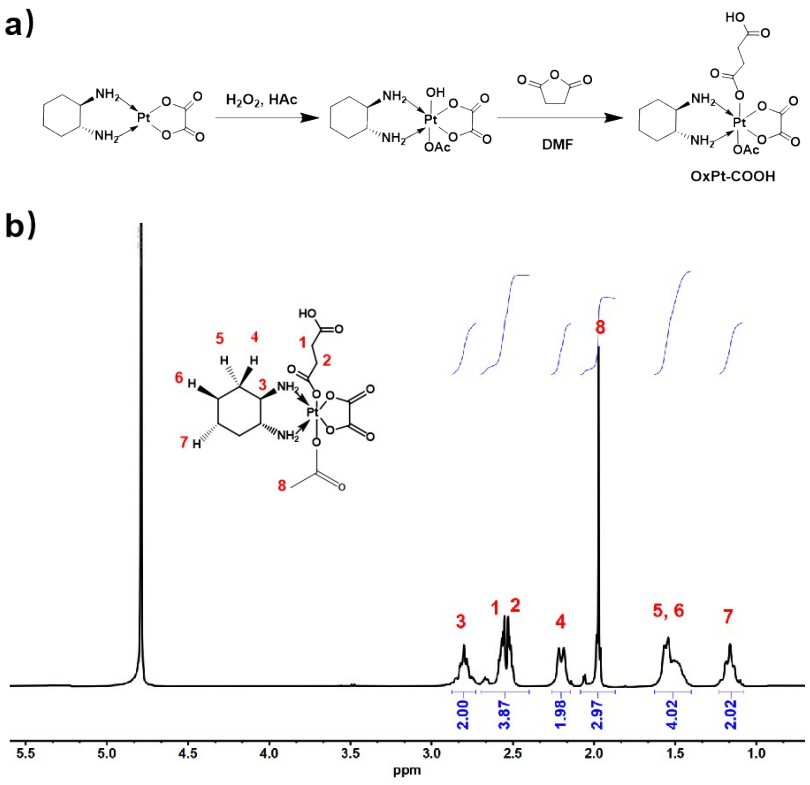


**Figure S3.** a) Synthetic scheme of OxPt-COOH. b) $^1$H NMR spectrum of OxPt-COOH in $D_2O$.

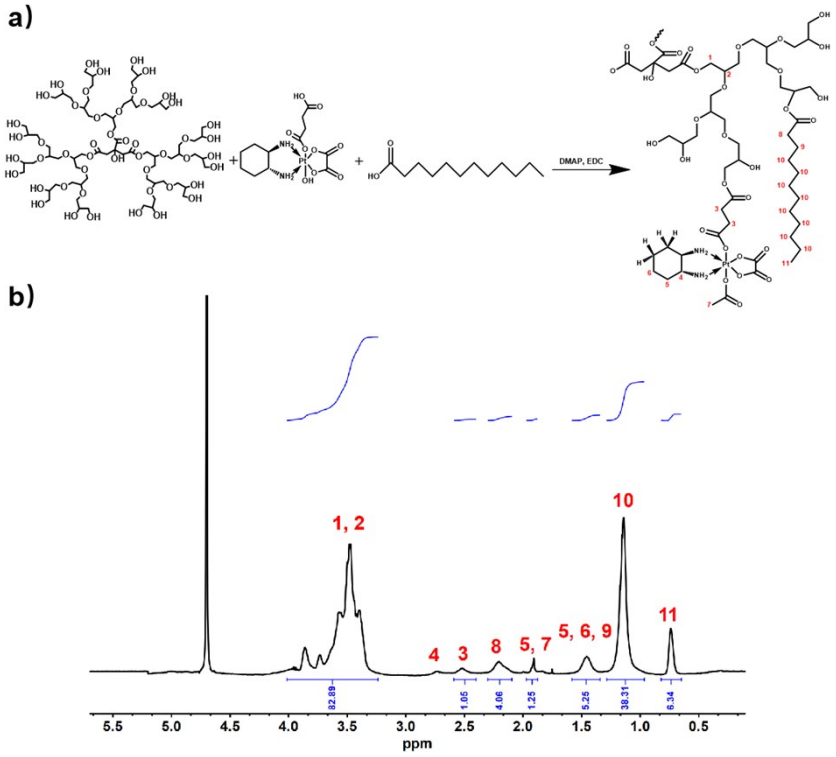


**Figure S4.** a) Synthetic scheme of PPL. b) $^1$H NMR spectrum of PPL in $D_2O$.

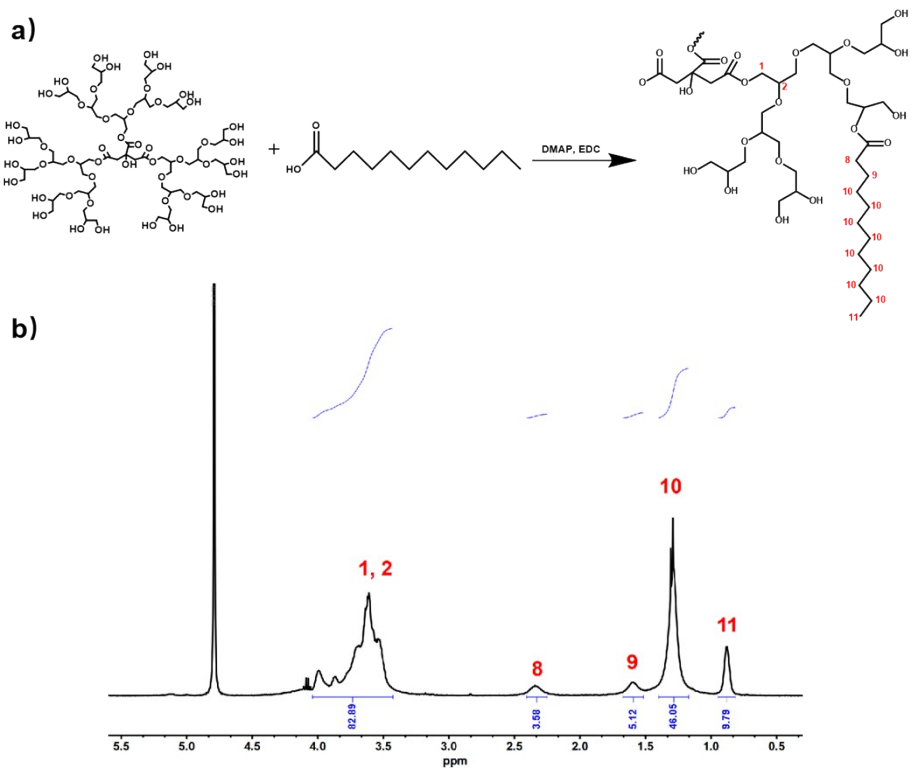


**Figure S5.** a) Synthetic scheme of PL. b) $^{1}H$ NMR spectrum of PL in $D_2O$.

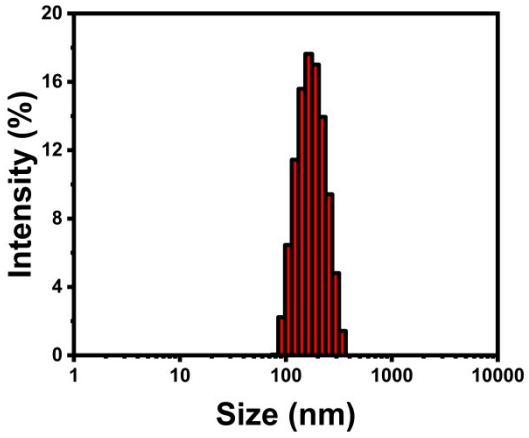


**Figure S6.** The hydrodynamic diameter of the nanomedicine **5-FU-LA@PL**.

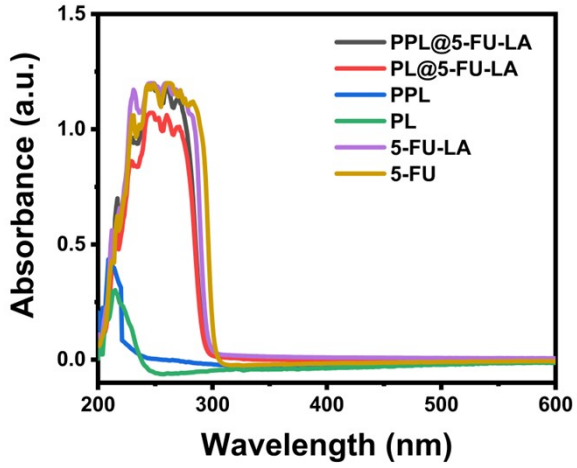


**Figure S7.** UV-vis spectra of **5-FU-LA@PPL**, **5-FU-LA@PL**, PPL, PL, 5-FU-LA and 5-FU.

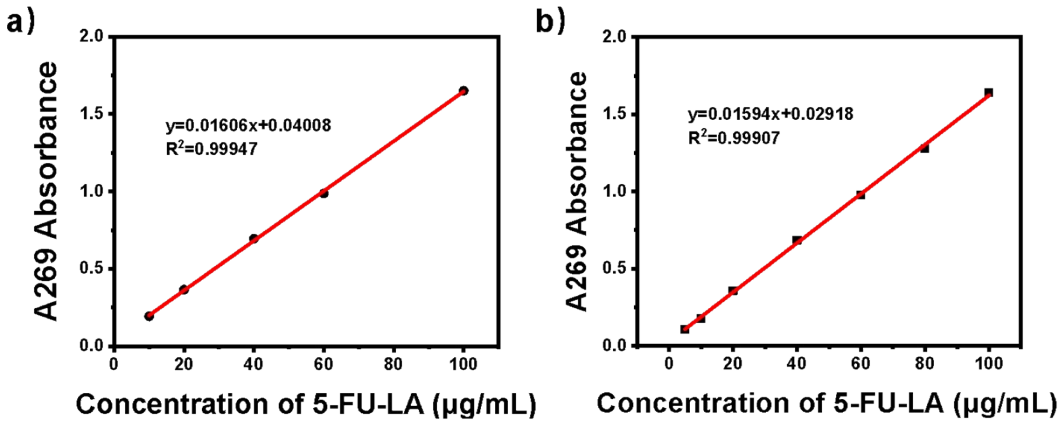


**Figure S8.** The standard curve of 5-FU-LA in a) DMSO and b) 5% Tween 80 solution.

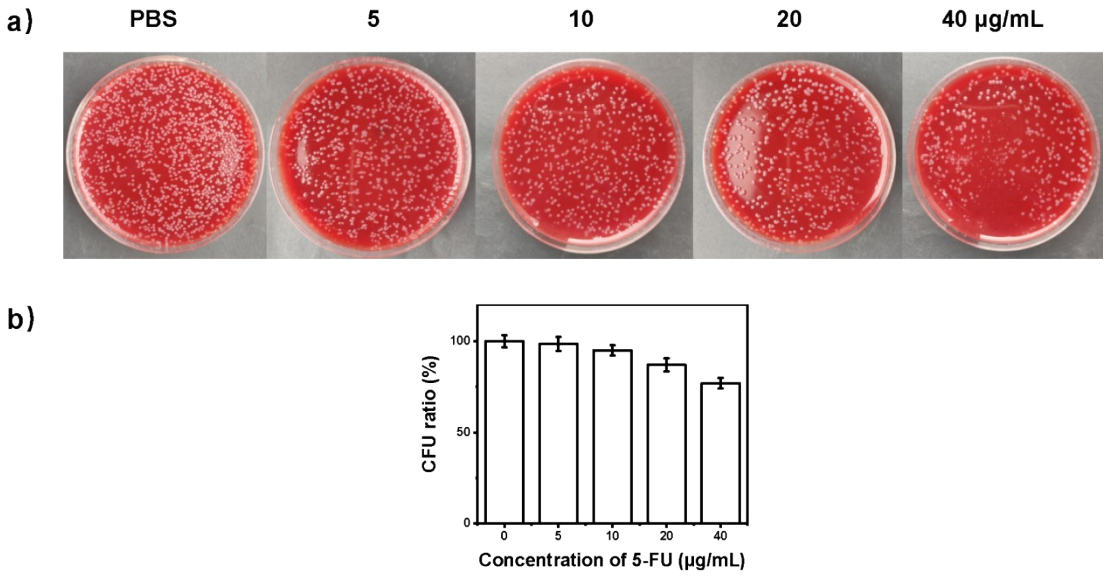


**Figure S9.** a) The spread plate and b) quantitative analysis of the antibacterial activity of *Fn* treated with different concentrations of 5-FU.

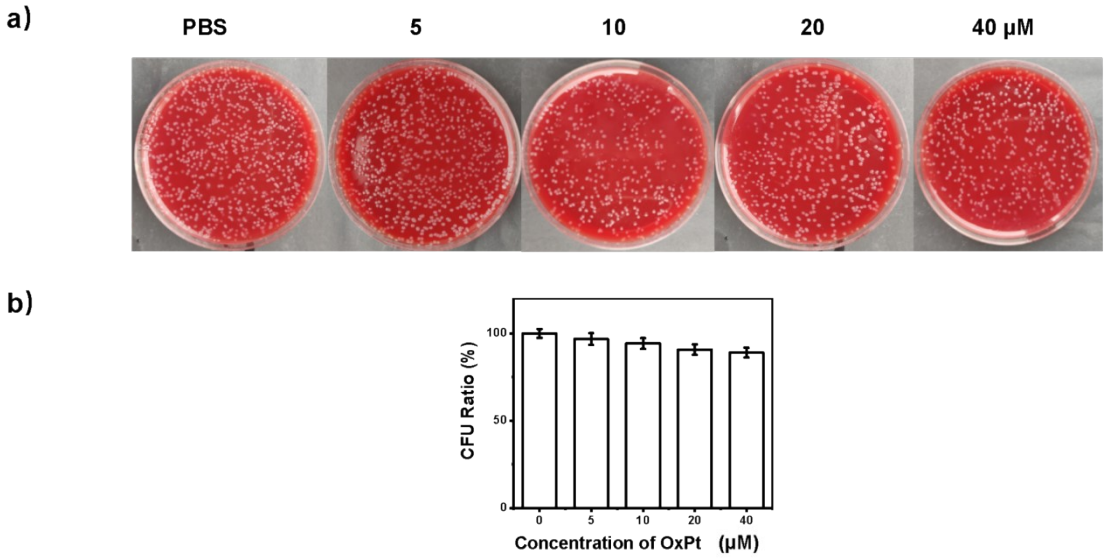


**Figure S10.** a) The spread plate and b) quantitative analysis of the antibacterial activity of *Fn* treated with different concentrations of OxPt.

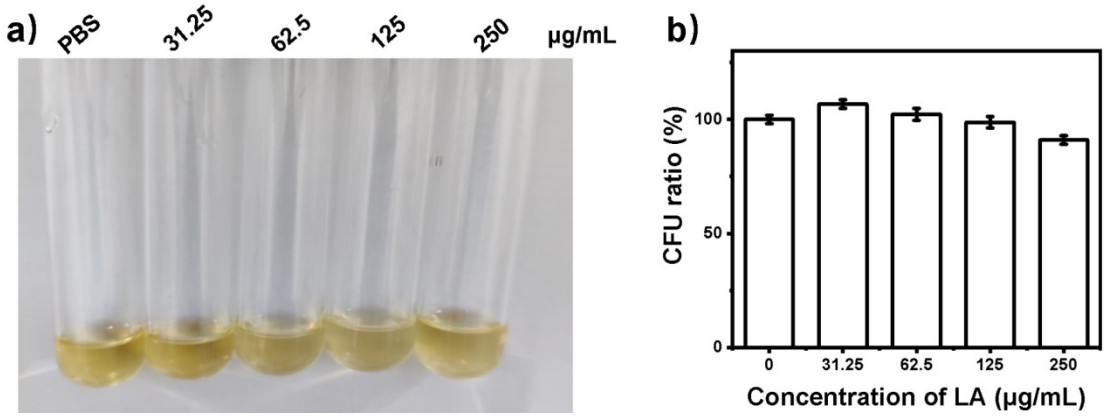


**Figure S11.** The antibacterial activities of *C. butyricum* under treatment of **5-FU-LA@PPL** at varied concentrations.

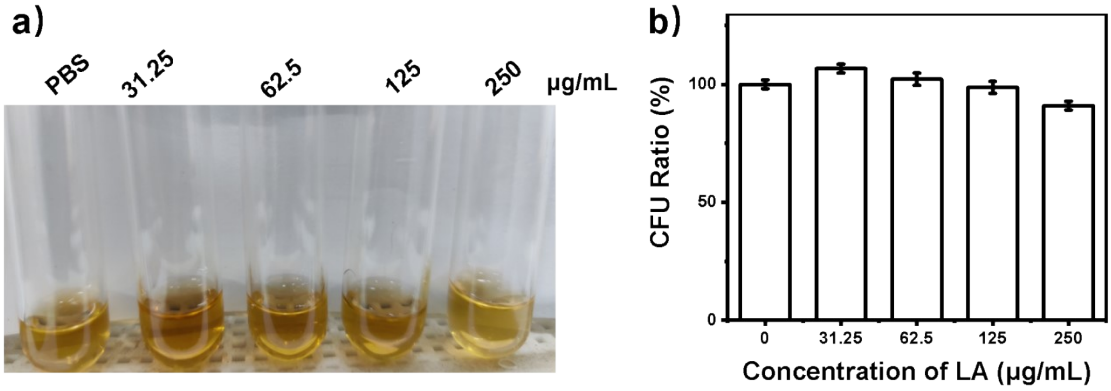


**Figure S12.** The antibacterial activities of *C. butyricum* under treatment of **5-FU-LA@PL** at varied concentrations.

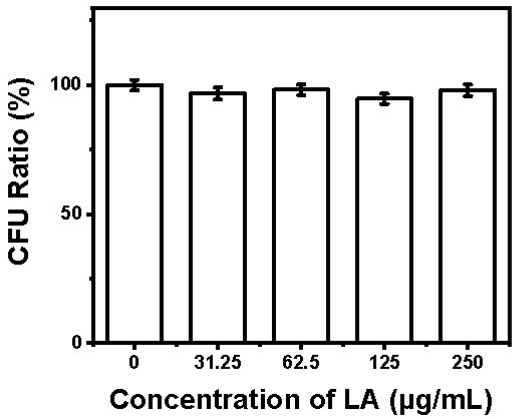


**Figure S13.** The quantitative analysis of the antibacterial activity of *E. coli* under treatment of **5-FU-LA@PPL** at varied concentrations of LA.

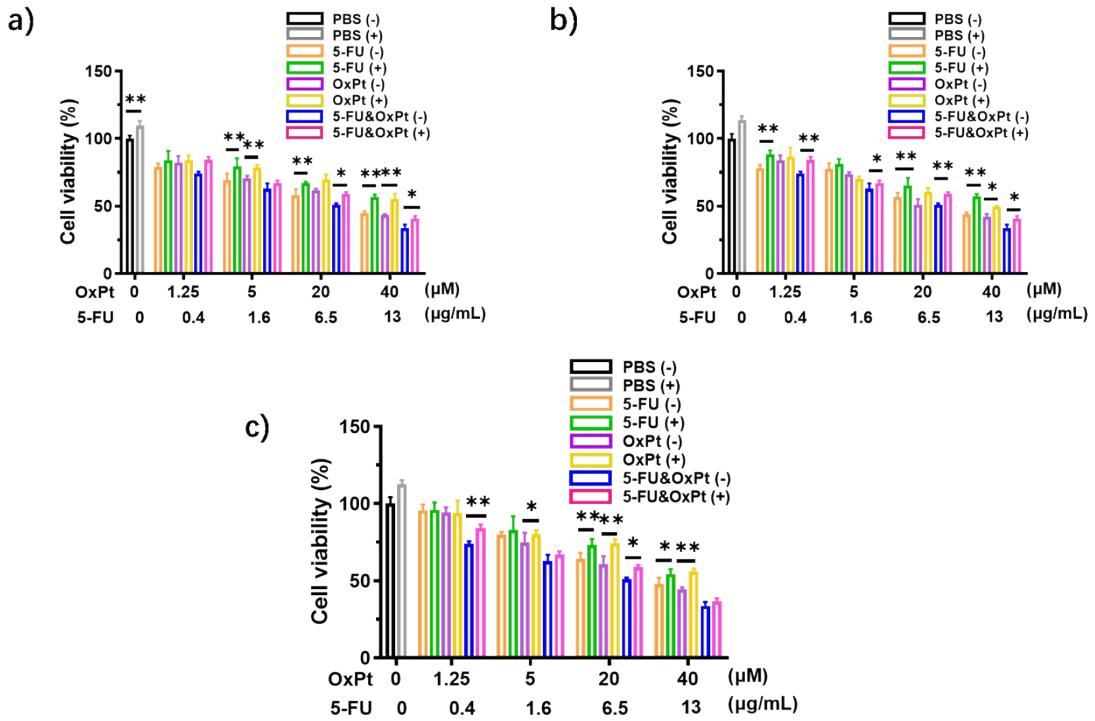


**Figure S14.** Cell viability of a) HT29, b) HCT116 and c) CT26 under treatment of PBS, 5-FU, OxPt, and 5-FU&OxPt at varied 5-FU and Pt concentrations, wherein (+) and (-) are referred to incubation microenvironment in presence and absence of *Fn*, respectively. **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

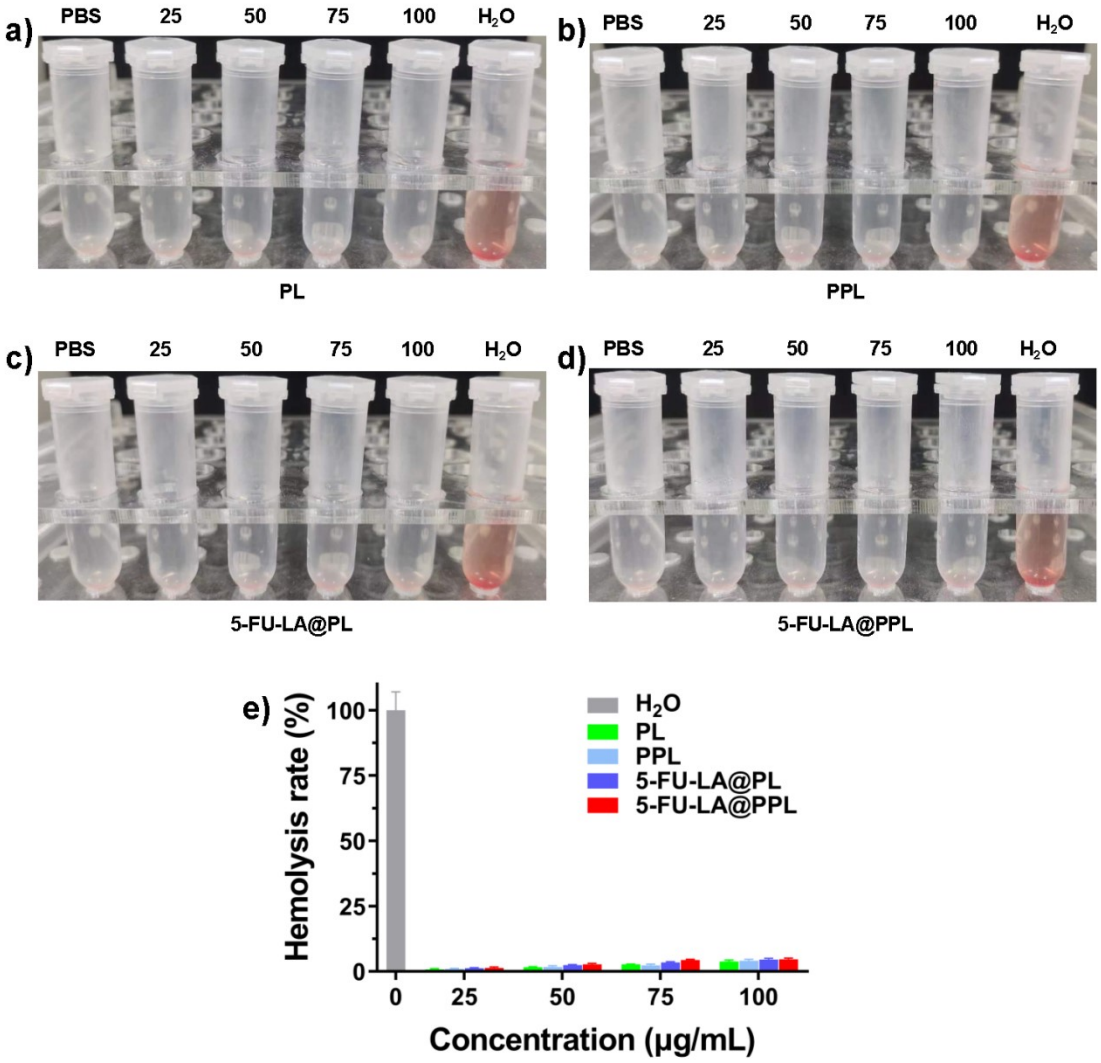


**Figure S15.** Hemolysis photographs of a) PL, b) PPL, c) **5-Fu-LA@PL** and d) **5-Fu-LA@PPL** respectively and hemolysis rates of different components at different concentrations (μg/mL) with 6% sheep red blood cell.

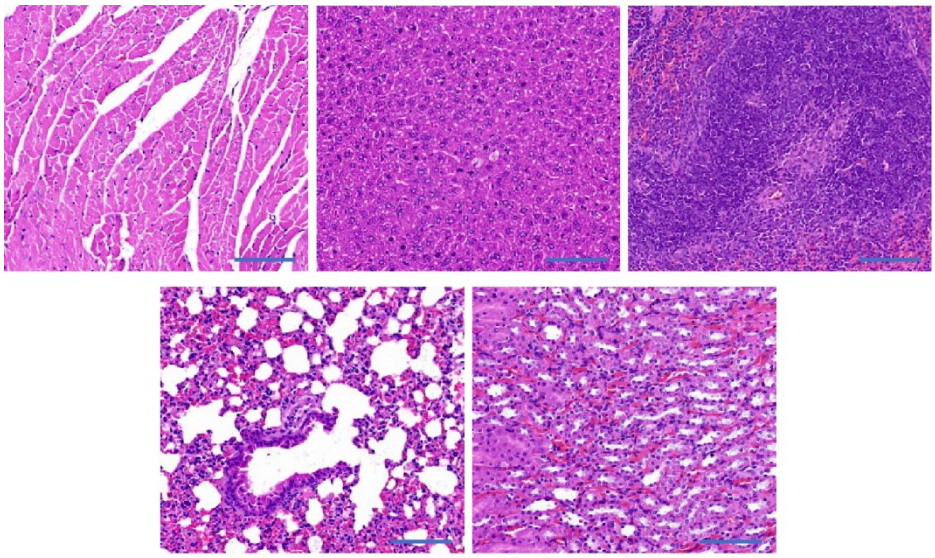

**Figure S16.** Hematoxylin and eosin (H&E) staining for the major organs (heart, liver, spleen, lung and kidney) of tumor-bearing mice under treatment of **5-FU-LA@PPL** incubation in presence of *Fn*. Scale bars: 100 µm.

## References


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