Supporting information

Bacteria-targeted photothermal therapy for combating drug-resistant bacterial infections

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Experimental Section

Material

Ti_3AlC_2 clay was obtained from Shandong Xiyan New Material Technology Co., Ltd, Lithium fluoride (99%) was obtained from Alab (Shanghai) Chemical Technology Co., Ltd., tris (hydroxymethyl) aminomethane (Tris) and Dopamine hydrochloride (DA-HCl) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd., Hydrochloric acid was obtained from Chengdu Kelong Chemical Co., Ltd., The peptide CAEKA (Cys-Ala-Glu-Lys-Ala, ≥95.0%) was obtained from Nanjing GenScript Biotechnology Co., Ltd., Calcein/PI Cell Viability/Cytotoxicity Assay Kit was purchased from Beyotime Biotechnology. CCK-8 Cell Proliferation and Cytotoxicity Assay Kit was obtained from Beijing Solarbio Science & Technology Co., Ltd.

Synthesis of monolayer-$Ti_2C_3T_x$ MXene

The $Ti_2C_3T_x$ MXene was prepared by etching Ti_3AlC_2 with LiF/HCl. Simply, 40 mL 9 M hydrochloric acid was prepared by diluting 36.0%~38.0% (wt%) hydrochloric acid (~12 M) with deionized water in a polytetrafluoroethylene beaker and then 2 g Lithium fluoride was added into under stirring and maintained this state for 1 h to make Lithium fluoride dissolve completely. Then, 1 g Ti_3AlC_2 powder was added into the above mixed solution slowly under stirring and in ice bath, the etching reaction was conducted for 24 h~36 h at 40℃. When the reaction was finished, the resultant was centrifuged at 3500 rpm for 5 min and washed several times with deionized water up to the pH value was above 6 and collect the sediment. The obtained sediment was then dispersed in a certain amount of deionized water and sonicated for 2 h under N_2 protection. Finally, the mixture was centrifuged at 3500 rpm for 60 min and the suspension was collected. The $Ti_2C_3T_x$ MXene powder was obtained through vacuum freeze-drying.

Synthesis of MXene@Polydopamine (MP)

The MXene@Polydopamine (MP) composite was synthesized by in situ oxidative polymerization of dopamine on the surface of $Ti_2C_3T_x$ MXene nanosheets under alkaline condition. Simply, 20 mg $Ti_2C_3T_x$ MXene powder was firstly dispersed in 80 mL deionized water, then 0.12114 g Tris base (10 mM, pH = 8.5) was added into the above $Ti_2C_3T_x$ MXene dispersion under stirring to adjust the pH value. Subsequently, 20 mL 5 mg/mL DA-HCl aqueous solution was injected into the above mixed solution slowly and the reaction was conducted under stirring and room temperature for 12 h. When the reaction was finished, the resultant was centrifuged at 8000 rpm for 5 min and washed with deionized water for several times to remove unreacted DA molecules. Finally, the obtained sediment was dispersed

deionized water with certain volume, and the concentration of the obtained MP colloidal solution was confirmed by freeze drying 10 mL sample dispersion.

Synthesis of MXene@Polydopamine@Peptide (MPP)

MXene@Polydopamine@Peptide (MPP) was synthesized via Michael addition or Schiff base reaction between polydopamine and sulfydryl or amino of Peptide CAEKA in alkaline condition[60]. Firstly, 20 mL 1.65 mg/mL MP dispersion was prepared by diluting original sample solution prepared in previous section, 0.05 g Tris was added into under stirring and the oxygen was removed by bubbling with N_2 for 3 h, then 20 mL 5 mg/mL Peptide CAEKA aqueous solution was injected into the mixture slowly via peristaltic pump, the reaction was conducted under protection of N_2 for 48 h. When the reaction was finished, the resultant mixture was centrifuged at 8000 rpm for 5 min and washed with deionized water for several times to remove unreacted Peptide molecules.

Characterization method

The X-ray diffraction (XRD, X'pert Powder, PANalytical) was performed to investigate the changing situation of crystal structure of samples with scan angle range from 3° to 90°. The morphology and structure of samples were observed via thermal field emission scanning electron microscope (SEM, Netherlands FEI, NOVA NANOSEM 430) equipped with the Oxford X-Max 80 energy dispersive spectrometer (EDS), transmission electron microscope (JEOL, JEM 1400 Plus) with an acceleration voltage of 120 kV and atomic force microscope (XE-100), respectively. The chemical structure and composition were recorded by Fourier transform infrared spectrometer (Thermo Fisher Scientific, Nicolet IS50-Nicolet Continuum) and X-ray photoelectron spectrometer (Thermo Fisher ESCALAB XI+). The UV-Vis-NIR spectrum was obtained by using a UV-VIS-NIR Spectroscopy (Lambda950). Nanoparticle size

potentiometer (Zetasizer Nano ZS90, Malvern Instruments Ltd.) was used to study the sample size and surface zeta potential. Photothermal conversion performance was observed via handheld temperature infrared thermometer (Wuhan Guide Sensmart Tech Co., Ltd., B256V) and NIR fiber-coupled laser (Changchun Lairui Photoelectric Technology Co., Ltd., LR-MFJ-808/2000mW).

Photothermal conversion performance of MPP

To investigate the photothermal conversion property of MPP, the UV-Vis-NIR absorption spectrum was firstly performed to study the light absorption capacity of MPP. Then, the temperature variation trend of MPP dispersion with time (Temperature-Time curve) under illumination of NIR-light with wavelength of 808 nm was recorded via handheld temperature infrared thermometer.

Bacterial culture

A single bacterial colony was picked up with a 100 μL pipette tip and dispersed in 3 mL of liquid LB medium, and incubate in a shaker (200 rpm, 37 °C) for 12 h. Then the culture is expanded at a ratio of 1:100 for another 8 h under the same conditions as above. After the culture, 1 mL of bacterial suspension was taken to centrifugate (5000 rpm, 10 min) and washed two times with sterile phosphate-buffered saline (PBS) to remove bacterial metabolites. Finally, the obtained bacterial sediment was redispersed in 1 mL of sterile PBS for use.

Photothermal antibacterial performance of MPP

The *E. coli*, *S. aureus* and methicillin-resistant *staphylococcus aureus* (MRSA) were selected as the representatives of Gram-negative, Gram-positive and drug-resistance bacteria to investigate the in vitro photothermal antibacterial performance of MPP. Briefly, 100 μL MPP and $Ti_3C_2T_x$ MXene colloidal solution with different concentration (100 μg/mL, 80 μg/mL, 60

μg/mL, 40 μg/mL) was mixed with 100 μL bacteria suspension (about 2.0×10^7 CFU/mL) in 96-well plate and cocultured for 30 min in the dark. Then, the MPP + NIR group was illuminated with 808 nm NIR laser (0.7 W/cm^2) for 5 min, and the final temperature was recorded with handheld temperature infrared thermometer. The $Ti_3C_2T_x$ MXene + NIR group was illuminated with 808 nm, 0.7 W/cm^2 NIR laser up to the bulk temperature is identical to the corresponding MPP + NIR group with same sample concentration. After illumination, 20 μL mixture was taken into 180 μL sterile PBS and after gradient dilution for 10^3 times, 100 μL diluent was spread on agar plate and cultured in biochemical incubator for 12 h at 37℃. The number of bacterial colonies was record by Image J software.

MRSA-targeting effect of MPP detected by SEM

The MPP dispersion (0.5 mL, 40 μg/mL) was mixed with MRSA suspension (0.5 mL, about 2.02×10^7 CFU/mL) in a 1.5 mL centrifuge tube and cocultured for 3 h. After incubation, the mixture was centrifuged at a low revolving speed (2000 rpm) for 5 min and washed with sterile PBS for several times to remove the MPP that don't combine to the surface of MRSA. The collected MPP-MRSA hybrid sediment was immobilized with 2.5% glutaraldehyde for 12 h at 4℃, and then successively dehydrated with 30%, 50%, 70%, 80%, 95%, 100% ethyl alcohol. Finally, the sample was dried in dryer for about 4 h, and then observed by SEM.

In vitro biocompatibility of MPP

CCK-8 assay: The in vitro cytotoxicity of MPP was determined by Cell Counting Kit-8 (CCK-8), and L929 cells (P8 generation) was selected as the experimental subject. Firstly, L929 cells were inoculated in 96-well plate with the cell density of 2000 cells per well and cultured for 16~24 h in CO_2 incubator (37℃, 5% CO_2), then the samples ($Ti_3C_2T_x$ MXene and MPP) in complete medium (containing 89% RPMI Medium1640 (1×) (gobic), 10% fetal bovine serum

(FBS) and 1% Penicillin-Streptomycin (gobic)) with different concentration (20, 40, 60, 80, 100 μg/mL) were added into the wells with 100 μL per well and cocultured for 3 days in CO_2 incubator. Washed with PBS for 2 times to remove the fuscous samples, then 100 μL culture medium and 10 μL CCK-8 reagent (Solarbio) were added into the above wells and cocultured in CO_2 incubator for 4 h. Finally, the absorbance at 450 nm was detected with microplate reader (DLJ-100D) and the survival rate of cell was calculated according to the following formula:

$$Cell\ viability\ (\%) = \frac{A_S - A_B}{A_{CTRL} - A_B} \times 100\%$$

where A_S is the absorbance of experimental group which containing samples, complete medium, L929 cells and CCK-8 agent; A_B is the absorbance of blank group which only containing complete medium and CCK-8 agent; A_{CTRL} is the absorbance of control group which containing L929 cells, complete medium, and CCK-8 agent.

Live and dead cells staining: The L929 cells were inoculated into 6-well plate with cell density of 60000 cells per well and cultured for 16~24 h in CO_2 incubator (37℃, 5% CO_2), then the culture medium was removed and 3 mL sample solution ($Ti_3C_2T_x$ MXene, MPP was dispersed in complete medium, respectively) with different concentration (20, 40, 60, 80, 100 μg/mL) were added into the corresponding well and subsequently cultured in CO_2 incubator for 24 h. Then, the supernatant complete culture was removed from every well and washed with sterile PBS for 2 times. 0.5 mL 0.05% EDTA-pancreatic ferment was added into every well to digest the adherent L929 cells for 80 s. Subsequently, 1 mL complete medium was added to terminate digestion. Then centrifuged (1000 rpm, 5 min) to collect L929 cells and washed with 1 mL sterile PBS, The obtained L929 cells were resuspended in 1 mL Calcein AM/PI and cultured for 30 min (37 ℃) under dark, centrifuged (1000 rpm, 5 min) and washed with sterile PBS, the

collected cells were resuspended in 20 μL PBS and then transferred to glass slide, after sealing with a coverslip, the fluorescence image was obtained with fluorescent slide scanner (PA53 FS6 SCAN) at wavelength of 490 nm and 545 nm.

Hemolytic activity: The sterile defibrillated rabbit blood was obtained from Nanjing SenBeiJia Biological Technology Co., Ltd. The obtained blood was firstly centrifuged (3000 rpm, 10 min) and washed with sterile PBS for 3 times to collect hemocyte, subsequently the collected blood cells were redispersed in sterile PBS to form blood cell suspension (5 wt%). Then, the blood cell suspension was mixed with sample ($Ti_3C_2T_x$ MXene and MPP) colloid solution with certain volume and concentration in 1.5 mL centrifuge tube, and the final concentration of samples was 200 μg/mL, 100 μg/mL, 50 μg/mL, 25 μg/mL and 12.5 μg/mL respectively. The mixture was cultured at 37℃ for 2 h, and then centrifuged to collect supernatant to detect optical density value at 540 nm. At the same time, Triton X-100 solution (0.2%, v/v) and PBS were selected as positive control and negative control, respectively. Hemolysis rate was calculated according to the following formula:

$$Hemolysis\ rate\ (\%) = \frac{A_S - A_N}{A_P - A_N} \times 100\%$$

where A_S, A_N and A_P are the optical density value of sample group, PBS group and Triton X-100 solution group, respectively.

In vivo antibacterial performance and biocompatibility

All animal experiment procedures were performed in accordance with the Guidelines for care and Use of Laboratory Animals of Institute of Biological and Medical Engineering, Guangdong Academy of Science and approved by the Animal Ethics Committee of Institute of Biological and Medical Engineering, Guangdong Academy of Science (K2022-01-012-015). The female

mice (Balb/c, 8~9 weeks, 19~22 g) were purchased from Zhuhai BesTest Bio-Tech Co, Ltd. as the object to evaluate the in vivo antibacterial performance and biocompatibility of MPP. In consideration of the remarkable responsiveness of MPP to NIR light and the outstanding penetrating ability of NIR light for biological tissue, we selected the subcutaneous infection of mice as the therapy model. The mice were firstly divided into 4 groups (PBS, Van, $Ti_3C_2T_x$ MXene + NIR and MPP + NIR group, 3 mice per group), next unhaired on the back after narcotizing and sterilizing with 75% medical alcohol, then 100 μL MRSA suspension (suspended in sterile PBS) was subcutaneously injected (denote as -1 day). After 24 h, the subcutaneously infectious model was established (0 day), 100 μL sterile PBS, Van solution, $Ti_3C_2T_x$ MXene colloidal solution and MPP colloidal solution with concentration of 100 μg/mL were subcutaneously injected into the infectious site respectively according to the group. For the MPP + NIR group, the infectious site was irradiated with 808 nm near-infrared laser (1 W/cm^2) for 5 min, and the temperature of infectious site was recorded by handheld temperature infrared thermometer. The $Ti_3C_2T_x$ MXene + NIR group was irradiated under the same laser condition up to the final temperature of infectious site was identical to the MPP + NIR group. On the first day (1 day), the second treatment was conducted, the apparent state of infectious site and weight of mice were recorded every day from zero day (0 day) to ninth day (9 day). At the end of the course of treatment, the whole blood was obtained for blood biochemical analysis and the all mice were killed by prolapsing cervical spine after anesthesia, the skin tissue of infectious site was resected and then homogenized to form homogenate diluent for coating plate and colony counting. The skin tissue around the infectious site and the main internal organs (heart, liver, spleen, lung and kidney) were collected, followed by immobilizing with 10% Neutral Buffered Formalin for 24 h, dehydrating with ethyl alcohol with different concentration

(75%, 85%, 90%, 95%, 100%), embedding with paraffin, slicing, dyeing with Hematoxylin and eosin (H&E), Mason tricolor for clinicopathologic analysis.

Statistics Analysis

All experiments were carried out in triplicate and data were expressed as mean ± standard deviation (SD). Difference between the control and the experimental groups were analyzed by One Sample t Test, and different characters indicate statistical difference at *P < 0.05, **P < 0.01 and ***P < 0.001 level.

原子百分比（%）	肽 CAEKA	$Ti_3C_2T_x$ 2DM	MPP
O 1s	20.74	13.66	13.76
C 1s	56.80	41.35	77.87
N 1s	14.10	1.29	5.9
Ti 2p	-	20.46	1.25
S 2p	2.76	-	0.43
F 1s	-	16.98	0.53

Figure S1. Atomic content of C 1s, O 1s, N 1s, S 2p, F 1s and Ti 2p obtained from XPS

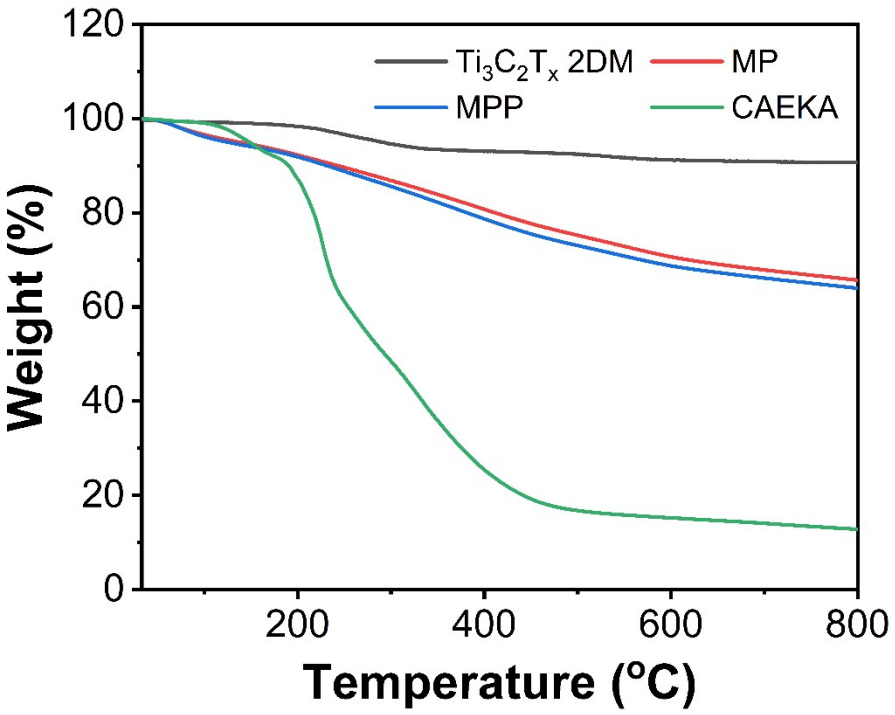

Figure S2. TGA curves of $Ti_3C_2T_x$ 2DM, MP, MPP and peptide CAEKA under nitrogen atmosphere.

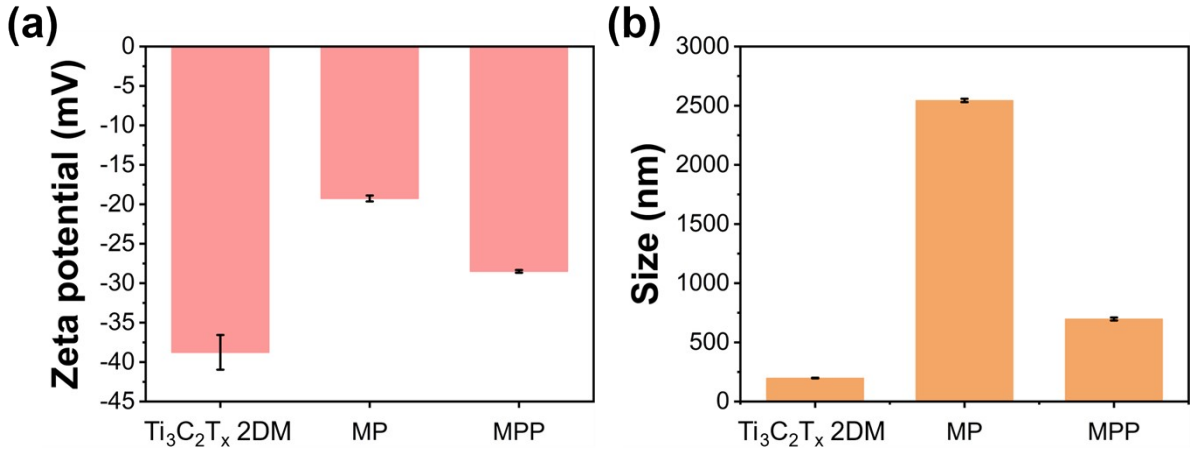

Figure S3. Zeta potential (a) and plate diameter size (b) of $Ti_3C_2T_x$ 2DM, MXene@PDA (MP) and MXene@PDA@Peptide (MPP).

Element	C	Al	P	S	Ca	Ti
MRSA	78.8	0.41	1.05	0.27	0.24	/
MRSA+MPP	85	0.78	1.7	0.39	1.24	1.73

Figure S4. The mass fraction of each element in the MRSA and MRSA + MPP groups

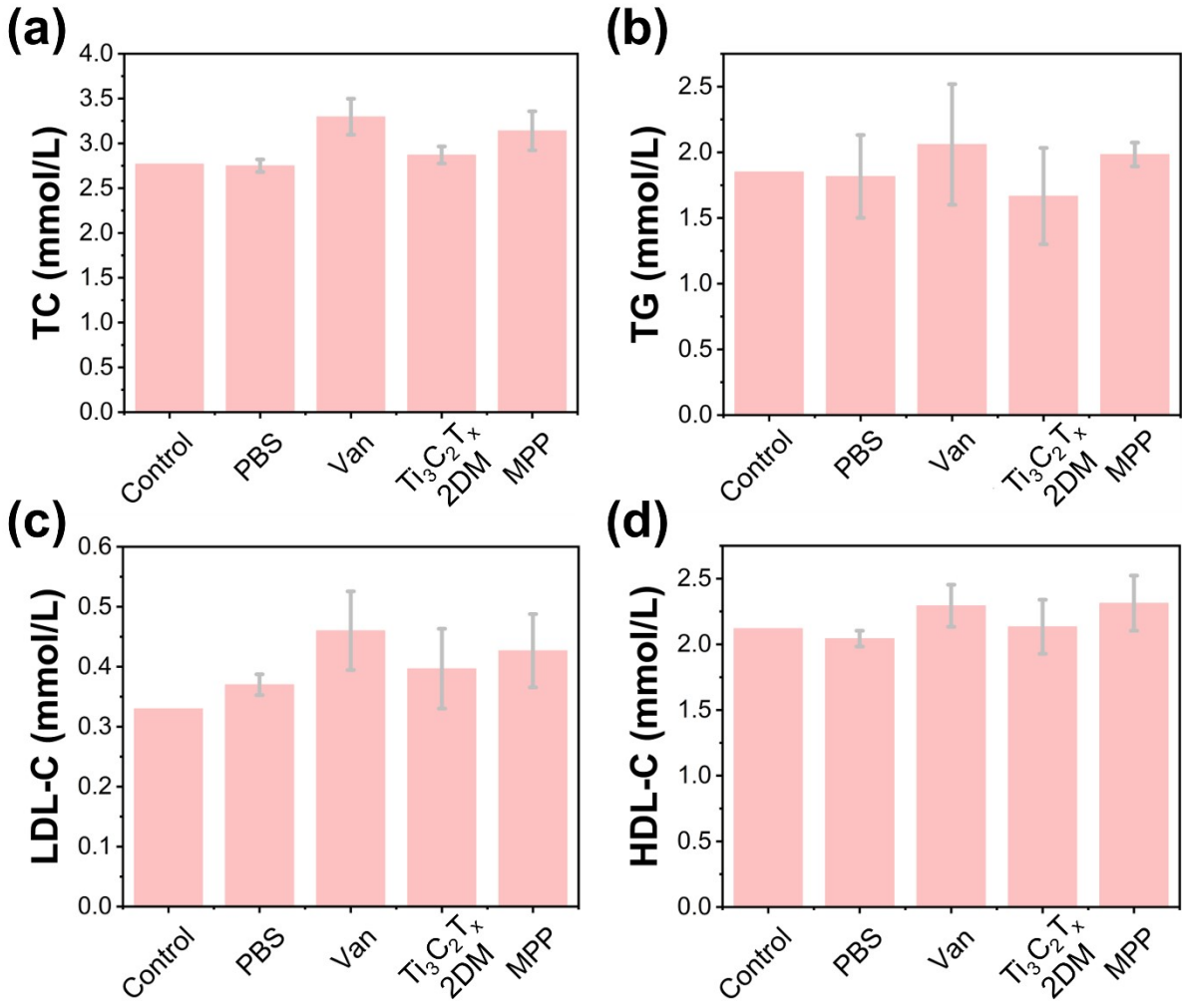

Figure S5. The changing situation of four types of blood fat for mice in every group after treating with different samples. (a) TC, serum total cholesterol; (b) TG, triglyceride; (c) LDL-C, low density lipoprotein cholesterol; (d) HDL-C, high density lipoprotein cholesterol.

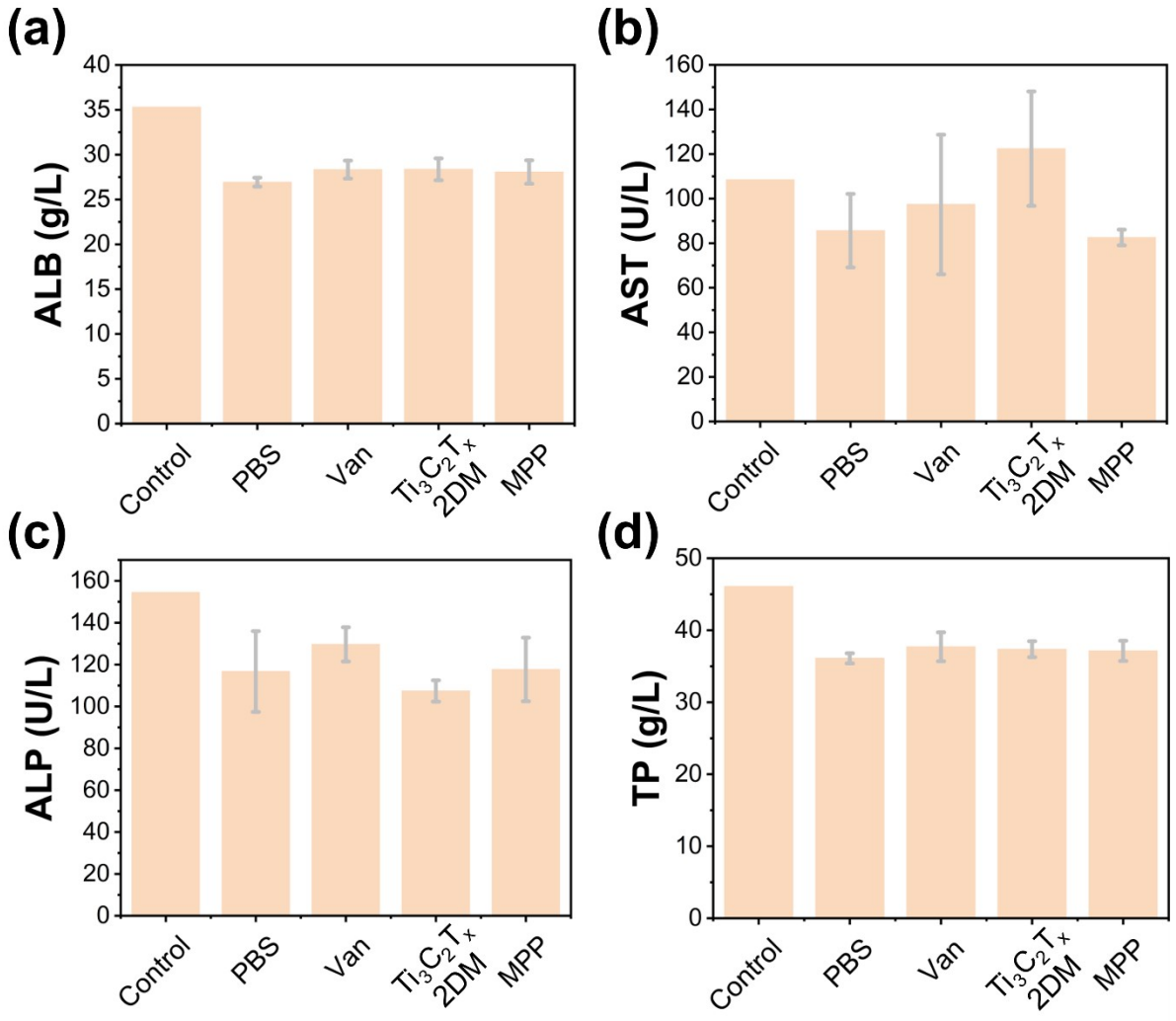

Figure S6. The changing situation of liver function index for mice in every group after treating with different samples. (a) ALB, serum albumin; (b) AST, aspartate aminotransferase. (c) ALP, alkaline phosphatase. (d) TP, serum total protein.

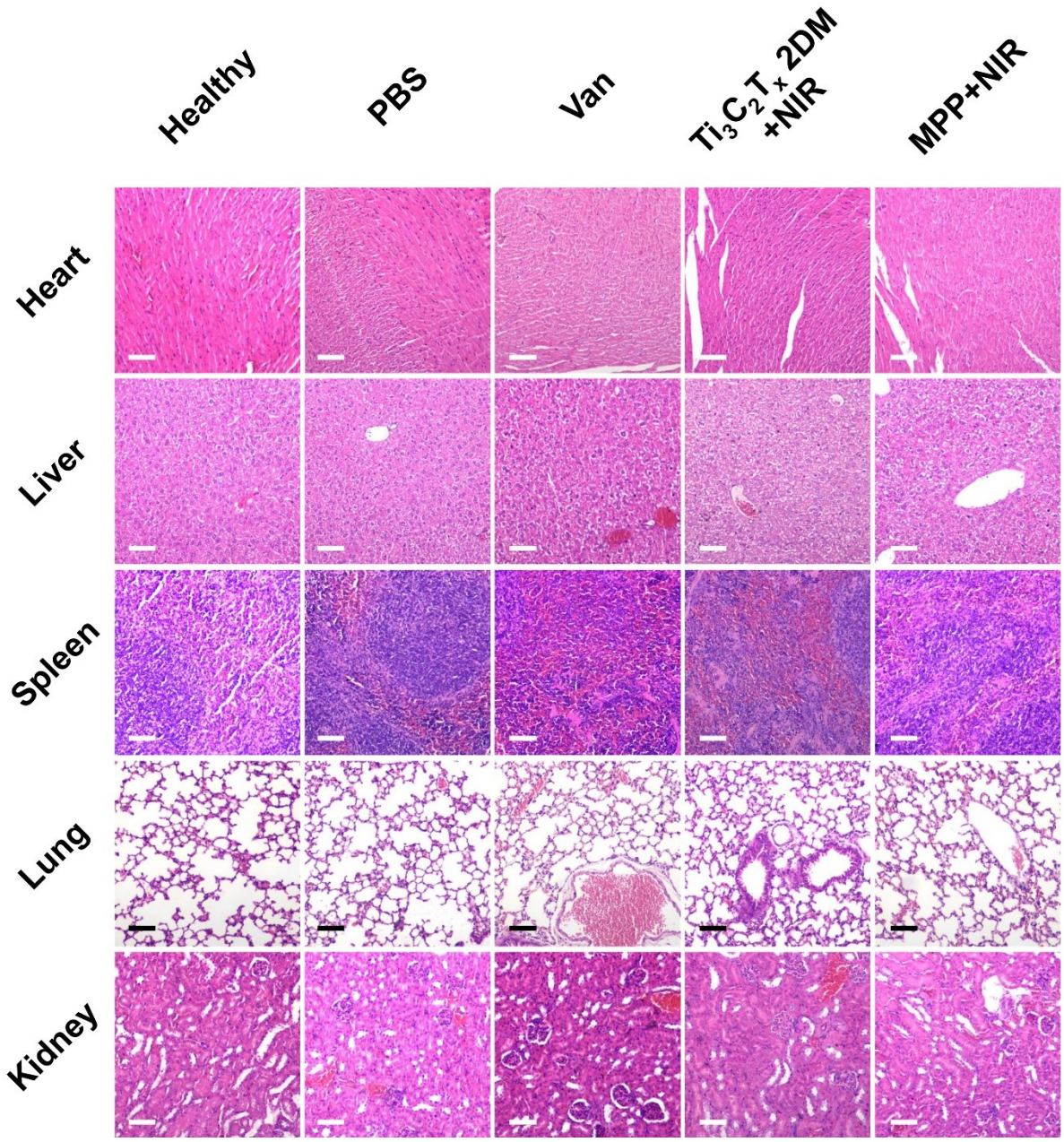

Figure S7. H&E-stained tissue slice of major organs (heart, liver, spleen, lung and kidney) for mice after treating with different sample. The scale bar is 100 μm.