Supplementary Information

EDTMP ligand-enhanced water interaction endowing iron oxide nanoparticles with dual-modal MRI contrast ability

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Experimental details

Bioactivity assay

The bioactivity of as-synthesized samples was determined by Alkaline phosphatase (ALP) activity assay based on the manufacturer's instructions.[1] Alkaline phosphatase (ALP) staining was applied to qualitatively assess the activity of ALP in MC3T3-E1 cells exposed to nanoparticles. 2×10^4 cells were firstly seeded into each well of the 24-well plate and incubated with DMEM for 24 h. Then, the original medium was substituted with fresh medium containing nanoparticles at the concentration of 100 μg/mL, and the cells were exposed to magnetic nanoparticles for other 24 h. Finally, the medium containing samples was replaced by fresh normal culture medium. When cultured for 7 days, the cells were washed three times with PBS, fixed with 4% paraformaldehyde (PFA) for 15 min, and washed with PBS again. Thereafter, the cells were immersed in 500 μL ALP dye for at least 12 h at room temperature under dark conditions. The extra ALP dye was washed away with PBS and then the purple color intensity was observed by a light microscope (TE2000U, Nikon). The ALP relative activity was tested by Alkaline phosphatase detection kit (Beyotime Biotechnology, Inc). After treated with nanoparticles for 7 days, MC3T3-E1 cells were washed with PBS for three times and split by adding 200 mL of RIPA cell lysis solution, freezing at -80°C for 25 min and thawing at 37°C. Then, p-nitrophenol phosphate substrate and BCA solution were added, followed by incubation in the dark for 30 min at 37°C. The absorbances at 405 nm (OD_{405}) and 562 nm (OD_{562}) were read using a multifunction microplate scanner. The corresponding ALP quantitative evaluation was calculated according to the equation OD_{405}/OD_{562}. The cells incubated with normal culture medium was employed as control group (TCP).

Serum analysis and histological evaluation

After MRI scanning, mice were anesthetized by chloral hydrate and the blood samples were collected for biochemical analysis. The Fe concentration in rat blood was measured using inductively coupled plasma-optical emission spectroscopy (ICP-OES,

Thermo Jarrell Ash, USA). Then, the liver, kidney and spleen were dissected and fixed in 4% PFA for histological evaluation. The fixed tissues were embedded in paraffin to cut into sections. After gradient dehydration, tissue sections were stained with hematoxylin and eosin and Prussian Blue Iron Stain Kit. Finally, tissue compatibility was observed by a digital slice scanner (Pannoramic DESK, P-MIDI, P250, 3D HISTECH, Hungary). Normal mice were used as control.

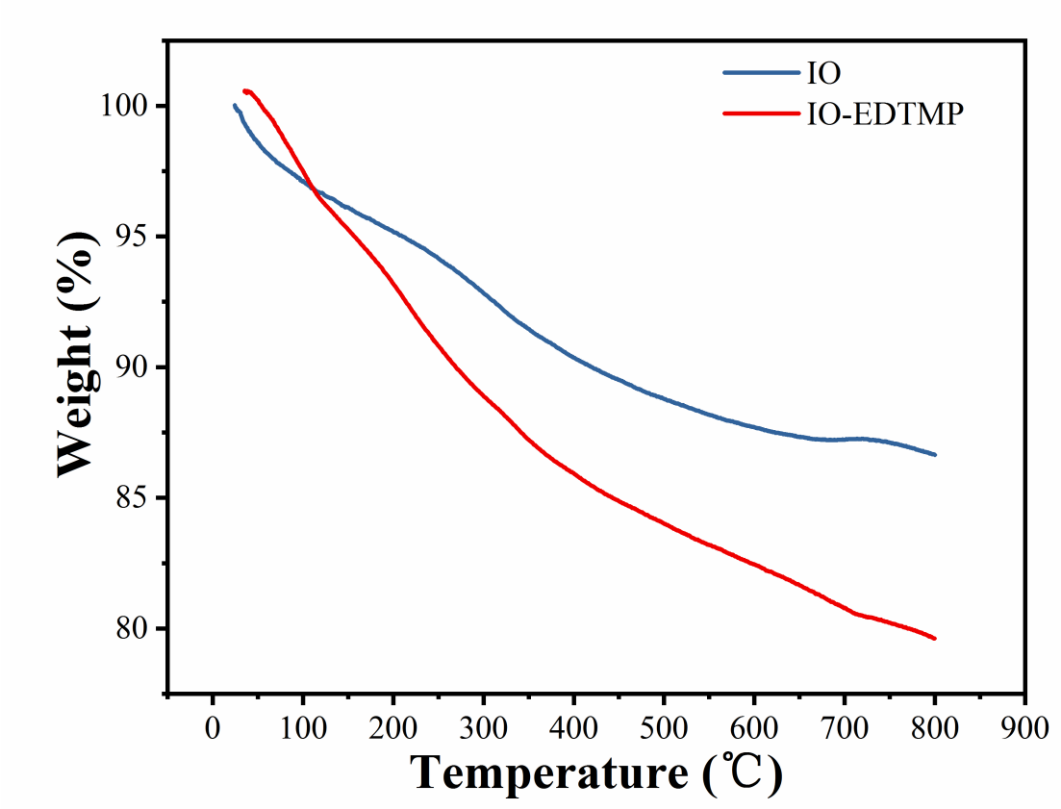

Figure S1. TGA curves of IO and IO-EDTMP NPs.

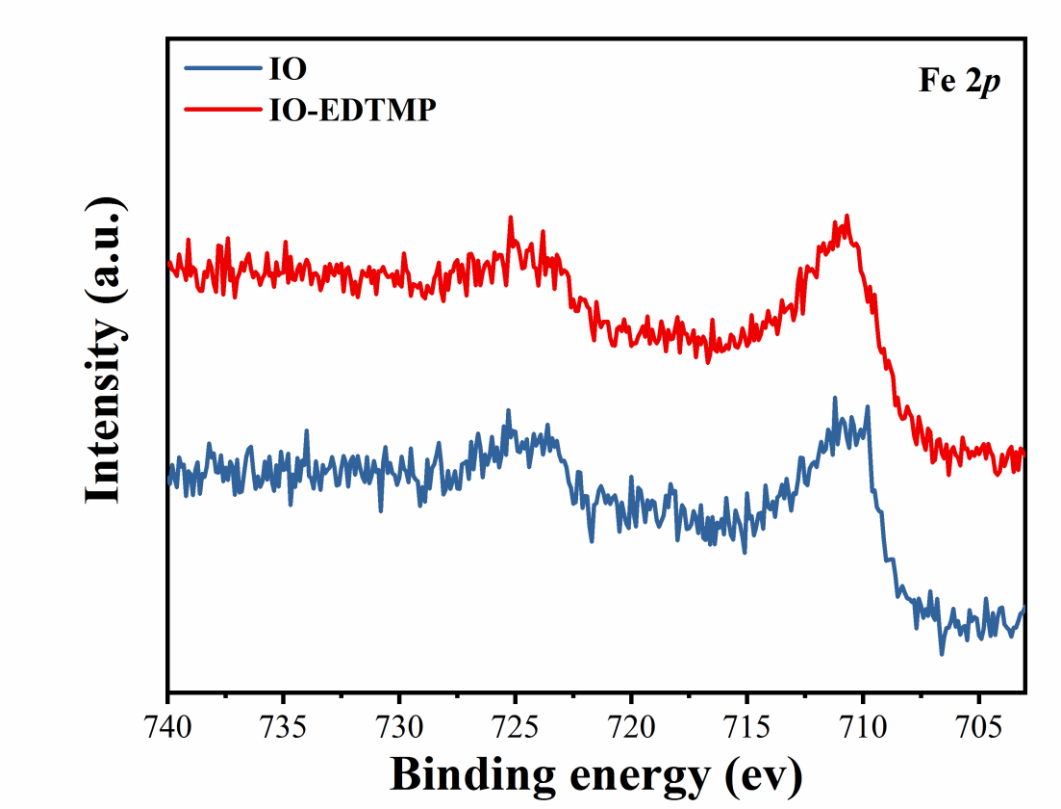

Figure S2. High-resolution Fe 2p XPS spectra of IO and IO-EDTMP NPs.

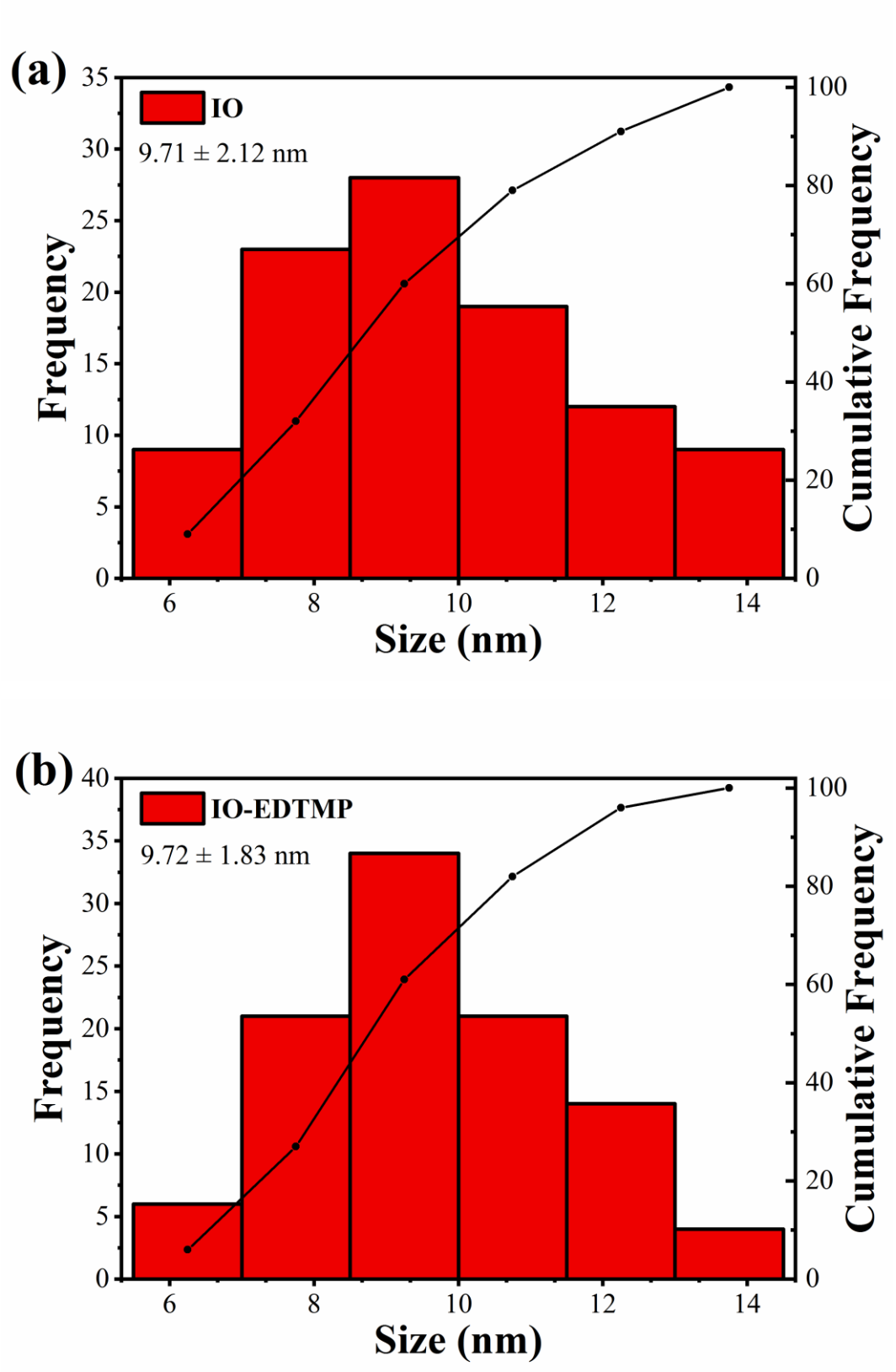

Figure S3. Statistical analysis of size distribution of (a) IO NPs and (b) IO-EDTMP NPs measured from TEM images.

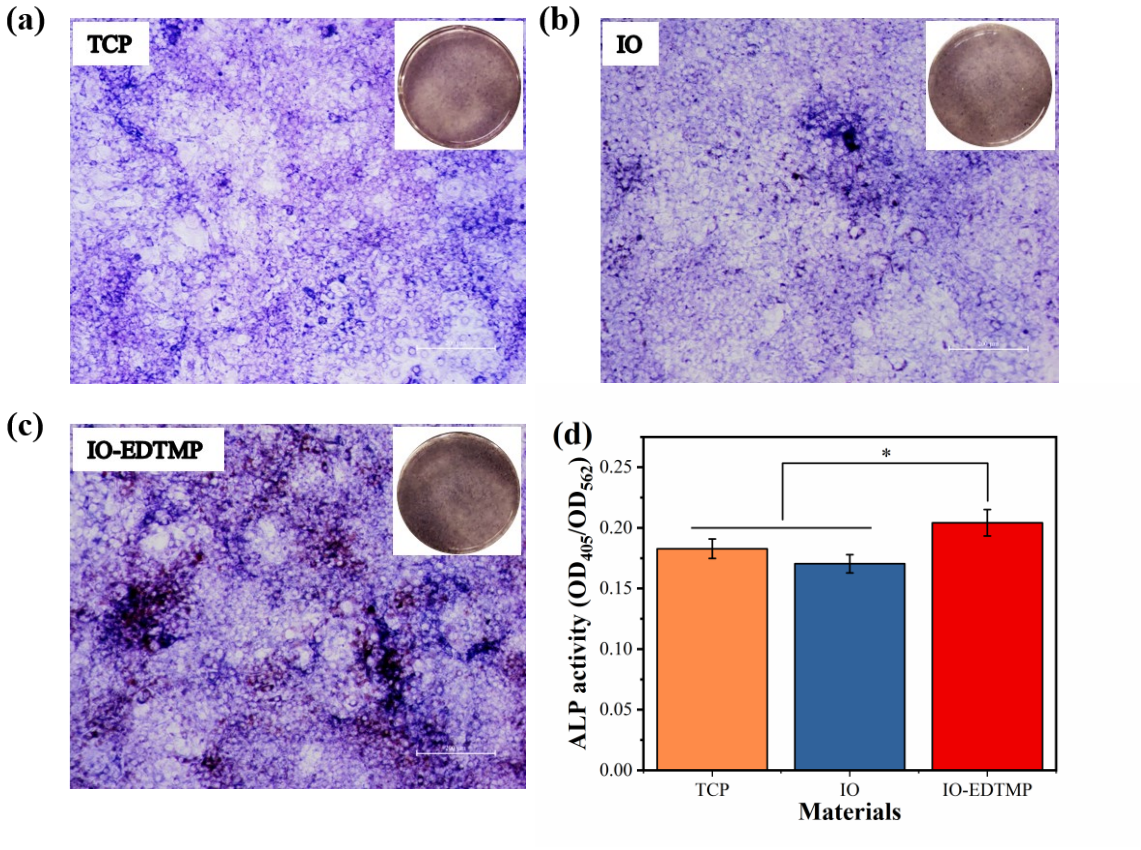

Figure S4. (a-c) ALP staining and (d) the corresponding quantitative evaluation of cells treated with different materials for 7 days. Scale bar: 200 μm. * indicates a statistically significant difference, *$p < 0.05$.

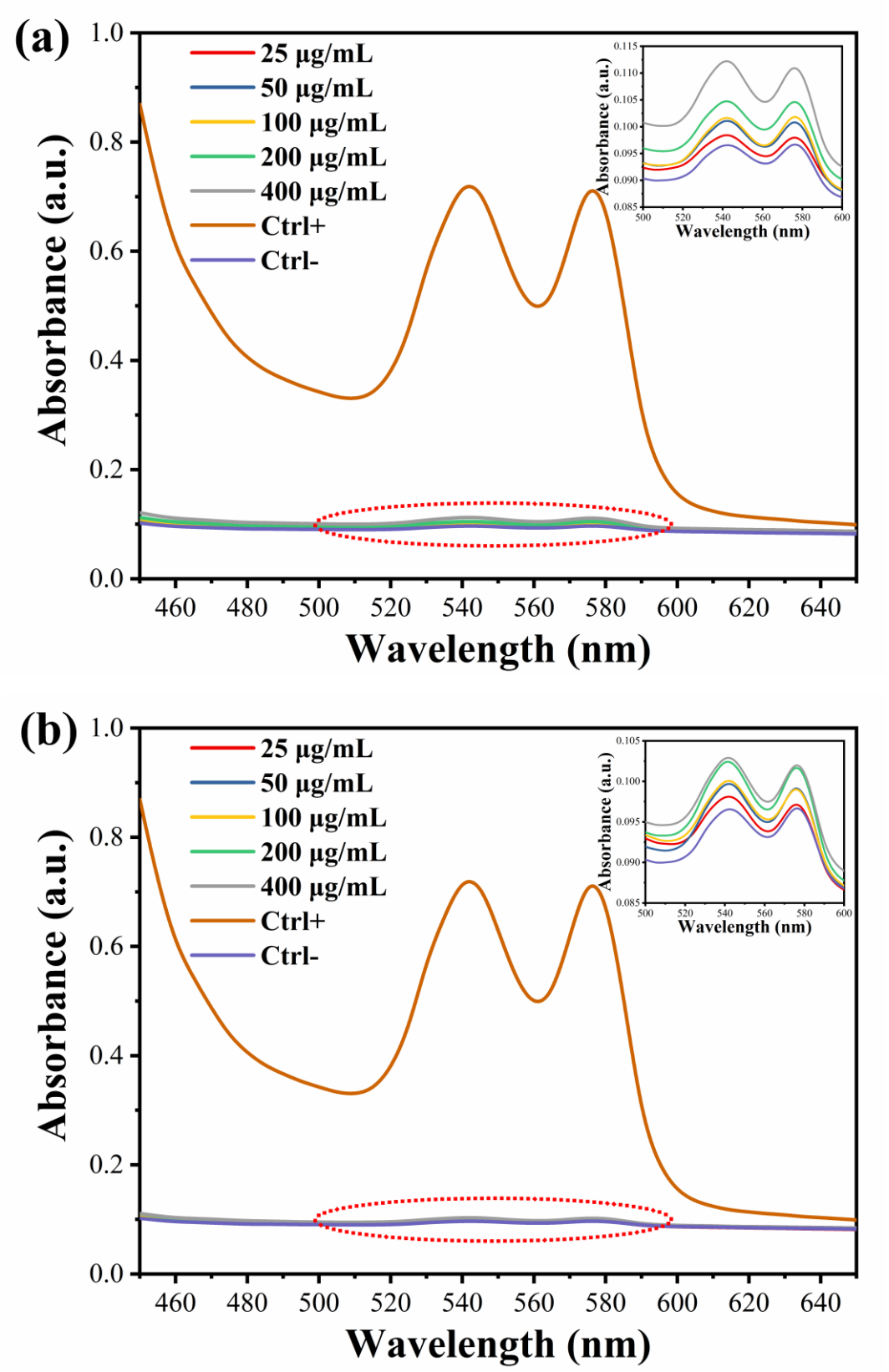

Figure S5. Hemolytic activity of (a) IO NPs and (b) IO-EDTMP NPs.

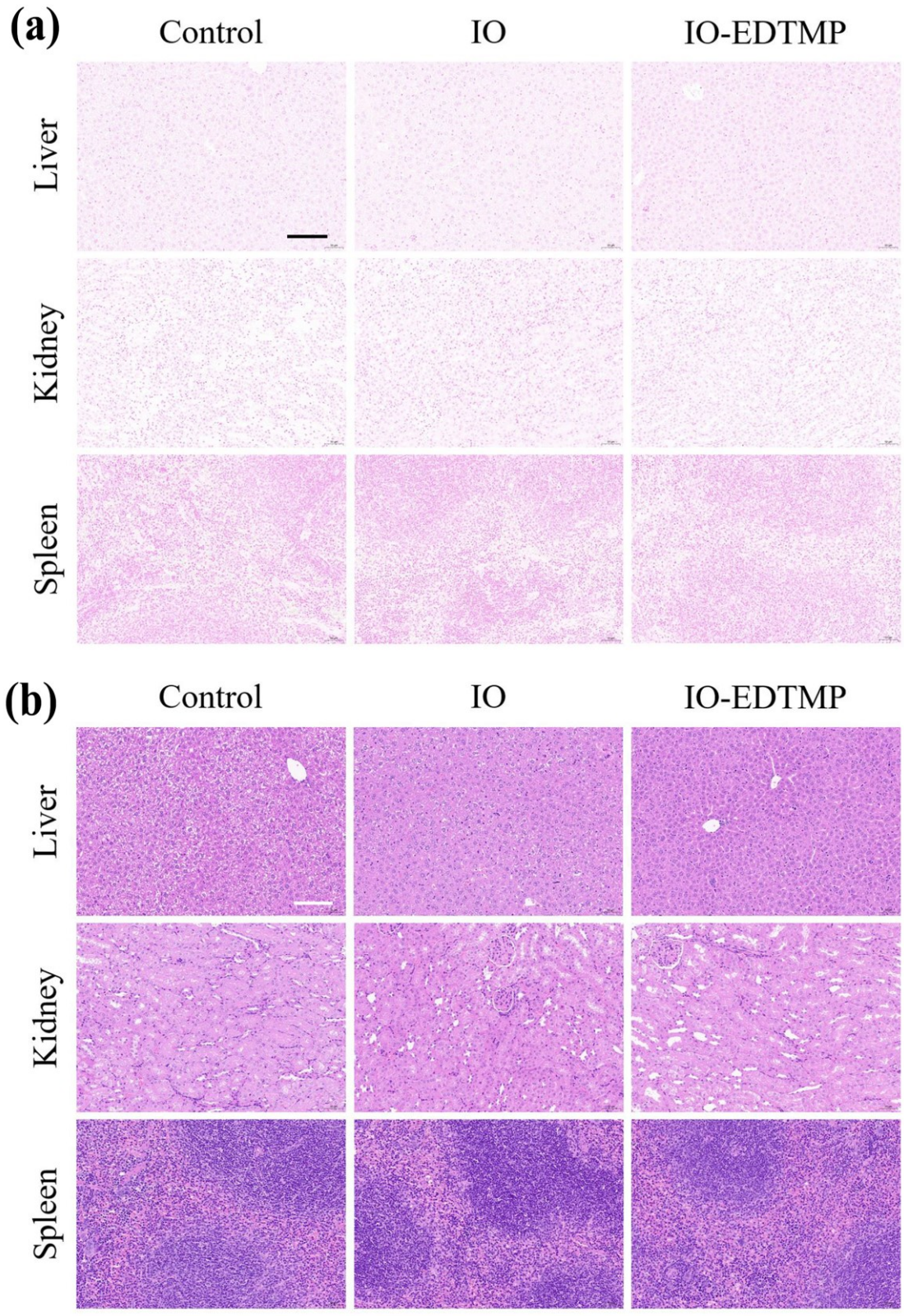

Figure S6. Histological evaluation of the liver, kidney and spleen excised from normal mice (control) and the mice suffered intravenous administration of IO and IO-EDTMP NPs. (a) Prussian Blue staining. (b) Hematoxylin and eosin staining. Scale bar: 100 μm.

Table S1. Serum analysis of normal mice (control) and the mice suffered intravenous administration of IO and IO-EDTMP NPs

Parameter	Control (n = 4)	IO (n = 4)	IO-EDTMP (n = 4)
	mean ± SD	mean ± SD	mean ± SD
Total protein, g/L	54.6 ± 1.4	48.5 ± 2.1	50.8 ± 1.1
ALT, U/L	58.3 ± 16.2	104.3 ± 21.3	76.0 ± 15.4
ALP, U/L	235.7 ± 33.9	320.8 ± 55.1	309.0 ± 32.2
Urea mmol/L	6.5 ± 1.8	9.9 ± 0.5	8.5 ± 0.7
Creatinine, μmol/L	37.7 ± 2.3	39.4 ± 2.1	41.5 ± 2.7
Albumin, g/L	29.7 ± 1.3	27.9 ± 0.9	28.5 ± 0.4
Iron, μmol/L	41.48 ± 7.54	32.48 ± 4.74	40.98 ± 1.88

Abbreviations: ALT, alanine transaminase; ALP, alkaline phosphatase.

References

[1]. P. Wang, L. Hao, Z. Wang, Y. Wang, M. Guo, P. Zhang, *ACS Appl. Mater. Interfaces*, 2020, 12, 49464-49479.