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Ultrasmall Calcium-enriched Prussian Blue Nanozymes Promote Chronic Wound Healing by Remodeling Wound Microenvironment

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Methods

1. Cell cultures and CCK-8 Analysis

HUVEC cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) medium (Gibco, China) containing 1% penicillin G sodium/streptomycin sulfate and 5% fetal bovine serum (FBS) (Gibco, China). The survival rate of HUVECs following various treatments was determined using CCK-8. After HUVECs were exposed to various concentrations of CaPB NPs and incubated for 24 h, HUVECs were washed three times using sterile buffer and subsequently incubated with 90 μL of fresh culture medium and 10 μL of CCK-8 solution. Then, HUVECs were exposed to 37 °C for 2 h, and the absorbance at 450 nm was analyzed with a microplate reader to quantify the cell viability.

2. Detection of intracellular ROS

The generation of intracellular ROS was identified by DCFHDA detector. DCFH can be converted by the intracellular ROS into fluorescent DCF, which serves as a ROS level indicator. Cells were pre-treated with H_2O_2(1mM) for 2 h in the 6-well. Next, the cells were treated with CaPB NPs and USPBs separately for 4 h. Then, the cells were treated with 10 μM DCFH-DA at 37 ℃ for 30 min in the incubator. After washing with PBS three times, DCF fluorescence staining photos were imaged by fluorescence microscopy (Ex/Em = 488/525 nm). ImageJ software was utilized to calculate the respective fluorescence intensity, and the results are presented as percentages after normalization to the control.

3. Hemolytic activity test of CaPB NPs and USPBs

Red blood cells (RBC) from fresh mouse blood were treated individually with different concentrations of CaPB NPs and USPBs for an hour at 37 °C. Then the supernatant was collected through centrifugation (1500 rpm, 10 minutes), the absorbance at 540 nm was determined using a microplate reader. The negative or positive control, respectively, was RBC treated with PBS or H_2O.

4.Detection of stability of CaPB NPs

To study the stability of CaPB NPs, the dialysis bag containing CaPB NPs (100 mg) was immersed in solution with PBS or ultra purified water. After 24 h, the ion concentrations in the solution were quantified by ICP-OES and the corresponding NPs were characterized by FTIR and XPS spectroscopy, respectively.

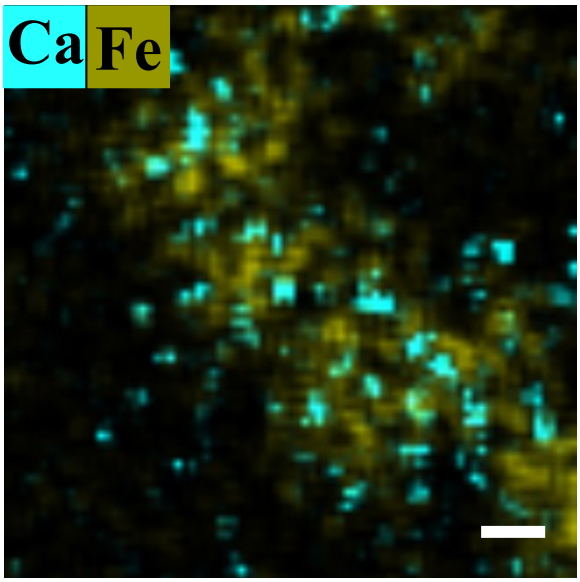

Fig S1. EDS mapping of CaPB NPs. The scale bar represents 20 nm.

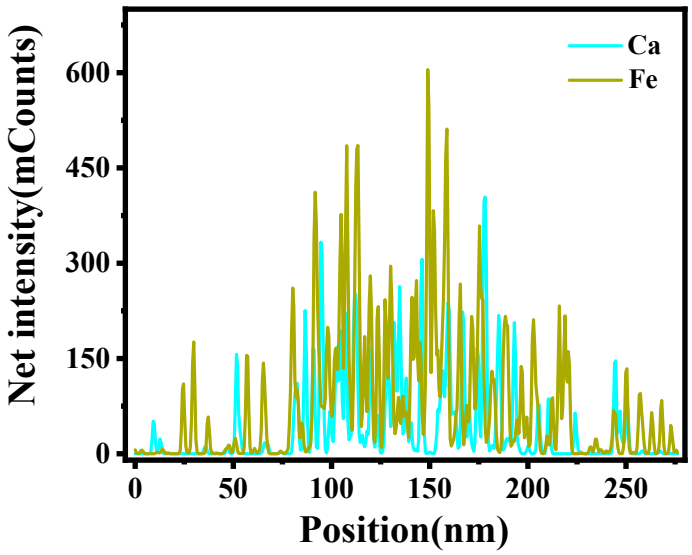

Fig S2. The corresponding line-scan TEM-EDS elemental distribution curves of Ca, Fe in CaPB NPs.

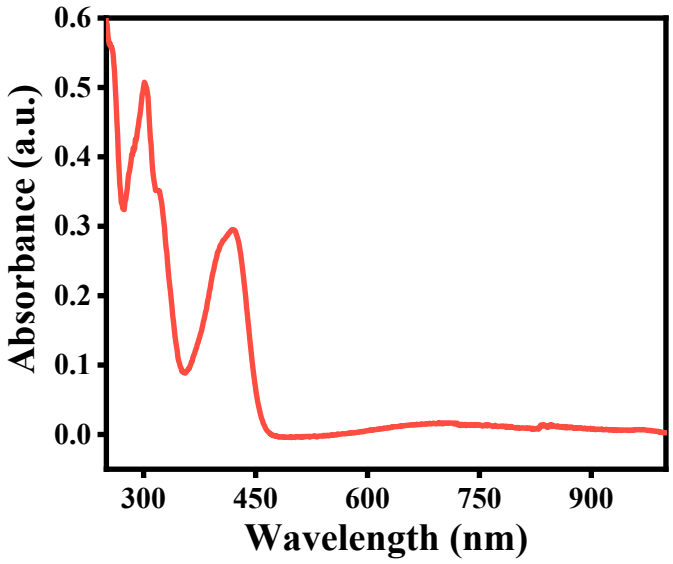

Fig S3. Absorption spectrum of CaPB NPs.

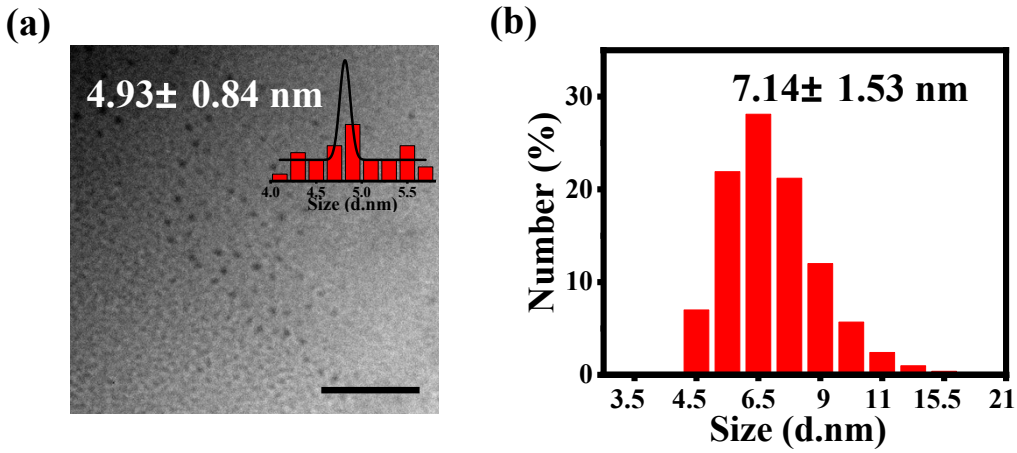

Fig S4. (a) TEM image of USPBs. The scale bar represents 50 nm. Inset represented the size distribution analyzed by Nano measure software. (d) DLS data of USPBs.

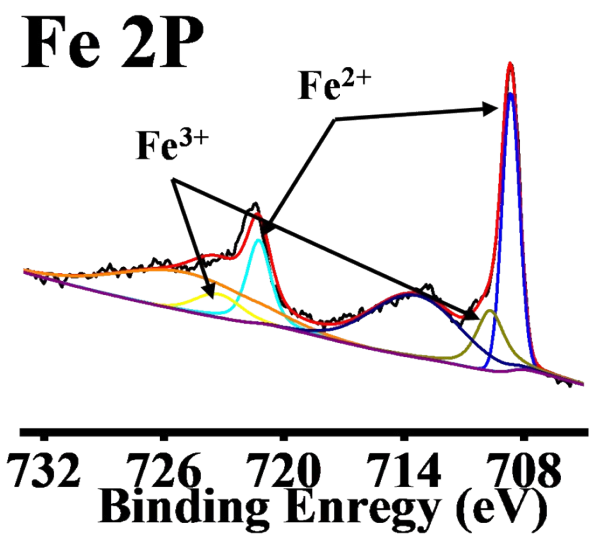

Fig S5. High resolution XPS spectra of USPBs in the Fe 2p region.

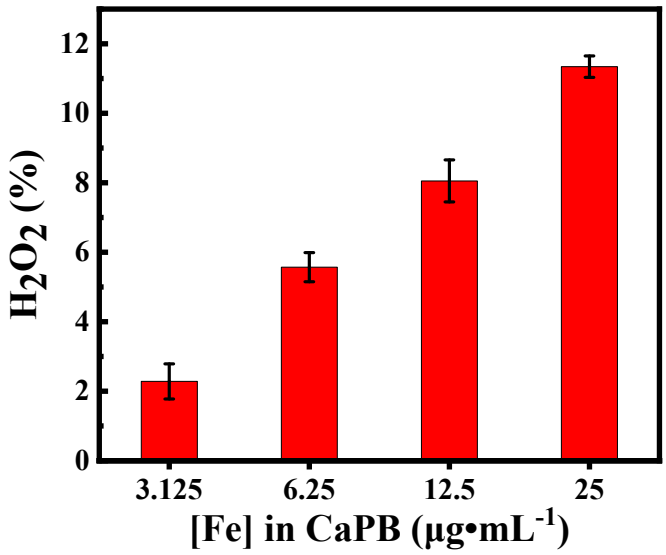

Fig S6. H_2O_2 degradation catalyzed by CaPB NPs through CAT mimics.

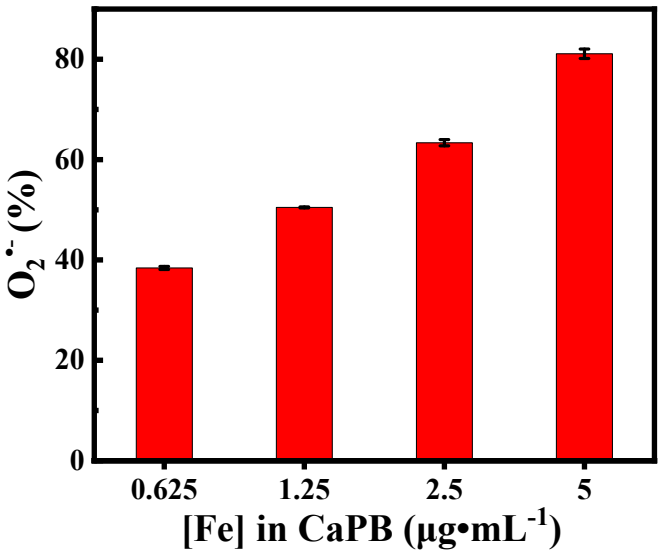

Fig S7. The $O_2^{\bullet-}$ scavenging rate of CaPB NPs.

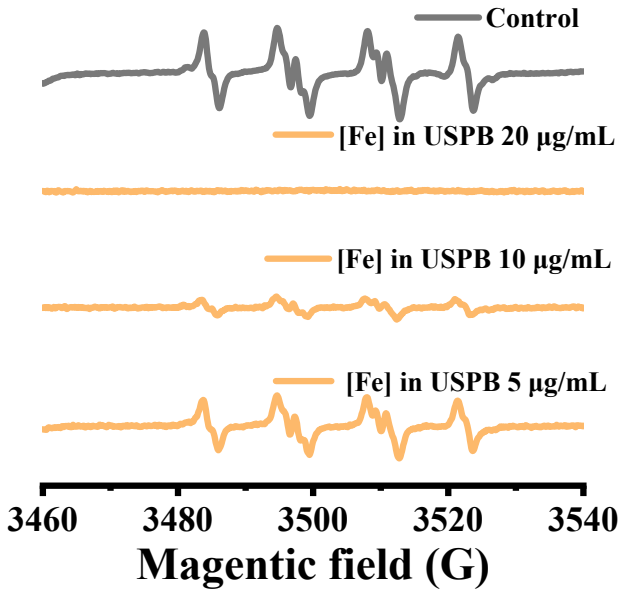

Fig S8. ESR spectra of USPBs for determining the SOD-like activity.

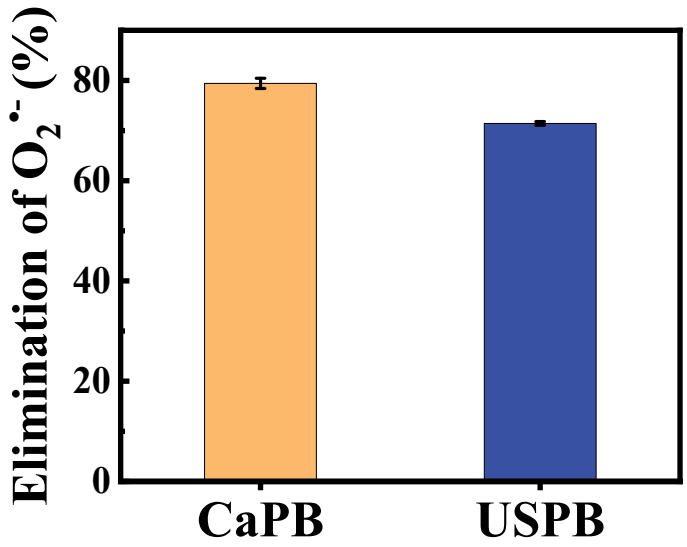

Fig S9. The $O_2^{\bullet-}$ scavenging rate of CaPB NPs and USPBs.

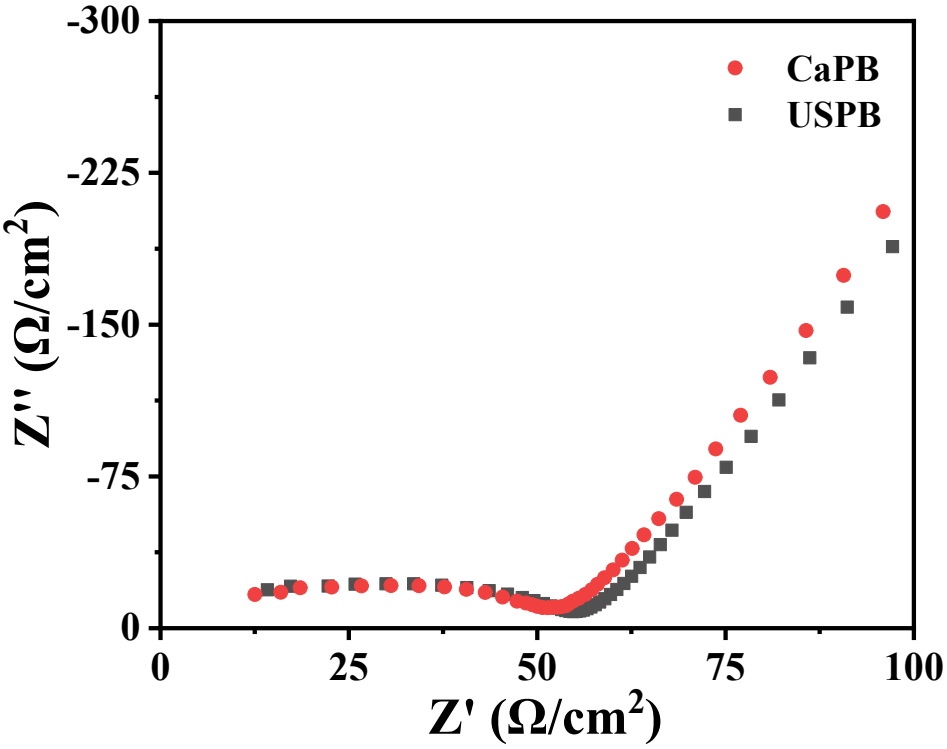

Fig S10. Electrochemical impedance spectroscopy plots of CaPB NPs and USPBs.

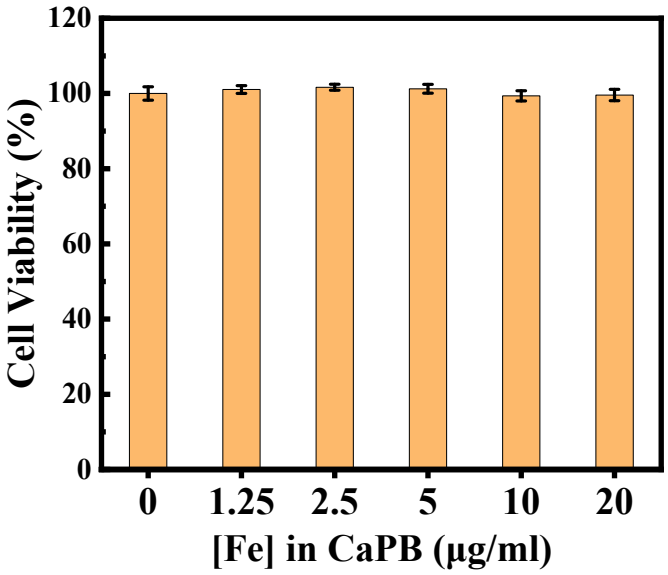

Fig S11. The cytotoxicity of CaPB NPs in HUVEC cells (n = 3, data are shown as mean ± SD).

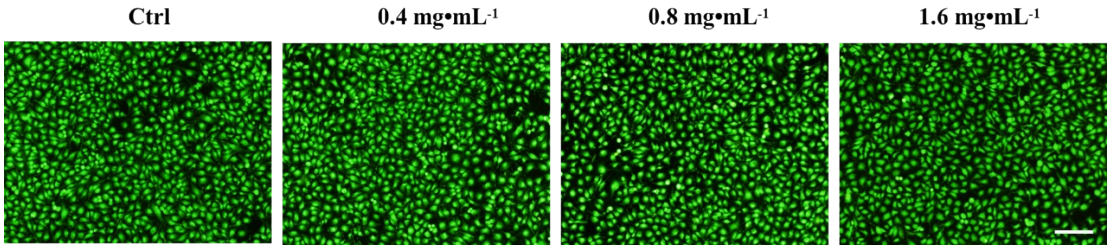

Fig S12. Fluorescent microscopic images of the CaPB NPs-treated HUVEC cells stained with SYTO 9 and PI, respectively. The scale bar represents 50 μm.

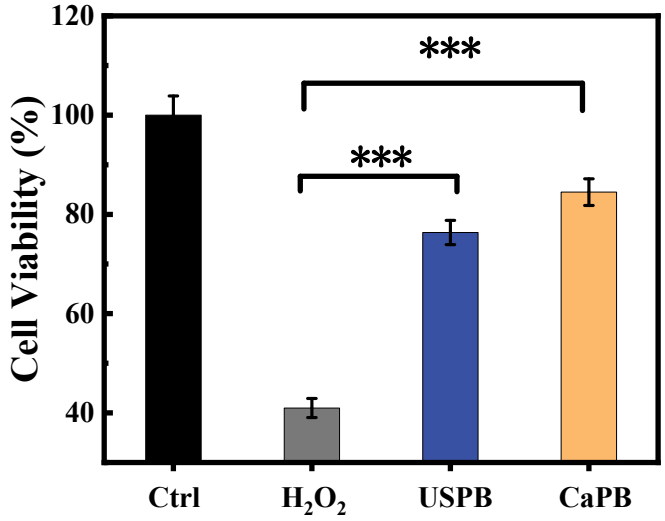

Fig S13. Viability of HUVEC cells after different treatments. Data are shown as mean ± SD (n = 3). *p<0.05, **p<0.01, ***p<0.001.

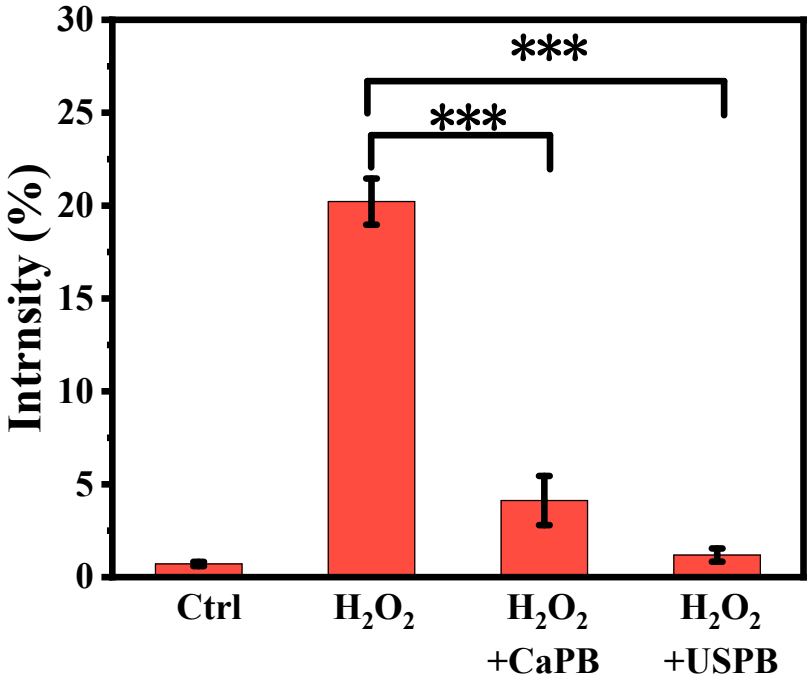

Fig S14. Quantitative analysis of the ROS levels using imageJ software. Data are shown as mean ± SD (n = 3). *p<0.05, **p<0.01, ***p<0.001.

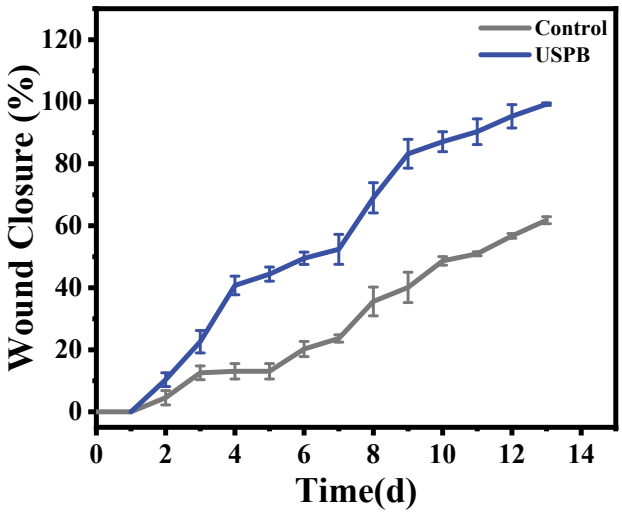

Fig S15. Quantification of wound closure rate. Data are shown as mean ± SD (n = 3). **p*<0.05, ***p*<0.01, ****p*<0.001.

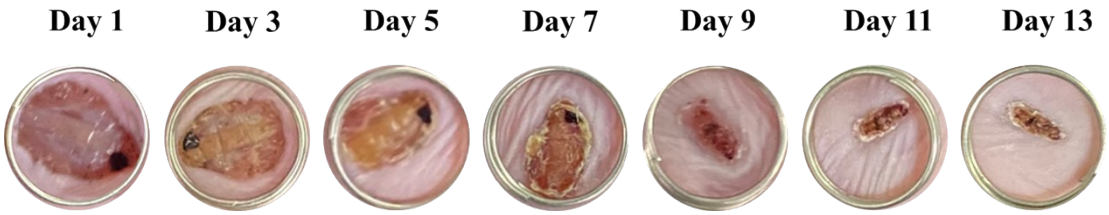

Fig S16. Photographs of the wound with the treatment of USPBs.

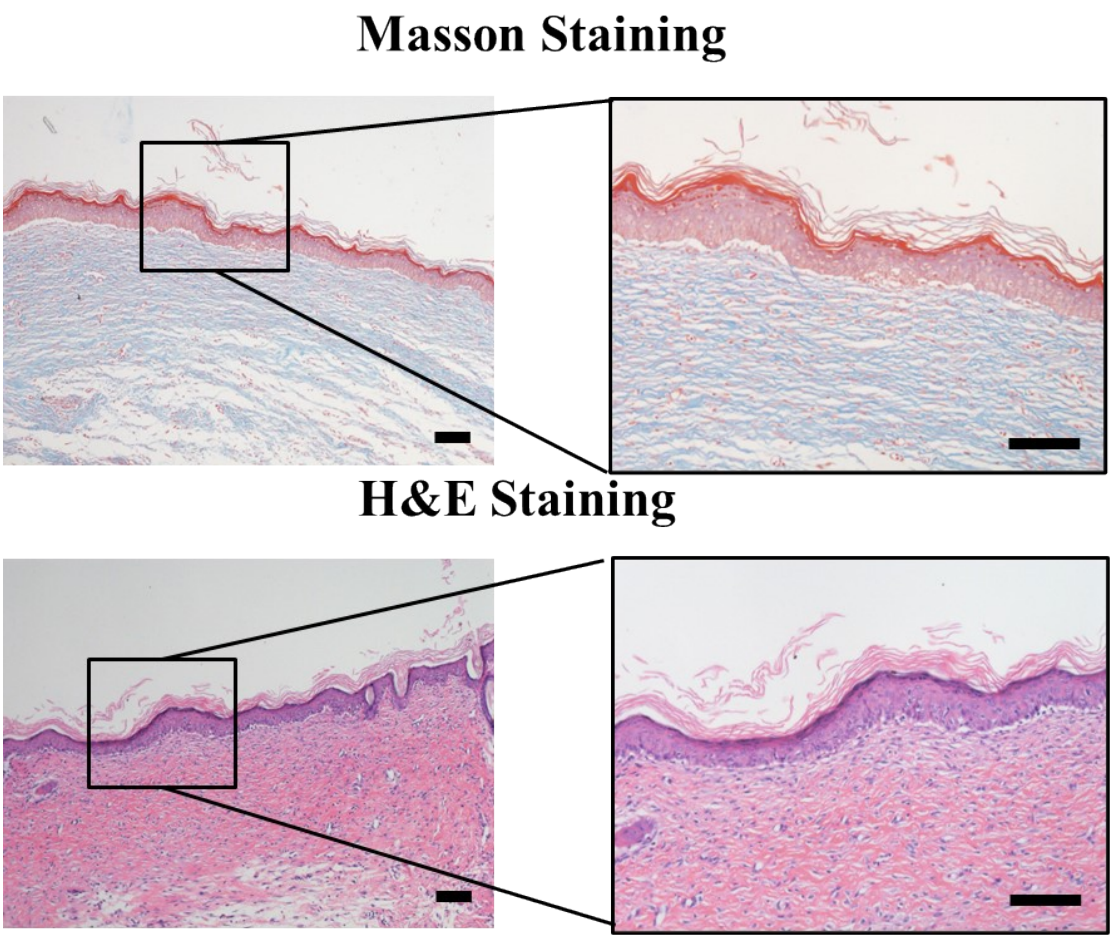

Fig S17. Masson staining and H&E staining of wound tissues with the treatment of USPBs. The scale bar represents 100 μm.

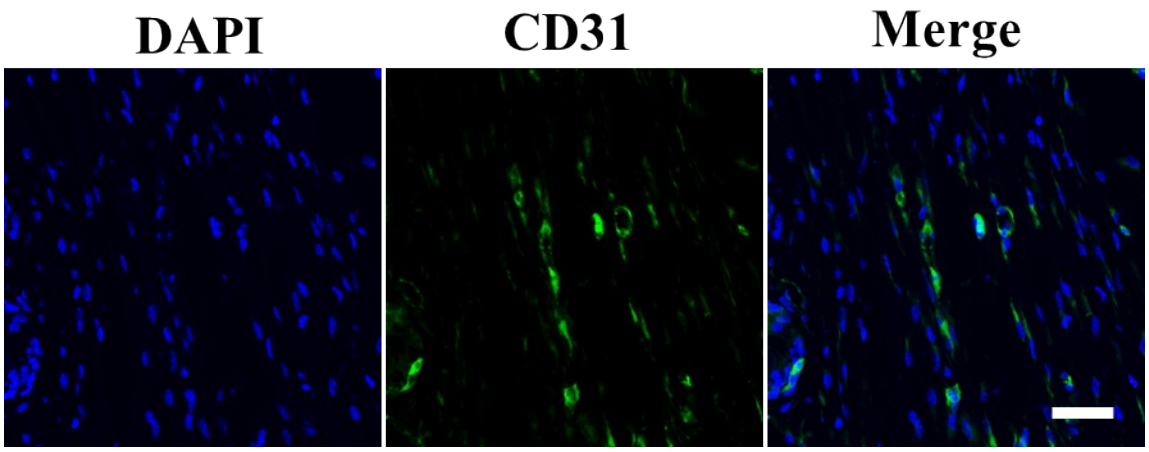

Fig S18. Representative confocal images of immunofluorescence staining for CD31 with USPBs treatments. The scale bar represents 50 μm.

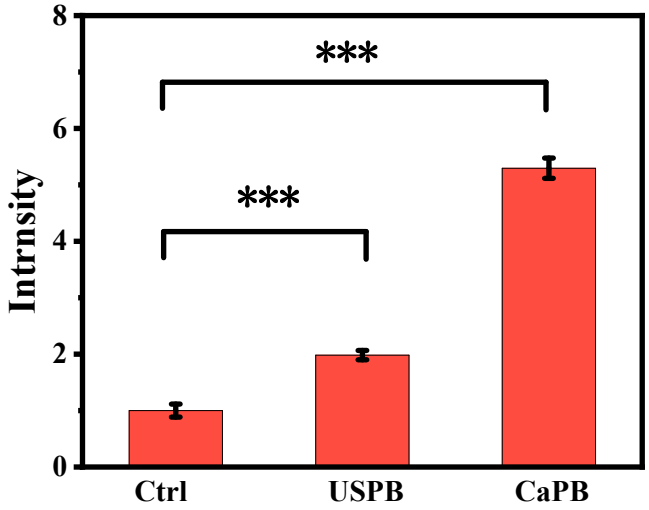

Fig S19. Quantitative analysis of the relative coverage area of CD31 for different groups. Data are shown as mean ± SD (n = 3). *p<0.05, **p<0.01, ***p<0.001.

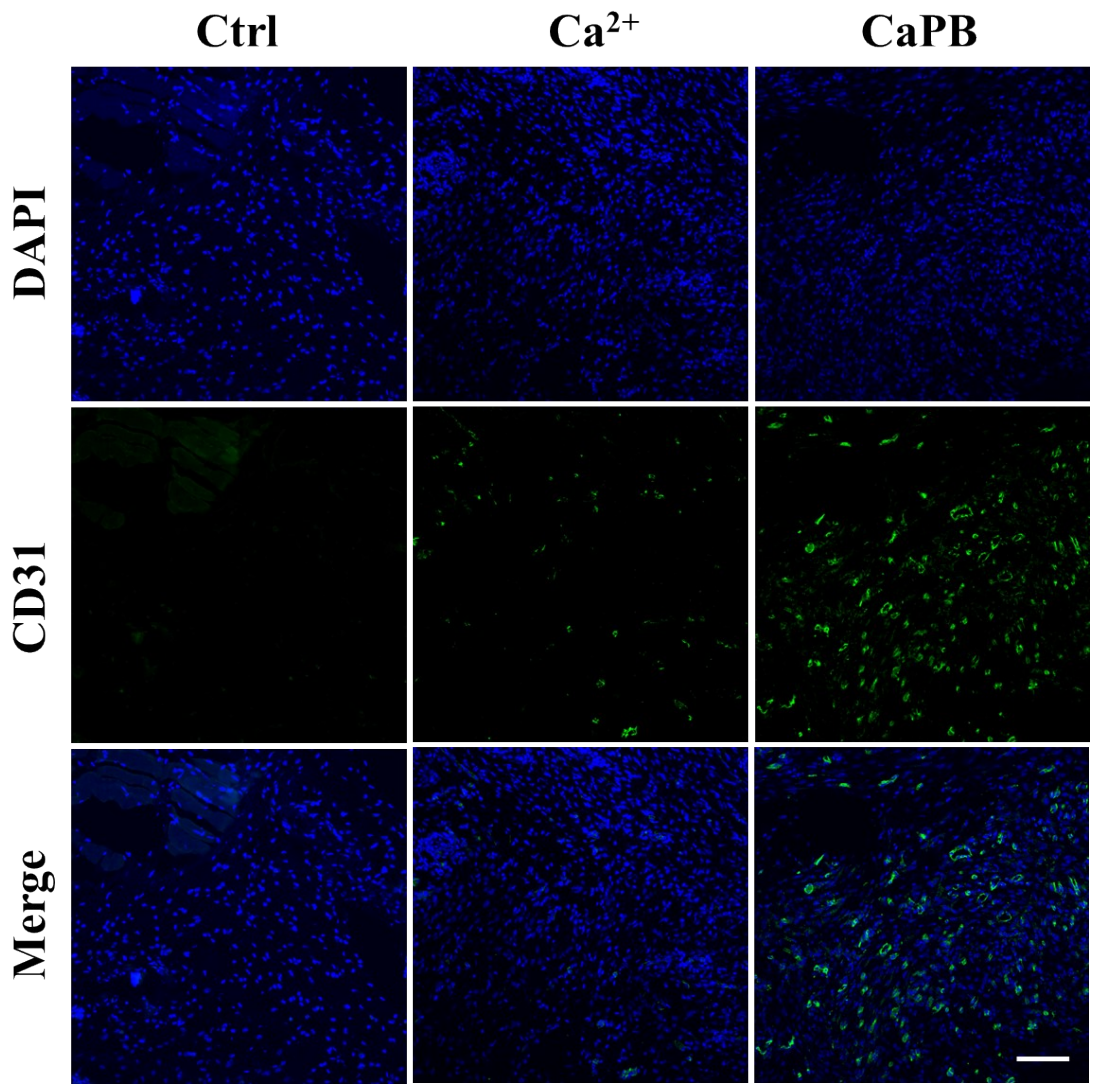

Fig S20. Representative confocal images of immunofluorescence staining for CD31. The scale bar represents 100 μm.

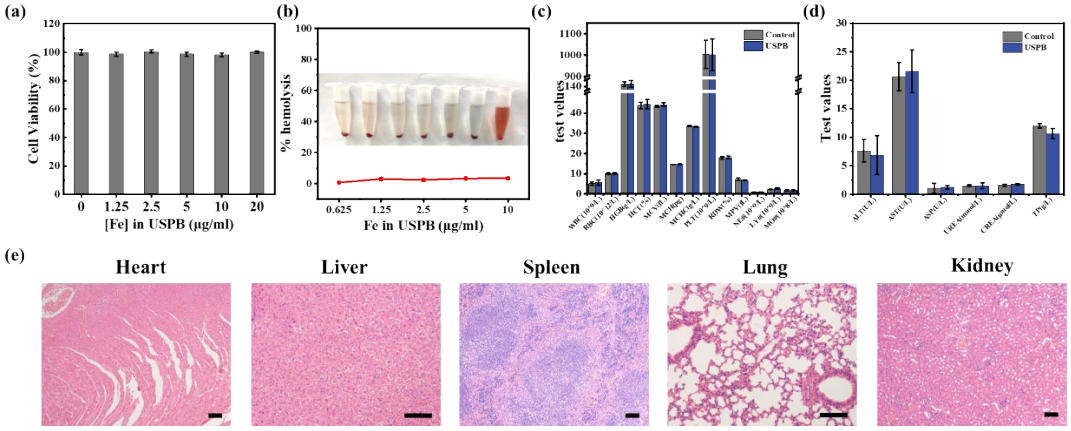

Fig S21. Biosafety evaluation of USPBs *in vitro* and *in vivo*. (a) Cell viability of HUVEC cells treated with different concentrations of USPBs for 24 h. Data are shown as mean ± SD (n = 3). **p*<0.05, ***p*<0.01, ****p*<0.001. (b) Hemolysis quantification of USPBs. (c) Blood routine analysis of healthy mice after treatment with USPBs. Data are shown as mean ± SD (n = 3). **p*<0.05, ***p*<0.01, ****p*<0.001. (d) Mice blood biochemical parameters at 13 days after treatment with USPBs. Data are shown as mean ± SD (n = 3). **p*<0.05, ***p*<0.01, ****p*<0.001. (e) H&E staining images of the major organs of mice after treatment with USPBs (13 days after treatment). The scale bar represents 100 μm.