Supporting Information

The pH-responsive ZC-QPP hydrogel for synergistic antibacterial and antioxidant treatment to enhance wound healing

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Supplementary Experiment Section

Materials and Reagents: NaOH (AR, 95%), $Ce(NO_3)_3 \cdot 6H_2O$ (99.95%, metals basis), Chitosan quaternary ammonium salt (QCS, degree of substitution, 95%), MB (AR, 70%), $FeSO_4$ (AR, 90%), and 1,1-diphenyl-2-picryl-hydrazyl (DPPH, AR, 96%) were purchased from Macklin Biochemical Co., Ltd (Shanghai, China). $Zn(AC)_2 \cdot 2H_2O$ (AR, ≥99.0%), ammonium hydroxide ($NH_3 \cdot H_2O$, 28.0-30.0% w/w NH_3) and PEG 2000 (CP) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Polyvinylpyrrolidone (PVP, powder, average M_w ~ 58000) was bought from Aladdin Chemistry Co., Ltd (Shanghai, China). Boric acid was bought from Shanghai Titan Scientific Co., Ltd. FBS were obtained from Every Green, Zhe Jiang Tian Hang Biotechnology Co., Ltd (Zhejiang China). Trypsin were obtained from Gibco, Grand Island, New York, USA. Medium is obtained from BasalMedia, Shanghai Titan Scientific Co., Ltd (Shanghai, China). CCK-8, Calcein-AM/PI Kit, ROS detection Kit and DCFH-DA were obtained from Beyotime Biotechnology (Shanghai, China).

Characterization: A transmission electron microscope (JEOL JEM 2100F) was used to obtain the TEM element mapping and observe morphology of the sample. Malvern Zeta-sizer (nano ZS90) was used to test the hydrated particle size of different samples. X-ray diffraction (XRD) patterns was measured by Rigaku X-ray powder diffractometer (ULTIMA IV). The ultraviolet-visible absorption spectrum was measured on the TU-1810 spectrophotometer. The content of elements was measured by coupled plasma atomic emission spectrometry (ICP-OES).

The evaluation of cell biocompatibility： The cytotoxicity of ZnO, ZC and ZC-QPP hydrogels on L929 cells were tested by the Cell Counting Kit-8 (CCK-8). Firstly, L929 cells were incubated in 96-well plates (1×10^4/well) at 37 °C and 5% CO_2 for 24 hours. Afterward, DMEM medium (~pH 7.4) at 50 μg·mL^{-1} of ZnO, ZC and ZC-QPP hydrogel (containing 15% ZC NCs) was used to replace the original medium, respectively. After 24 h by co-incubating, the cell viability was then determined by CCK-8. Live/dead cell assays were performed using the Calcein AM/PI double-staining kit. Same culture environment as above, L929 cells were first incubated in 12-well plates (1×10^5/well) for 24 h so that cells grew completely adherent to the wall. The cells were then incubated

with ZnO, ZC and ZC-QPP hydrogel ($\mu g \cdot mL^{-1}$) for 24 h Then cells were washed several times with PBS and further treated with PBS solution containing Calcein-AM (10 μL) and PI (10 μL) and stained at 37 °C for 30 minutes in the dark. Finally, cell morphology was observed and images were taken using confocal. Live/dead cells displayed green fluorescence and red fluorescence respectively under confocal fluorescence microscopy.

Bacterial culture and in vitro antibacterial: The antibacterial assays were measured by Gram-negative (E. coli) and Gram-positive (S. aureus) bacteria. Specifically, E. coli and S. aureus on the agar medium were firstly transferred to sterilized Luria Bertani (LB) medium and incubated in a shaker (180 rpm, 37 °C) for 24 hours. Colonies were then collected by centrifugation and diluted to 3×10^6 CFU/mL with LB medium (~pH 7.0). The control group, ZnO group, ZC group, QPP hydrogel group and ZC-QPP hydrogel group were co-incubated with the bacterial suspension. After 1 h, the bacteria were collected by centrifugation and fixed with 4% formaldehyde solution. The above treated bacteria were dehydrated with ethanol solution. Finally, the bacterial inhibition capacity of the material was verified by observing the bacterial state through the electron scanning microscopy (SEM). The bacterial suspensions of E. coli and S. aureus were logarithmically diluted to 0.2 Abs of OD_{600} nm, after which the blank, ZnO, ZC, QPP hydrogel and ZC-QPP hydrogel materials were co-incubated with 20 mL of the bacterial suspension at 37 °C and the OD values were measured by spectrophotometer at different times.

Supplementary Figures

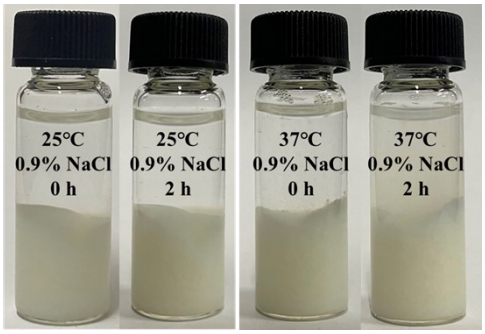

Fig. S1. The photos of ZC-QPP hydrogel at 25 °C and 37 °C in physiologically environment (0.9% NaCl).

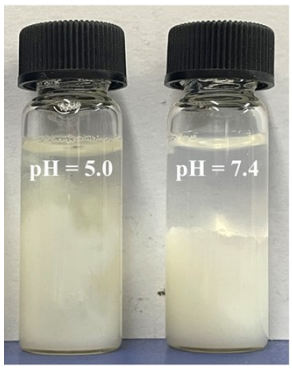

Fig. S2. The morphology of ZC-QPP hydrogels after adding different PBS (pH 5.0 and 7.4) at 15 minutes.