Electronic Supplementary Material (ESI) for Dalton Transactions.
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Supplementary Information

Oxygen-Carrying Acid-Responsive Cu/ZIF-8 for Photodynamic Antibacterial Therapy against Cariogenic *Streptococcus mutans* Infection

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Addition of Experimental Details

Characterization of OCZCH NPs

The microscopic morphology and dimensions of OCZCH were observed by TEM. Equipped with an energy dispersive Scanning electron microscope on TEM was used to study the elemental distribution nanoparticles.

XRD with Cu Kα radiation (λ = 1.54) was used to characterize the crystal structure of nanoparticles. The prepared OCZCH were characterized analytically using XPS with an incident light source of monochromatic Al Kα at an energy of 1486.6 eV, and the pass energy was set at 100 eV for the high-resolution spectra and 30 eV for the survey spectra.

The X-ray power level was 200 W (12.5 kV, 16 mA). The survey and high-resolution spectra acquisition time was 40–50 ms. The specific surface area and pore size distribution were measured by BET.

The generation of $^1O_2$ assay

DPBF was used as a ROS probe to measure the production capacity of $^1O_2$ according to the literature. The mixture of DPBF solution (5 mg/mL) and OCZCH was exposed to red-light irradiation (650 nm). The absorbance of DPBF at 410 nm was measured at intervals with a UV-VIS spectrometer under 650 nm light (120 mW/cm^2).

The loading and release rate of Ce6

The absorption peak intensity of solutions with different concentrations at 400 nm was taken as the ordinate, and the concentration of Ce6 was taken as the abscissa to draw the standard UV-vis absorption curve. 10 μg/mL Ce6 dispersed in Methanol was

prepared and diluted to 5, 2.5, 1.25, and 0.625 μg/mL, respectively. The peak intensities of Ce6 solutions with different concentrations at 400 nm were measured by UV-Vis. 1 mg OCZCH was completely decomposed with 50 μL concentrated hydrochloric acid and diluted with methanol. The absorption peak intensity at 400 nm was measured by UV-Vis. The concentration of Ce6 was calculated by substituting it into the standard UV-vis absorption curve to obtain the mass of Ce6. Then the following formula was used to calculate the loading rate of Ce6:

Ce6 loading rate (wt%) = loading Ce6 / carrier weight × 100%

The rapid acid-responsive ability

OCZCH was dispersed in phosphate-buffered saline (PBS) with pH 6.5 and 7.4, respectively. Centrifugation was performed at intervals, and the supernatant was taken to determine the absorbance of Ce6 at 400 nm, finally, the Ce6 release was calculated according to the free Ce6 standard UV-vis absorption curve to reflect the amount of Ce6 released. Then OCZCH was dispersed in PBS (pH 6.5 and pH 7.4) for 5 and 10 min, and the morphology of OCZCH was observed by SEM.

Cell culture

L929 mouse fibroblast cells were derived from the Cell Bank of Shanghai Chinese Academy of Sciences. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 1% penicillin-streptomycin and 10% fetal bovine serum. The cells were incubated at 37 °C in a 5% CO_2 incubator.

Dark cytotoxicity of OCZCH

The dark cytotoxicity was evaluated by CCK-8 assays. L929 cells were seeded in 96-well plates (8000 cells per well) with 200 μL of DMEM medium in each well for 24 h, then the cells were treated with OCZCH suspension prepared by DMEM medium at concentrations of 50, 25, 12.5, 6.25, 3.13, and 1.56 μg/mL for 24 h. The group without OCZCH nanoparticles was used as the control group, and the group without cells was used as the blank control group. After washing the cells with PBS 3 times, 20 μL CCK-8 solution was added to each well, and the cells were incubated in the cell culture incubator for 1 h. Finally, the absorbance at 450 nm was detected in a microplate reader and the cell viability was calculated. All measurements were conducted in triplicate, respectively.

Bacterial strain and culture

S. mutans (UA159) was provided by the Chinese Academy of Sciences, and the use of bacterial species was approved by the Institutional Review Board of Jilin University School of Stomatology.

Brain heart infusion (BHI) was used for planktonic *S. mutans* cultivation, and BHI supplemented with 1% sucrose (BHIS, *m/v*) was used for *S. mutans* biofilm cultivation. *S. mutans* were inoculated in BHI medium and incubated anaerobically (90% N_2, 10% CO_2) at 37°C until the bacteria were grown to the log phase. According to the optical density at a wavelength of 600 nm (OD_{600}), the concentration of the bacterial solution was adjusted to 2×10^6 CFU/mL) for subsequent experiments.

Determination of minimum inhibitory concentration

The equal volumes (100 μL) of *S. mutans* suspension (2×10^6 CFU/mL) and different concentrations of OCZCH BHI medium solution were mixed in 96-well plates. The final concentration of bacteria was 1×10^6 CFU/mL, and the final concentration of OCZCH was 100, 50, 25, 12.50, and 6.25 μg/mL. The pure BHI medium was used as the blank control group, and the bacteria solution without OCZCH was used as the negative control group. The pure sample solution group was set to remove the color difference of the sample itself. After that, the plates were incubated in the dark for 15 min, and then the light group was placed under a 650 nm laser (120 mW/cm^2) for 5 min. At the same time, the group treated in the dark was kept in the dark. After the light exposure, the 96-well plates were cultured anaerobically in an incubator at 37 °C for 24 h, and the MIC values were observed.

Quantification of Biofilm Reduction

The biofilm formation of *S. mutans* after PDT was evaluated by crystal violet dye. After grouping and treatment according to the above method, the well plates were anaerobically cultured for 24 h. The culture medium was poured out and gently washed three times with PBS along the side wall of the 96-well plate to remove unattached bacteria. After drying at room temperature for 20 min, 2.5 % glutaraldehyde (150 μL/well) was added to the 96-well plate and fixed for 15 min. The glutaraldehyde was removed and washed again 3 times with PBS. Next, 60 μL of 0.1 % crystal violet dye solution was added to each well. The culture plate was placed in a shaker (60 RPM /min) for 10 min, and the crystal violet dye solution was aspirated. After washing with PBS, the bound dye in the biofilm was fully released using 200 μL of 95 % ethanol on

the shaker. Finally, the optical density at 600 nm measured by a microplate reader was used to quantify the amount of biofilm travel.

MTT assay

The metabolic activity of *S. mutans* biofilm was detected by MTT assay. The treated specimens were washed three times with PBS to remove loosely attached bacteria. MTT staining solution 20 μL (5 mg/mL) was added to each well of the 96-well plate and incubated for 20 min at 37 °C in an incubator. After that, DMSO was added, and the specimens were placed in a shaker in the dark for 20 min to fully dissolve the crystal methylzan deposited in the living bacteria. Finally, the absorbance at OD_{540} was measured by a microplate reader, and the metabolic activity of *S. mutans* biofilm was evaluated according to the measured OD value. The OD value is positively correlated with the metabolic activity of the biofilm.

Confocal laser scanning microscopy (CLSM)

To observe the inhibitory effect of OCZCH on *S. mutans* biofilm more clearly and directly, confocal laser scanning microscopy (CLSM) was used to observe the biofilm after staining. Sterile hydroxyapatite (HA) discs containing biofilm were gently rinsed with normal saline. After mixing SYTO 9 and propidium-iodide (PI) according to the instructions of Filmtracer™ LIVE/DEAD™ Biofilm Viability Kit, the HA discs were stained in the dark for 20 min. At the end of the staining, the excess dye solution was blotted with filter paper. The HA discs were placed in confocal dishes and placed under CLSM to avoid light for observation. All experiments were repeated three times. Images were reconstructed and analyzed using Imaris software.

Scanning electron microscope (SEM)

Preformed biofilm was treated as described above. The effects of OCZCH on the structure and morphology of *S. mutans* and its biofilm were observed by SEM. Sterile HA Discs were placed in 24-well plates, grouped, and treated as described above. The treated HA Discs were gently rinsed with PBS and then immersed in 2.5% glutaraldehyde and fixed at 4℃ overnight. Fixed samples were dehydrated with different concentrations of ethanol solution (30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, and 100%). The dehydrated HA discs were oven-dried, and glued to the sample stage for gold spraying when they were completely dry. Finally, the samples were observed and analyzed by SEM.

Inhibition of acid production

To verify the ability of OCZCH to inhibit acid production in *S. mutans*, we assessed this using an acid production assay. The groups were divided and treated as above. The pH of the supernatant of each group was measured with a pH meter and recorded as "pH_0". After 24 h of anaerobic incubation, the supernatant of each group was centrifuged again at 4 °C and the pH was measured as "pH_{24}". The change in pH before and after 24 h was recorded as "ΔpH". The inhibition rate of acid production by OCZCH on *S. mutans* was calculated using the following equation The inhibition rate of acid production by OCZCH was calculated using the following equation.

$\Delta pH = pH_0 - pH_{24}$

Acid production inhibition rate = (ΔpH control - ΔpH experimental) / ΔpH control × 100 %

Quantification of extracellular polysaccharides substance

As previously reported, EPS in extracellular polymers of *S. mutans* plaque biofilm was determined to use the phenol-sulphuric acid method. The biofilm was rinsed with PBS and dried at room temperature for 20 min. Sterile distilled water (40 μL), 6 % phenol (40 μL), and concentrated sulfuric acid (200 μL) were added to each well in turn. The wells were incubated for 30 min at room temperature and the absorbance at 490 nm was measured using an enzyme marker.

Statistical analysis

SPSS 22.0 software was used for statistical analysis. All experiments were repeated three times. Experimental data are presented as mean ± standard deviation (SD). The t-test was used to compare the mean values between the two groups. Data with $p < 0.05$ was statistically significant.

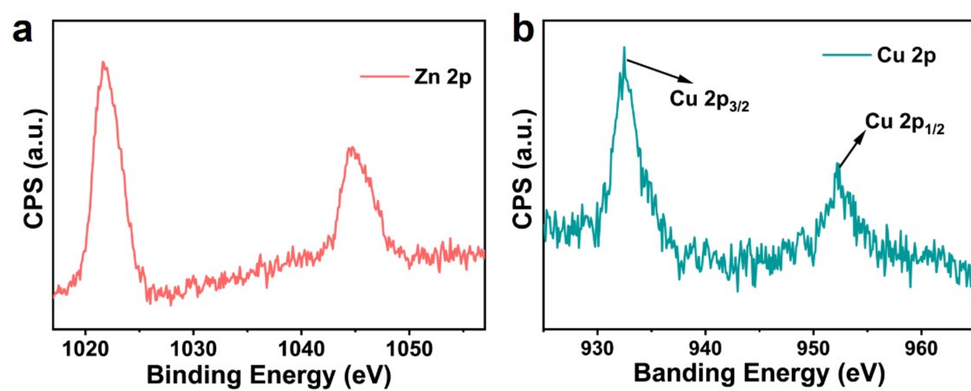

Figure S1. XPS survey spectra and high-resolution a Zn 2p, b) Cu 2p spectra of OCZCH

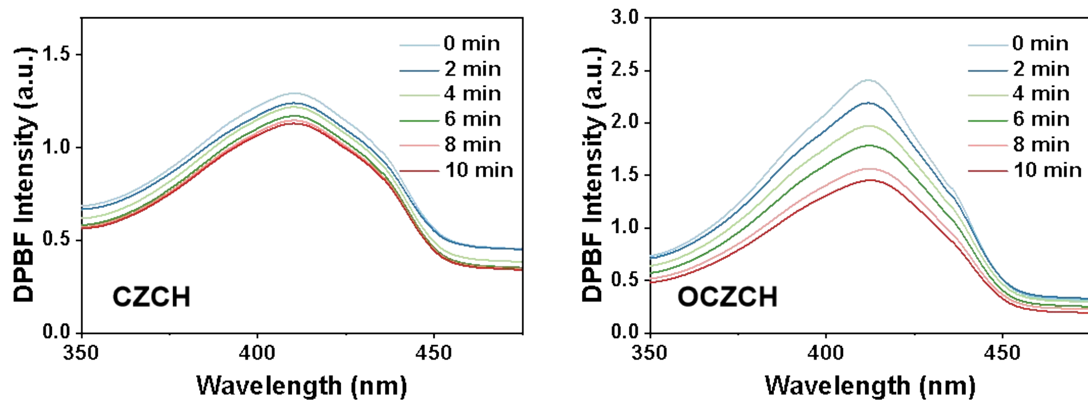

Figure S2. UV-vis absorption spectra of DPBF along with time.

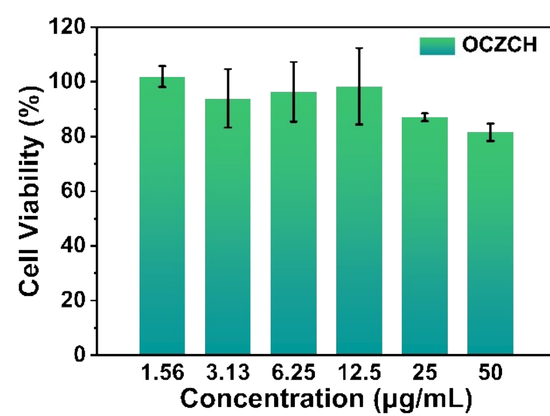

Figure S3. CCK-8 cell biocompatibility test of OCZCH with different concentrations.

Table S1. BET surface area, micropore volume and pore diameter of ZIF-8, Cu/ZIF-8, and CZC. Values are mean ± standard deviation.

Sample	BET surface area (m^2/g)	Micropore volume (cc/g)	Pore diameter (nm)
ZIF-8	1772	0.67	0.63
Cu/ZIF-8	1595	0.68	0.64
CZC	1213	0.63	0.62