Supporting Information

Iron-Substituted Bioglass Nanozyme for Alcohol Intoxication Alleviation

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Materials and methods

Materials

ε-Caprolactone (ε-CL, JUREN, China), glycolide (GA, Purac, Netherlands), stannous octanoate ($Sn(Oct)_2$, Sigma-Aldrich, Germany), and Poly(ethylene glycol) (PEG, M_w = 2000, Sinopharm, Shanghai, China) were used for hydrogel synthesizing. Tetraethyl orthosilicate (TEOS), calcium nitrate tetrahydrate (CN), iron nitrate nonahydrate ($Fe(NO_3)_3 \cdot 9H_2O$) and Dodecylamine (DDA) were purchased from Macklin (Shanghai, China). BALB/C rats (20-25 g, male) were obtained from Chengdu Dashuo Experimental Animal Co., Ltd.

Characterizations

The chemical structure of PCGA were characterized by ^1H NMR (Bruker, AVIII400 HD, Germany). The chemical structure, morphology and surface chemistry of BNZ were characterized by XRD (Bruker, INVENIO R, Germany) and SEM (Phenom, ProX, Netherlands).

Preparation of SiO_2-CaO Bioglass

First, 0.2224 g of DDA was dissolved in 30 mL of anhydrous ethanol. Then, 1 mL of ammonia solution (30%) was added to adjust the pH to 10, followed by the addition of 0.5904 g of CN (dissolved in 2 mL of water) under stirring. Subsequently, 2.216 mL of TEOS was added dropwise under continuous stirring, resulting in the formation of a white turbid solution. After 3 h of stirring, the white precipitate was collected by centrifugation (4000 rpm, 5 min), washed three times with water, and lyophilized to obtain SiO_2-CaO bioglass (BG) powder.

Synthesis of BNZ Nanozymes

100 mg of BG powder was dispersed in 30 mL of water via ultrasonication. Under constant stirring, 3 mL of aqueous TA solution (20 mg/mL) was added. After stirring for 2 h, the dispersion turned grayish-green. The precipitate was collected by centrifugation, washed and redispersed in 30 mL of water. Then, 1 mL of $Fe(NO_3)_3 \bullet 9H_2O$ solution (30 mg/mL) was added under stirring, causing the dispersion to immediately turn black. After another 3 h of stirring, the precipitate was separated by centrifugation, washed three times with water, and lyophilized to obtain a black

powder. Finally, the powder was calcined at 600 °C for 2 h under a nitrogen atmosphere to yield the BNZ nanozyme powder.

Fabrication of SiO_2@CaO_2 Core-Shell Structures

900 mg of $CaCl_2$ and 180 mg of PVP were dissolved in 90 mL of anhydrous ethanol. Then, 3 mL of 30% ammonia solution was added under stirring. Subsequently, 2 mL of 30% H_2O_2 was added dropwise (1 mL/min), leading to the immediate formation of a white precipitate. After stirring for 1 h, CaO_2 nanoparticles were collected by centrifugation and stored dispersed in ethanol. 100 mg of CaO_2 nanoparticles were dispersed in 80 mL of anhydrous ethanol. Then, 150 mg of PVP and 0.6 mL of NaOH (2 M) were added. Under stirring, 0.2 mL of TEOS was added dropwise, and the reaction proceeded for 3 h. The resulting precipitate was collected by centrifugation, washed, and lyophilized to obtain the SiO_2@CaO_2 powder.

Preparation of Hydrogel

13.64 g of PEG (M_w = 2000), 33.67 g of ε-CL, 2.7 g of GA, and 0.1 g of $Sn(Oct)_2$ were added to a 250 mL round-bottom flask. The flask was evacuated for 3 h and then immersed in an oil bath at 160 °C for 30 min, followed by further reaction at 130 °C for 48 h. After reaction was complete and the system had cooled to room temperature, the viscous liquid product was poured into hexane for purification. The resulting white waxy PGCL-PEG-PGCL copolymer was obtained after vacuum drying. 3 g of PGCL-PEG-PGCL copolymer was mixed with 10 mL of water and heated to 60 °C. After 20 min, PCGA was uniformly dispersed in water via vortexing. Upon cooling to room temperature, a freely flowing PCGA hydrogel was obtained.

Peroxidase-like Activities Assay of BNZ Nanozymes

The peroxidase-like activity was determined using TMB as substrate in HAc-NaAc buffer (0.1 M, pH 4.0) at 25 °C. Briefly, 500 μL of BNZ dispersion at varying concentrations was added to a mixture containing 500 μL of H_2O_2 (100 mM) and 500 μL of TMB (1.2 mM). The absorbance of the catalytic oxidation product of TMB (oxTMB) was measured at 652 nm using a UV-visible spectrophotometer to establish the relationship between nanozyme concentration and peroxidase-like activity. Steady-state kinetic analysis was performed by varying the concentrations of TMB and H_2O_2.

The Michaelis-Menten constant (K_m) was derived by fitting the data to the Michaelis-Menten equation:

$$v = \frac{v_{max}\,[S]}{K_m + [S]}$$

where v = ΔA/Δt represents initial reaction velocity (linear portion of absorbance change over time), v_{max} represents maximum reaction velocity, [S] represents substrate concentration and K_m represents Michaelis constant.

Evaluation of the Catalytic Activity of BNZ towards Ethanol and Ethanal

500 μL of ethanol or ethanal solution (100 mM) was added to a system containing 500 μL of H_2O_2 (100 mM) and 500 μL of BNZ (100 μg/mL). Samples were taken at 1, 2, 5, 10 and 20 min, respectively. The ethanol or ethanal concentration in the system was measured using an Elabscience Ethanol (E-BC-K891-M) and Ethanal (E-BC-K769-M) Colorimetric Assay Kits. The catalytic products of BNZ were further characterized by ^{1}H NMR spectroscopy.

Establishment of the Acute Alcohol Intoxication Model

All animal experiments were strictly conducted in accordance with the guidelines of the Ministry of Health of the People's Republic of China and were approved by the Animal Ethics Committee of Sichuan University. Twenty male BALB/C mice were fasted for 12 h and then randomly divided into four groups. PCGA and BNZ-P groups received oral gavage of 100 μL of PCGA hydrogel and BNZ-P hydrogel, respectively. The other two groups (serving as controls, Control and NaCl groups) received oral gavage of 100 μL of normal saline. After allowing the mice to adapt for 20 min, NaCl, PCGA, and BNZ groups received oral gavage of an ethanol liquid diet (5 g/kg body weight), while the Control group received an equivalent volume of normal saline. Approximately 10 μL of blood was collected from the submandibular vein at 30, 60, 120, 180, and 240 min. Blood ethanol and ethanal concentrations were measured using Elabscience Ethanol (E-BC-K891-M) and Ethanal (E-BC-K769-M) Colorimetric Assay Kits, respectively. The duration of anesthesia was recorded, and all mice were euthanized after 6 h. Blood, liver, and colon tissues were collected for subsequent physiological tests.

Establishment of the Chronic Alcohol Intoxication Model

Twenty male BALB/C mice were randomly divided into four groups to establish the mouse model of chronic and binge ethanol feeding (NIAAA model). Three groups (NaCl, PCGA and BNZ-P groups) were fed with Lieber-DeCarli liquid diet containing ethanol (5% wt), while control group (Control group) was fed with isocaloric control liquid diet without ethanol. After 5 days of feeding, PCGA and BNZ-P groups received oral gavage of 100 μL of PCGA hydrogel and BNZ-P hydrogel every morning, respectively. Control and NaCl groups received oral gavage of 100 μL of normal saline. On the morning of day 14, NaCl, PCGA, and BNZ-P groups received oral gavage of an ethanol liquid diet (5 g/kg body weight). Blood ethanol and acetaldehyde concentrations were measured at different time points. All mice were euthanized 6 h later. Blood, liver, colon, and fecal samples were collected for physiological tests.

Analyze of gut microbiota changes

Faecal samples were collected within 5 min after defecation on the morning of 14 d and stored at −80 °C. Microbial genome DNA was extracted from faeces by using the E.Z.N.A.® Soil DNA Kit according to the manufacturer's instructions, and the variable 3-4 (V4-v4) region of the 16S rRNA gene was PCR-amplified using barcoded 338F-806R primers (forward primer, 5'-ACTCCTACGGGAGGCAGCAG-3'; reverse primer, 5'-GGACTACHVGGGTWTCTAAT-3'). PCR amplification cycling conditions were as follows: initial denaturation at 95 ℃ for 3 min, followed by 27 cycles of denaturing at 95 ℃ for 30 s, annealing at 55 ℃ for 30 s and extension at 72 ℃for 45 s, and single extension at 72 ℃ for 10 min, and end at 4 ℃. The PCR product was extracted from 2% agarose gel and purified using the PCR Clean-Up Kit (YuHua, Shanghai, China) according to manufacturer's instructions and quantified using Qubit 4.0 (Thermo Fisher Scientific, USA). Purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina Nextseq2000 platform (Illumina, San Diego,USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Amplicon sequence variants (ASVs) were denoised and clustered by the DADA2 plugin in the Qiime2 (version 2020.2). Bioinformatic analysis of gut microbiota was carried out using the Majorbio Cloud

platform. The similarity among the microbial communities in different samples was determined by principal coordinate analysis (PCoA) based on Bray-curtis dissimilarity using Vegan v2.5-3 package. The PERMANOVA test was used to assess the percentage of variation explained by the treatment along with its statistical significance using Vegan v2.5-3 package.

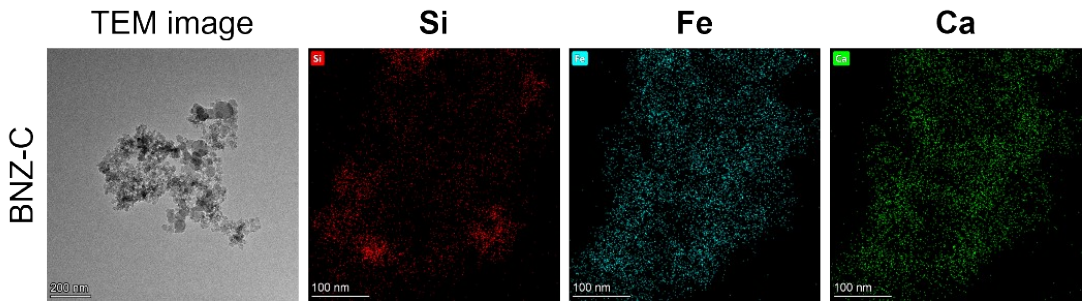

Figure S1. The TEM images and elemental mapping of BNZ-C.

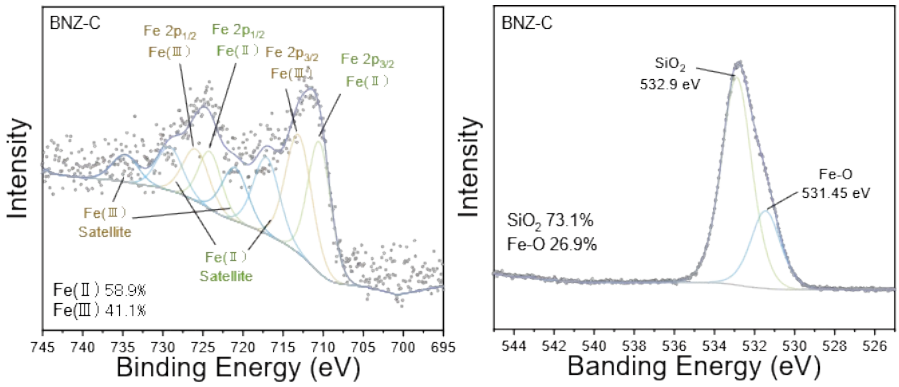

Figure S2. The XPS Fe $2p_{3/2}$ spectrum and O 1s spectrum of BNZ-C.

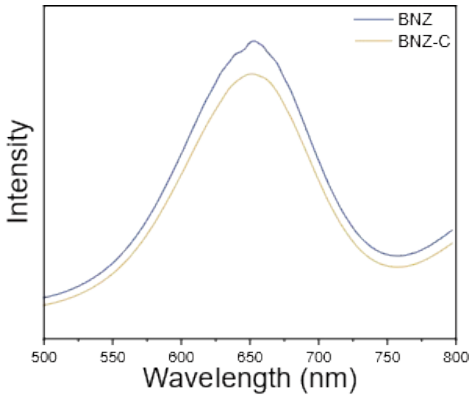

Figure S3. Catalytic efficiency of BNZ-C and BNZ tested by UV-vis spectra.

Table S1. Steady-state kinetic parameters of BNZ for TMB and H_2O_2 substrate.

Substrate	[E] (M)	K_m (mM)	V_{max} (μM s^{-1})	K_{cat}(min^{-1})	K_{cat}/K_m($M^{-1}min^{-1}$)
TMB	2.976×10^{-5}	0.1283	0.1128	0.2274	1.777×10^{3}
H_2O_2	2.976×10^{-5}	73.52	0.1985	0.4002	5.443

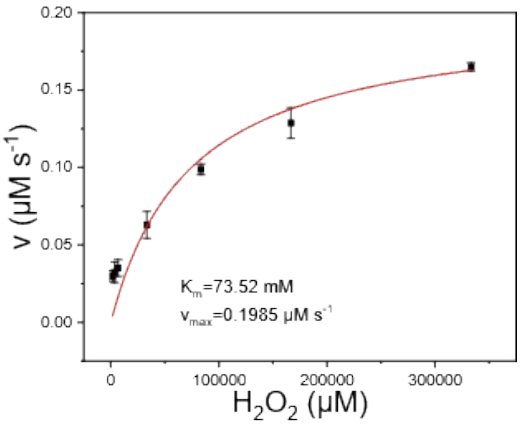

Figure S4. Michaelis–Menten curves of BNZ for H_2O_2 substrate.

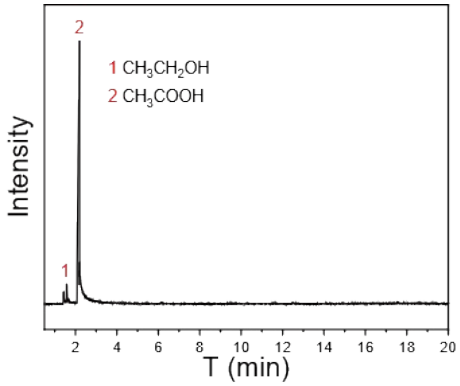

Figure S5. The GC-MS chromatograms of catalytic products of ethanol.

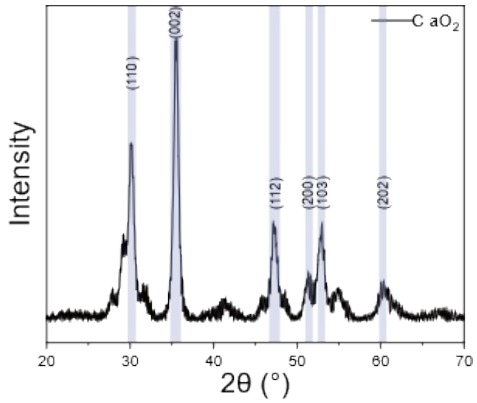

Figure S6. XRD spectra of CaO_2 nanoparticles.

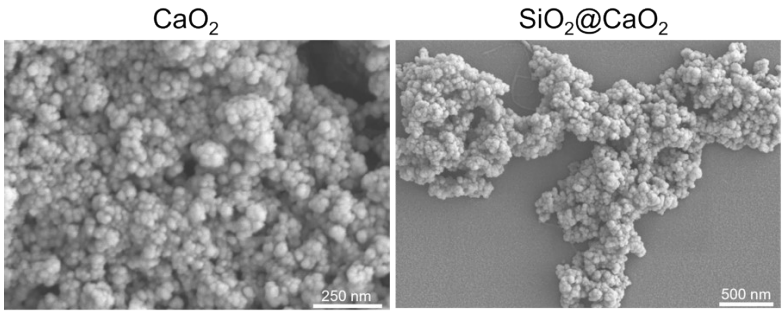

Figure S7. SEM images of CaO_2 and $SiO_2@CaO_2$.

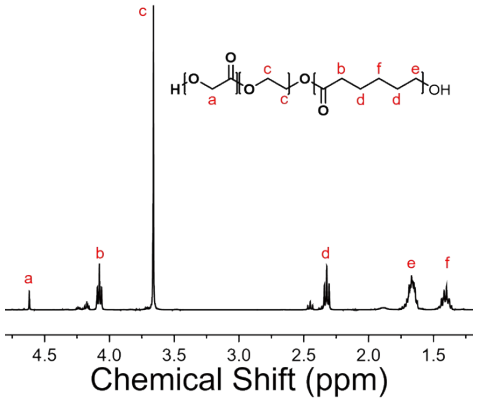

Figure S8. ^{1}H NMR of PCGA copolymer hydrogel.

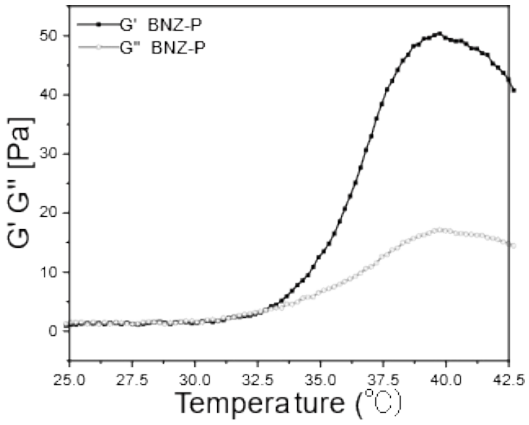

Figure S9. Rheological properties of BNZ-P thermosensitive hydrogel.

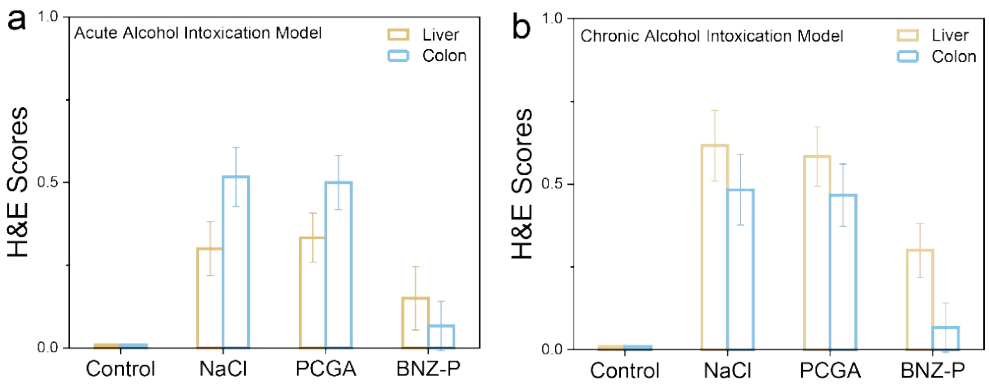

Figure S10. H&E scores of liver and colon in acute (a) and chronic (b) alcohol intoxication model.

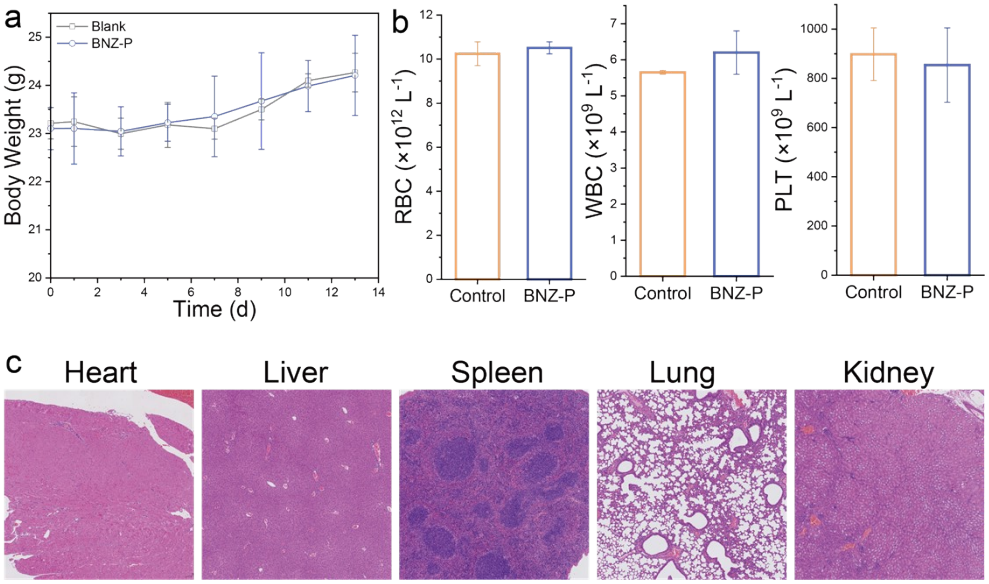

Figure S11. (a) Body weight changes, (b) complete blood count and (c) H&E-stained images of major organs following 14 days of administration.

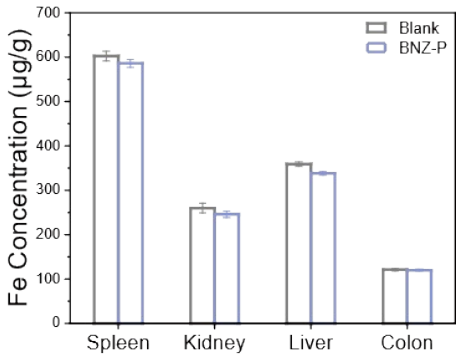

Figure S12. The iron content in major organs of mice following 14 days of administration.

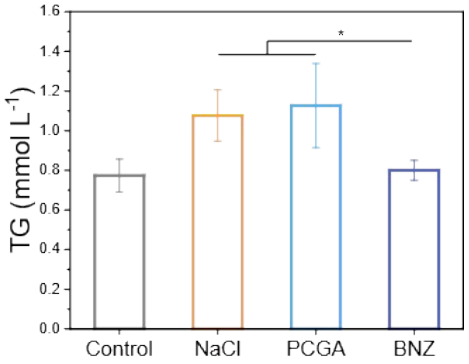

Figure S13. Triglyceride (TG) content in blood.

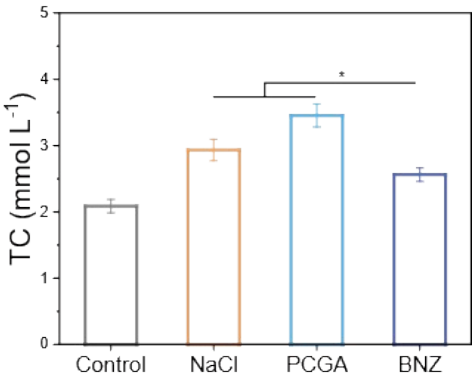

Figure S14. Total cholesterol content in blood.

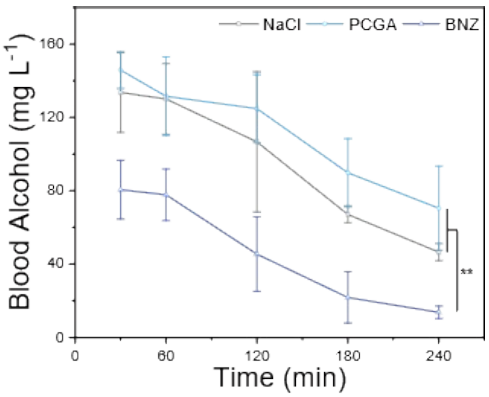

Figure S15. Mean concentrations of blood alcohol in alcohol-intoxicated mice.

Table S2. Standard of histological scoring of mice liver upon alcohol administration and gavage treatments of NaCl, PCGA and BNZ-P.

Histological features	Score	Description
Steatosis	0	Under 5%
	1	5-33%
	2	34-66%
	3	Over 66%
Lobular inflammation	0	No foci
	1	1-2 foci per ×20 field
	2	2-4 foci per ×20 field
	3	Over 4 foci per ×20 field
Hepatocellular ballooning	0	None
	1	Moderate
	2	Evident

Table S3. Standard of histological scoring of mice colon upon alcohol administration and gavage treatments of NaCl, PCGA and BNZ-P.

Histological features	Score	Description
Inflammation severity	0	None
	1	Minimal
	2	Mild
	3	Moderate
	4	Severe
Crypt damage	0	None
	1	Basal 1/3 damaged
	2	Basal 2/3 damaged
	3	Crypts lost; surface epithelium present
	4	Crypts lost and surface epithelium lost
Hepatocellular ballooning	0	None
	1	Minimal
	2	Mild
	3	Moderate
	4	Severe