Supplementary materials

NiCo LDHs nanozyme with selective antibacterial activity against Gram-negative bacteria for wound healing

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1. Experimental Sections

1.1. Kinetic analysis of peroxidase-like activity of NiCo LDHs

The kinetic data were obtained by measuring the absorbance at 417 nm in time-course mode. The detailed experiment process was carried out using 50 μL ABTS (10 mM), 100 μL H_2O_2 (0.02 M), 100 μL 0.5 mg mL^{-1} NiCo LDHs with different mole ratio in 2.75 mL water for 5 min at 37°C, with fixed ABTS and variation H_2O_2 or vice versa. Using the Lineweaver–Burk plot ($1/v = K_m/V_{max}$ $(1/[S] + 1/K_m)$) to obtain the Michaelis–Menten constant. Among the plots, v represents the initial velocity, V_{max} represents the maximal reaction velocity, [S] represents the substrate concentration. K_m represents Michaelis–Menten constant. K_m is an indicator of enzyme affinity for its substrate, the smaller the value of K_m, the stronger the affinity between the enzyme and the substrate.

1.2. Animal feeding conditions

The kunming mice purchased Hualan Biology, and the animal protocol was reviewed and approved by Henan Normal University, Institute of Animal Care and Use Committee, which conformed to the NIH Guide for the Care and Use of Laboratory Animals. The mice were kept at the Experimental Animal Center of Henan Normal University. Feeding conditions were set at a temperature (24 ± 3 ˚C) and humidity (35 ± 5%) with 12 h day/night cycle; mice were free to get food and water.

1.3. Membrane depolarization

Firstly, the cultured bacteria were added to 96-well plates, followed by the addition of $DiSC_3(5)$ (0.2 μM) staining, and the change of fluorescence intensity ($\lambda_{excitation}$=622 nm, $\lambda_{emission}$=673 nm) was measured by a multi-mode enzyme marker until it stabilized. After that, PBS, H_2O_2 (0.02 M), Ni_4Co_6 LDHs (0.5 mg/mL) and Ni_4Co_6 LDHs+H_2O_2 were added to different groups, and then the fluorescence intensity changes were continued to be measured until stabilization.

2. Supplementary Figures

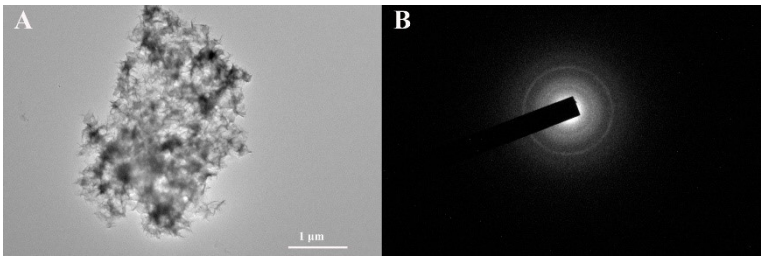

Fig. S1 (A) TEM image and (B) Selected Area Electron Diffraction (SAED) pattern of Ni_4Co_6 LDHs.

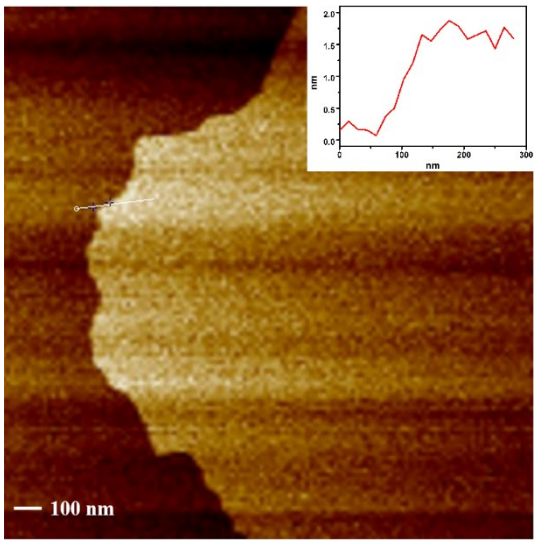

Fig. S2 AFM image of $Ni_{10}Co_0$ LDHs.

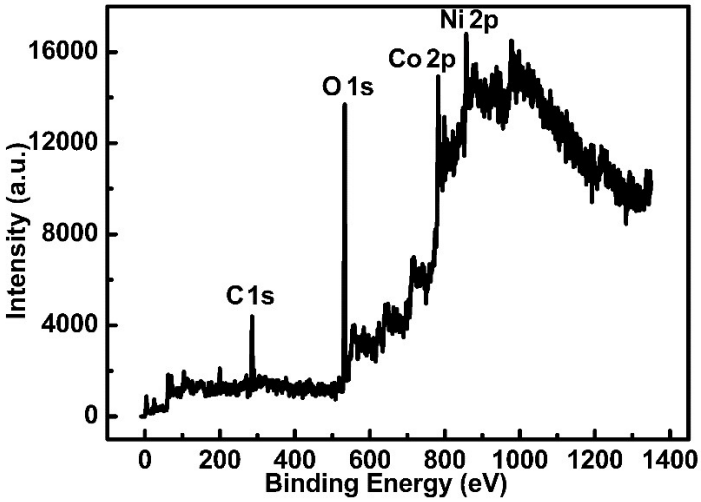

Fig. S3 XPS spectra of NiCo LDHs.

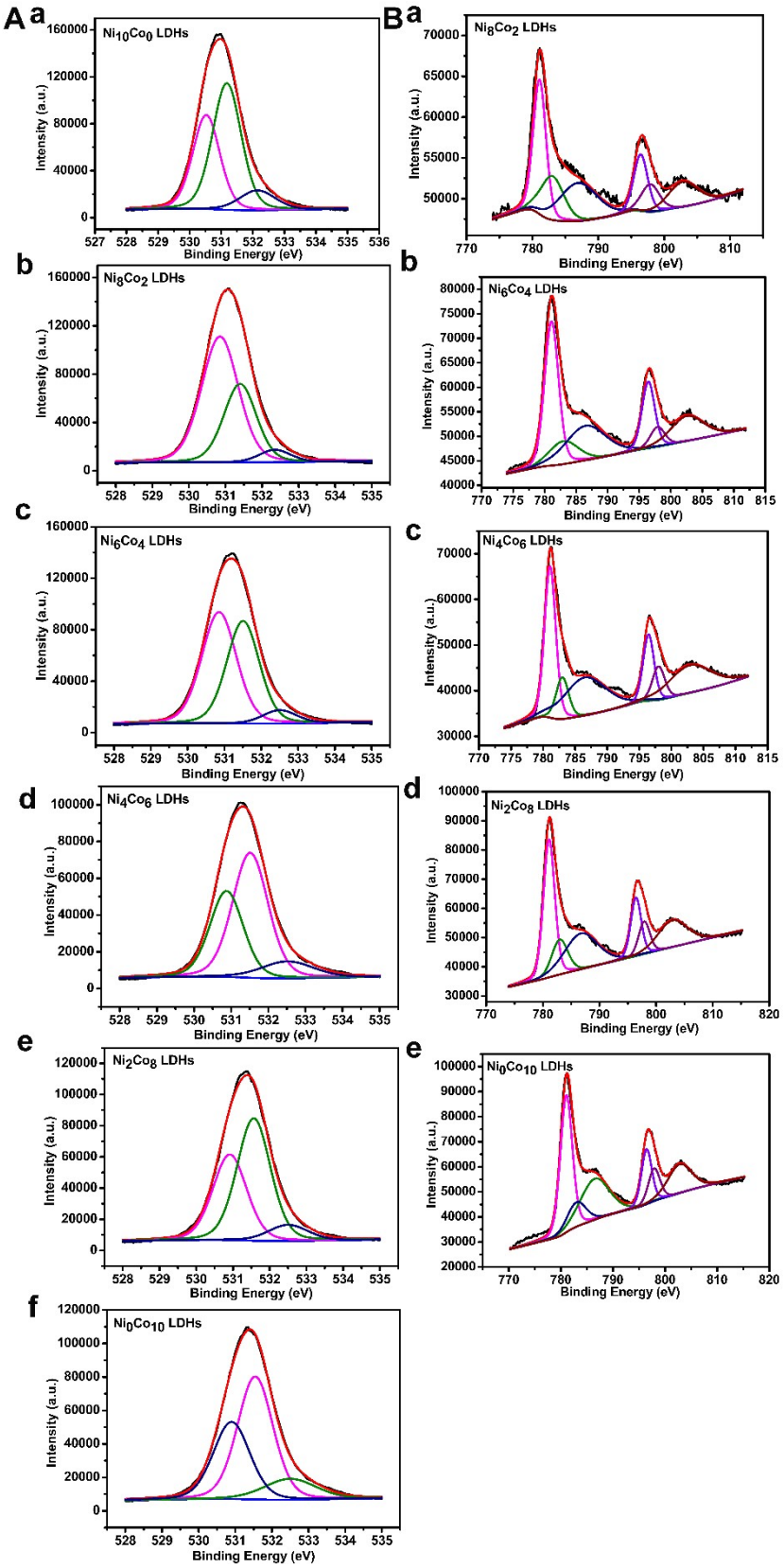

Fig. S4 O 1s spectra (A) and Co 2p spectra (B) for different ratios of NiCo LDHs.

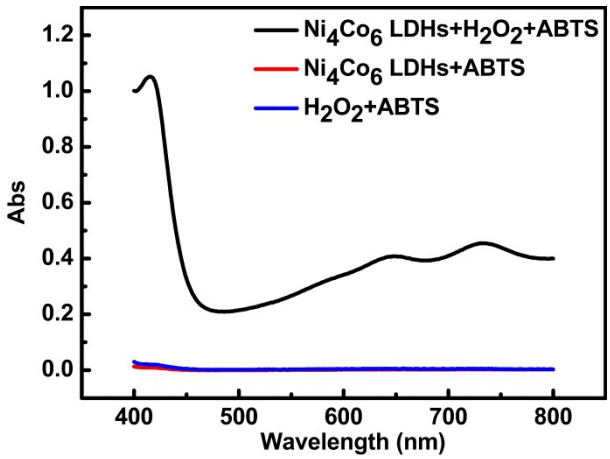

Fig. S5 Determination of the absorbance at 417 nm of the ABTS chromogenic system catalyzed by different systems in a neutral environment.

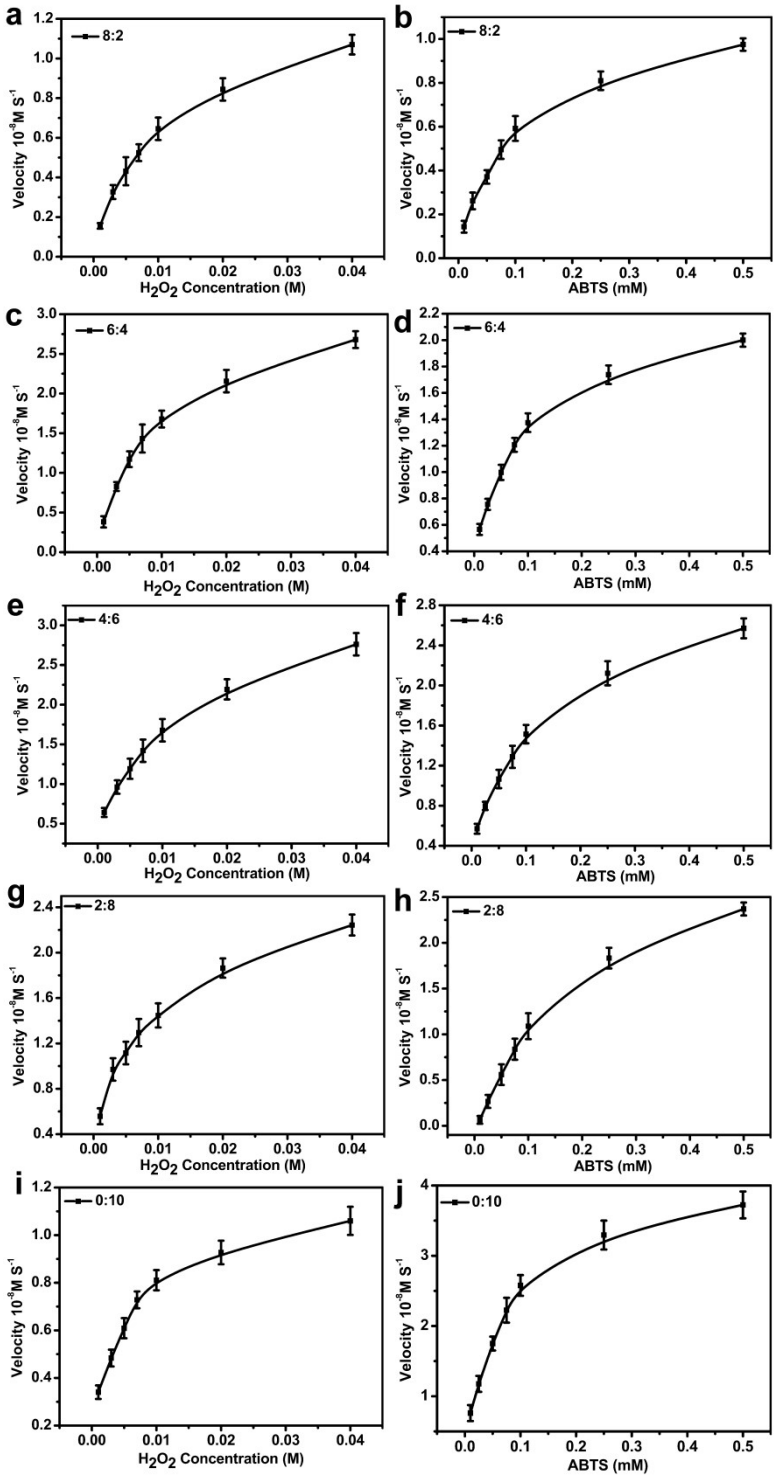

Fig. S6 The steady-state kinetic curves of NiCo LDHs with different molar ratios of Ni and Co by changing the concentration of the substrate H_2O_2 and ABTS.

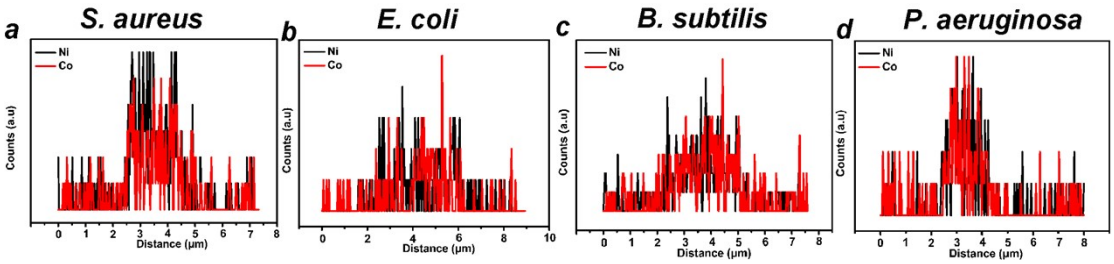

Fig. S7 The distribution of elements along the indicated lines for the samples.

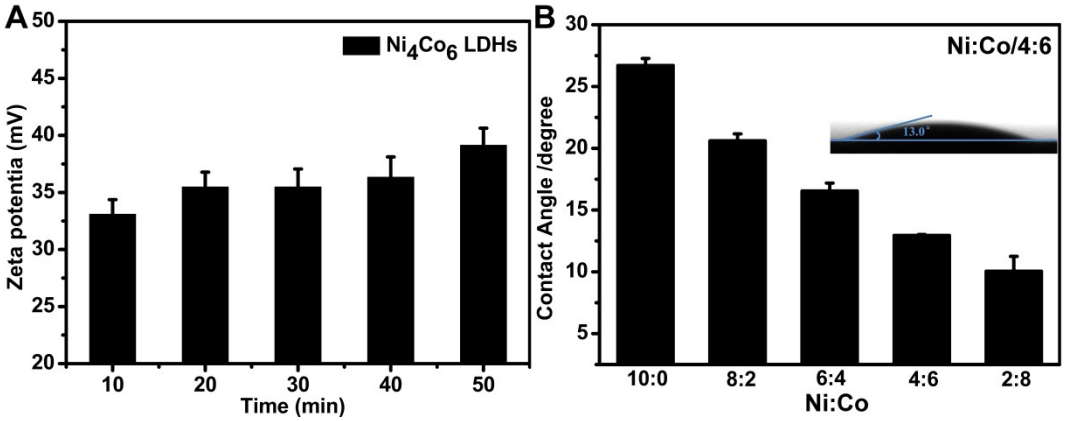

Fig. S8 (A) Time-changing potential of Ni_4Co_6 LDHs in aqueous solution. (B) Contact angle of NiCo LDHs with different molar ratios.

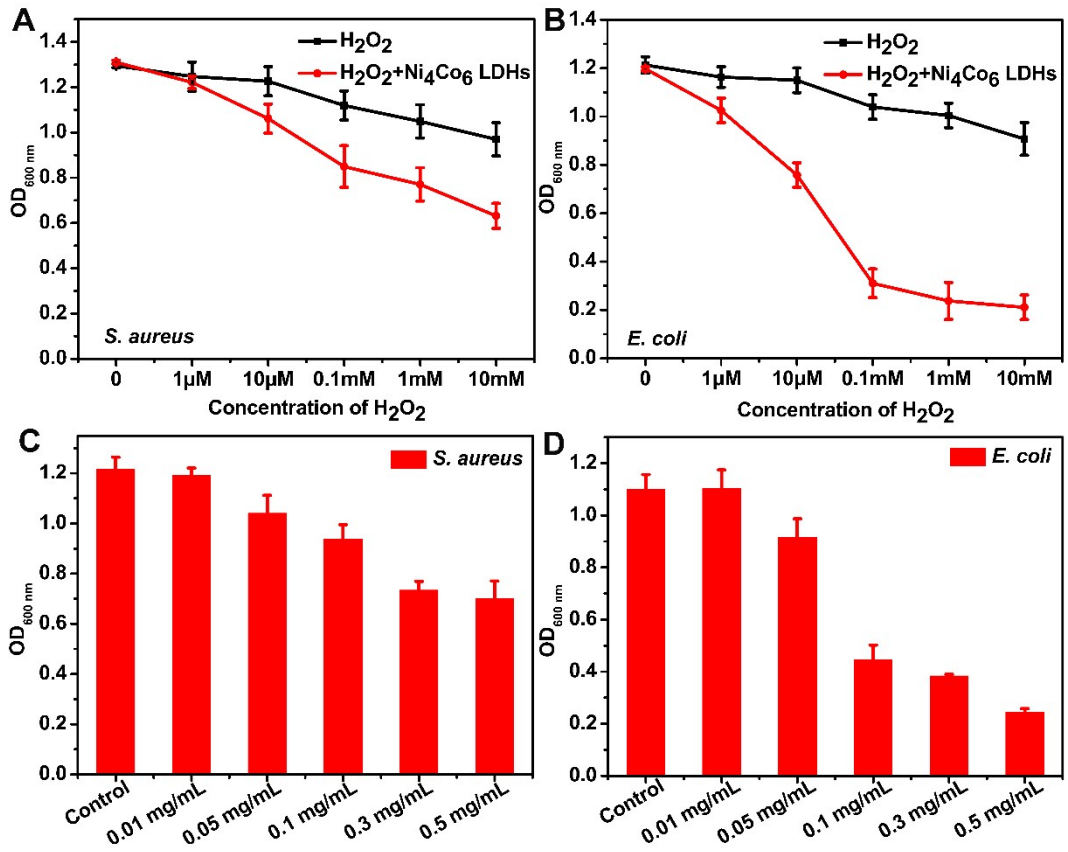

Fig. S9 OD $_{600nm}$ values of bacteria treated with different systems for (A) *S. aureus* and (B) *E. coli* under different concentrations of H_2O_2. MIC of NiCo LDHs nanozymes to (C) *S. aureus* and (D) *E. coli* .

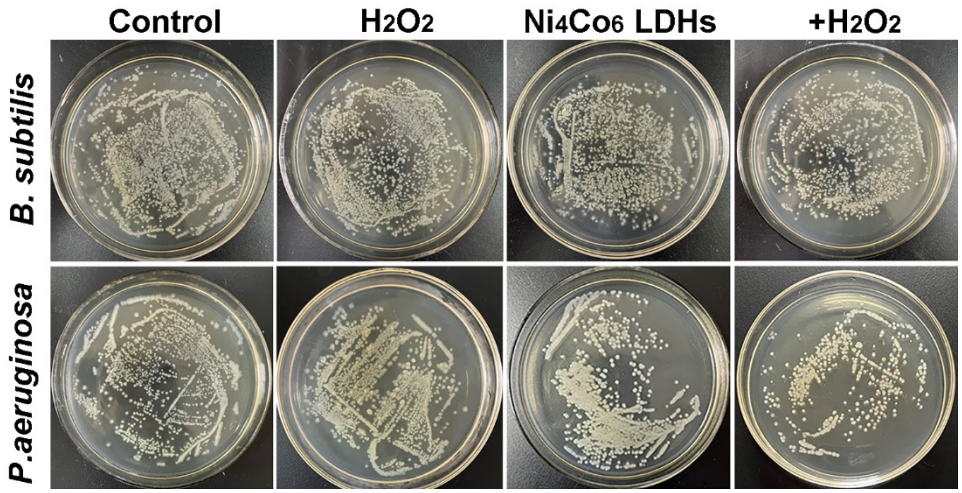

Fig. S10 Photographs of colonies of *B. subtilis* and *P. aeruginosa* on agar plates under different culture systems.

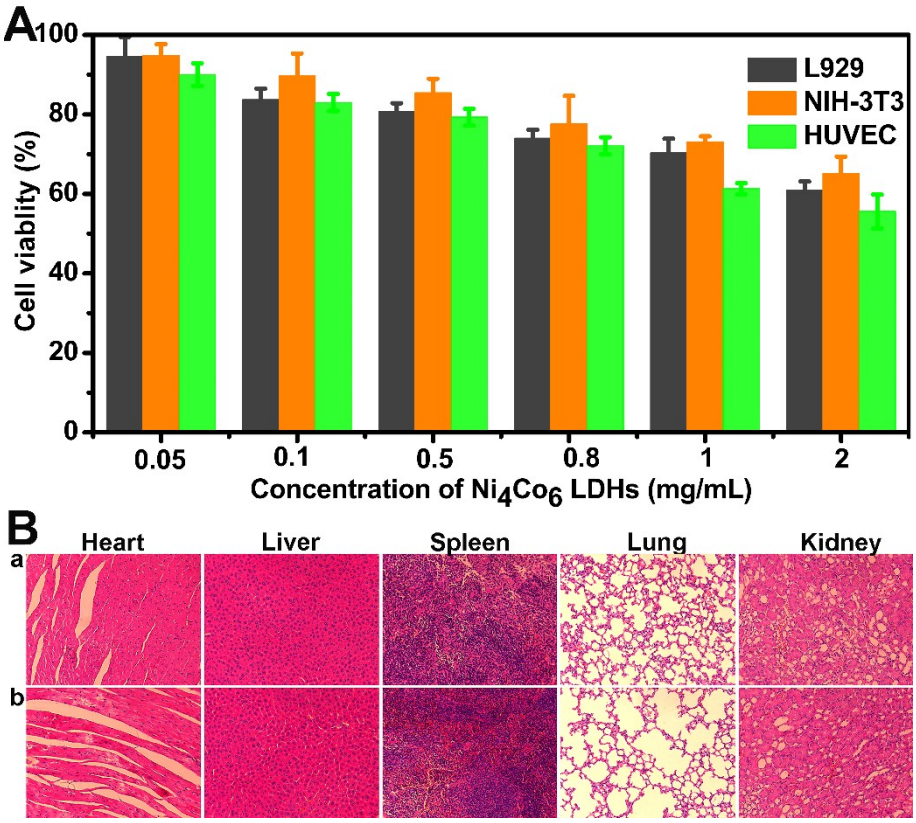

Fig. S11 (A) The cytotoxicity graph of different cells treated with different concentrations of Ni_4Co_6 LDHs. (B) (H&E) photos of Ni_4Co_6 LDHs on different organs of mice, (a) Control; (b) Ni_4Co_6 LDHs. All H&E images were obtained under magnification of 20.

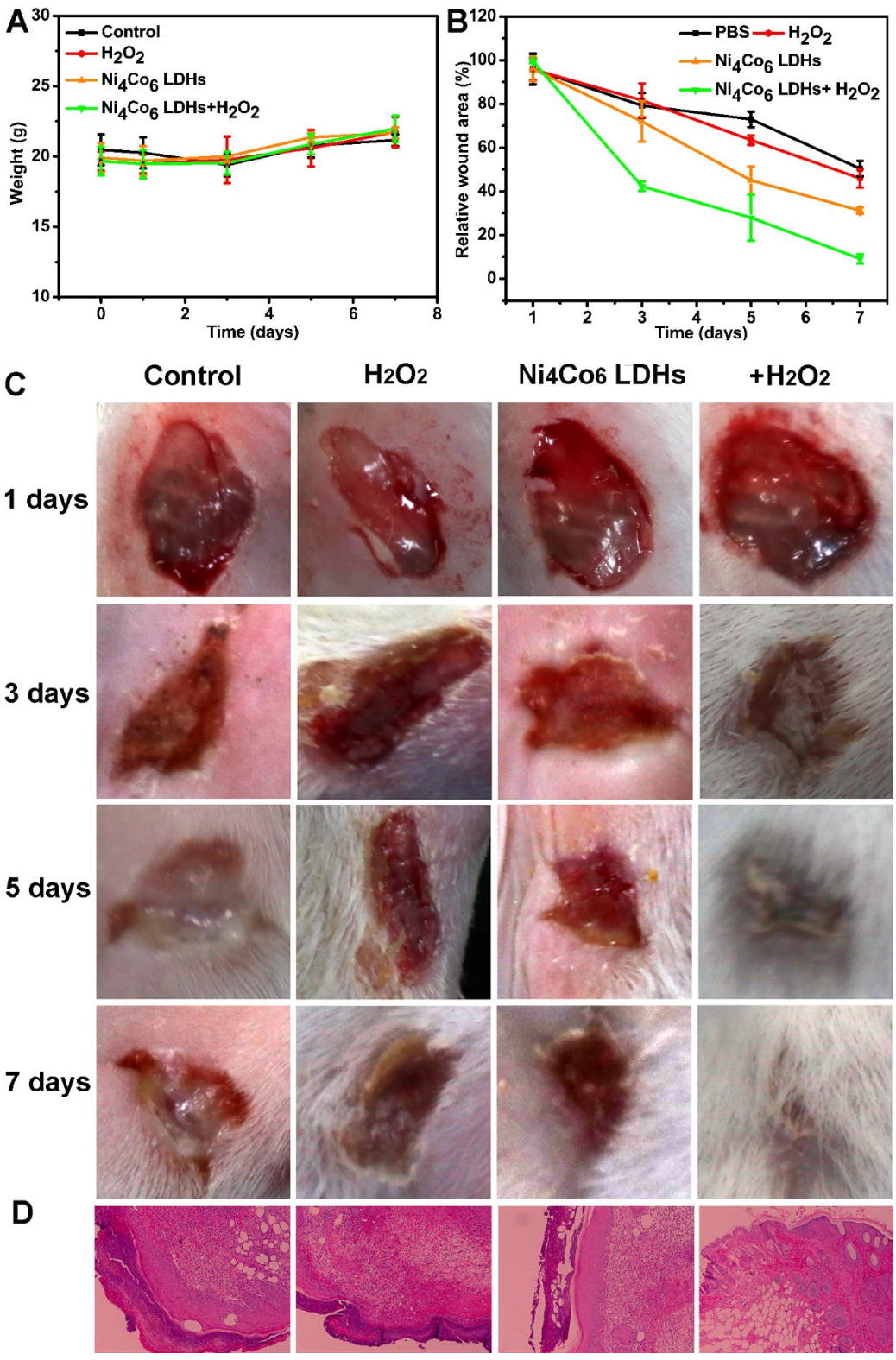

Fig. S12 Changes in (A) body weight and (B) wound area of mice with different treatments over time. (C) Photographs of mouse wounds after treatment with different systems and (D) H&E staining of wound tissue. All H&E images were obtained under magnification of 20.

3. Supplementary Tables

Table S1 Specific surface area and pore volume parameters of NiCo LDH samples with different molar ratio of Ni and Co.

Sample	$Ni_{10}Co_0$	Ni_8Co_2	Ni_6Co_4	Ni_4Co_6	Ni_2Co_8	Ni_0Co_{10}
Specific Surface Area ($cm^2\ g^{-1}$)	162.3	152.1	220.5	186.2	164.1	101.3
Pore Volume($cm^3\ g^{-1}$)	0.708	0.859	1.071	1.156	1.028	0.658