Supporting information

Ni Nanocrystals Supported on Graphene Oxide: Antibacterial Agents for Synergistic Treatment of Bacterial Infections

Ting Du[1#], Baojia Huang[1#], Jiangli Cao[1], Chunqiao Li[1], Jingbo Jiao[1], Zehui Xiao[1], Lifei Wei[1], Jing Ma[3], Xinjun Du[1]*, Shuo Wang[2]*

[1]State Key Laboratory of Food Nutrition and Safety, Key Laboratory of Food Nutrition and Safety, Ministry of Education, College of Food Science and Engineering, Tianjin University of Science and Technology, Tianjin 300457, PR China

[2]Tianjin Key Laboratory of Food Science and Health, School of Medicine, Nankai University, Tianjin 300071, PR China

[3]College of Life Science, Yangtze University, Jingzhou, Hubei 434023, PR China

1. Experimental section

1.1. Characterization

The morphology of the prepared NCNCs, GO and GO/NCNCs was determined by a Thermo Scientific Talos G2 200X transmission electron microscope (TEM). The crystalline structure was characterized by a Rigaku SmartLab 9000 X-ray diffraction (XRD) system. The chemical composition and chemical states were tested on X-ray photoelectron spectrometer (XPS, ThermoFisher ESCALAB 250Xi) and a Fourier transform infrared spectrometer (FT-IR, Thermofisher IS50 70). The content of Ni^{2+} on GO/NCNCs was conducted on Inductively coupled plasma mass spectrometer (Thermo Fisher Scientific ICAPQ, German). The magnetic performance of the as-prepared GO/NCNCs was performed on Lakeshore 7404 vibrating sample magnetometer (VSM) at ambient temperature. Zeta-potential assay were determined by Zetasizer-3000 (Malvern Instruments, UK). UV−vis absorption spectra measurement were performed on Thermo Evolution 300 spectrophotometer. The morphology changes of bacteria were characterized by Thermo Scientific Talos G2 200X transmission electron microscope (TEM) and FEI_Apreo scanning electron microscope (SEM). The cell live/dead tests and microscopic fluorescence images were recorded using an Olympus IX73 microscope (Japan).

1.2. In Vitro Cytotoxicity Assay

The cytotoxicity of GO/NCNCs nanocomposite was evaluated by using Vero cells as a test model. Briefly, Vero cells (100 μL, 5×10^4/well) were seeded in 96-well plates and cultured in a humidified incubator (37 ℃/5% CO_2) until reaching

monolayer confluence, followed by co-culture separately with 100 μL of GO, NCNCs and GO/NCNCs at different concentrations (1.0 mg/mL, 0.50 mg/mL, 0.25 mg/mL, 125 μg/mL and 62.5 μg/mL) for 12, 24, 36, and 48 h. Next, 20 μL MTT (5.0 mg/mL) was added to each well and incubated for 4 h. Finally, add 150 μL DMSO to each well to dissolve the crystal precipitate, and a microplate spectrophotometer (Epoch 2, Biotek, VT, USA) was used to record the absorbance value of each well at 490 nm.

1.3. Leakage of intracellular components and electrolytes

The integrity of bacterial cell membranes can be detected by measuring the leakage of intracellular compounds and electrolytes in the cells. In this study, the extracellular protein concentration of the bacterial suspension was detected using the Coomassie Brilliant Blue G-250 kit (Beijing Solarbio Science & Technology Co., Ltd. Beijing, China). Briefly, 20 μL of treated solution containing bacteria was added to 96-well plates, followed by adding 200 μL of protein working solution to each well plate and incubation at 37 ° C for 5 min. Finally, the protein leakage was evaluated by measuring the absorbance at 562 nm using an enzyme-linked immunosorbent assay (ELISA) microplate reader (Waltham, MA, USA).

The conductivity of the bacterial suspension was determined using a conductivity meter. Specifically, *S. aureus* cells (10^7 PFU/mL) were treated with GO/NCNCs nanocomposite (125 μg/mL) at 37 °C for 2 h, then centrifuged at 6000 rpm for 5 min to collect the supernatant and measure the conductivity.

1.4. Anti-oxidative effects of GO/NCNCs nanocomposite

After treating *S. aureus* cells (10^7 PFU/mL) with GO/NCNCs nanocomposite

(125 μg/mL) at 37 °C for 2 h, the MDA content, GSH, CAT and SOD activities in the cells were tested using the kits according to the manufacturers' instructions.

2. Results

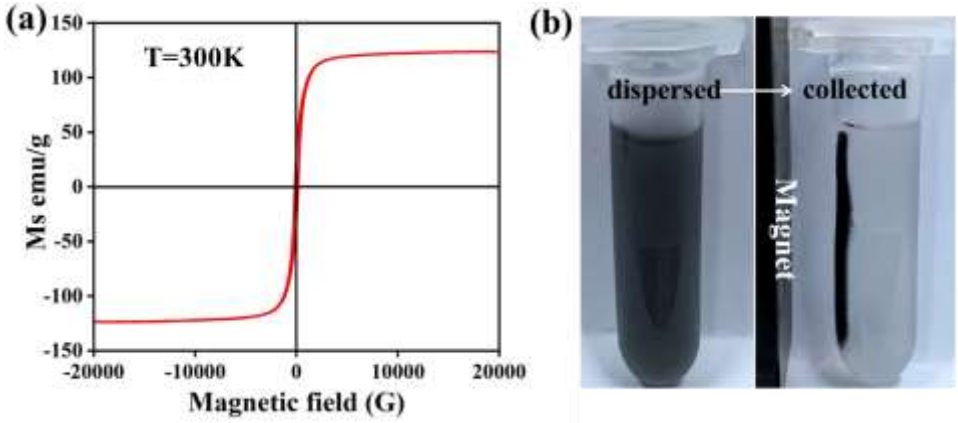

Figure S1. Magnetic hysteresis loop (a) and magnetic enrichment (b) of GO/NCNCs nanocomposites.

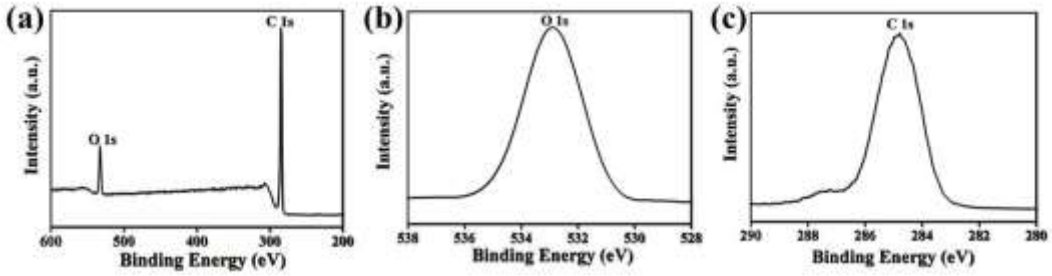

Figure S2. XPS survey spectra of GO. Overall spectrum (a), C 1s (b) and O 1s (c).

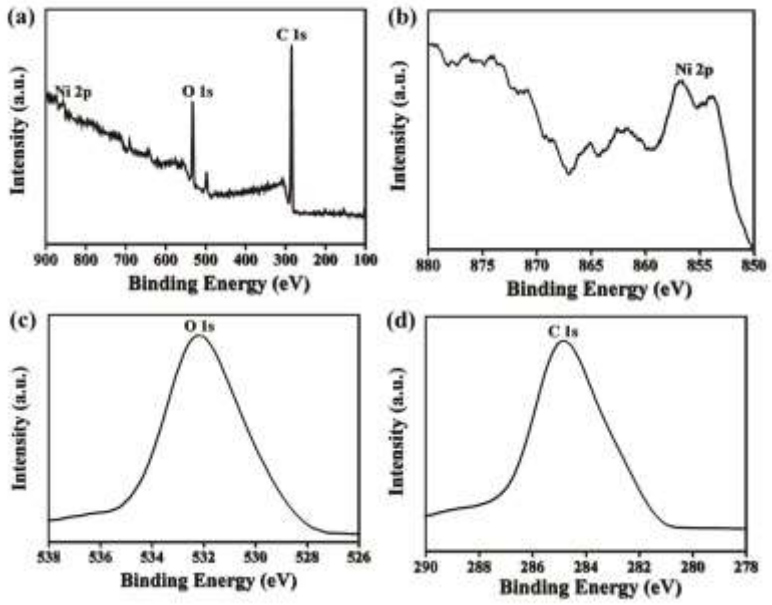

Figure S3. XPS survey spectra of NCNCs. Overall spectrum (a), Ni 2p (b), O 1s (c) and C 1s (d).

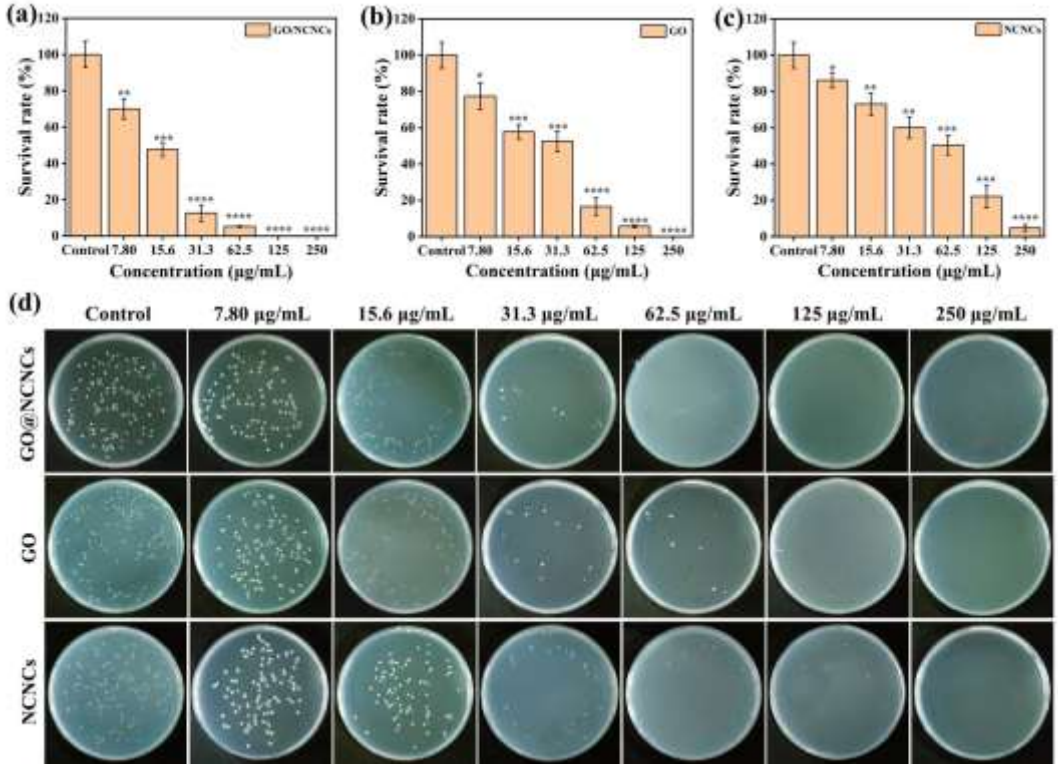

Figure S4. Dose-response to the survival rate of *E. coli*. The survival rate of *E. coli* treated with GO/NCNCs (a), GO (b) and NCNCs (c). Photographs of the agar plates of *E. coli* exposed or unexposed with various concentrations of GO/NCNCs, GO and

NCNCs (7.8-250 μg/mL) (d).

Table S1. Minimal inhibitory concentration of GO/NCNCs, GO and NCNCs against *S. aureus* and *E. coli*.

Bacteria	MIC (μg/mL)		
	GO/NCNCs	GO	NCNCs
S. aureus	125	500	500
E.coli	62.5	250	250

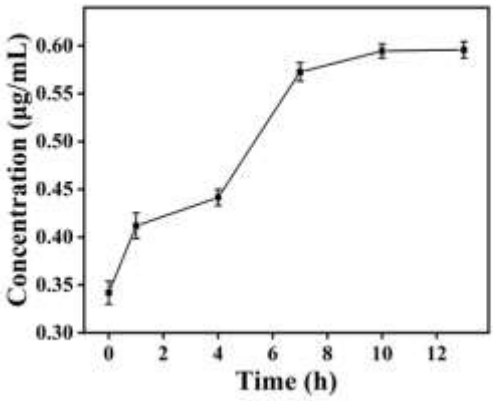

Figure S5. The leach of Ni^{2+} measured at different incubation time intervals under the treatment of the 125 μg/mL GO/NCNCs.

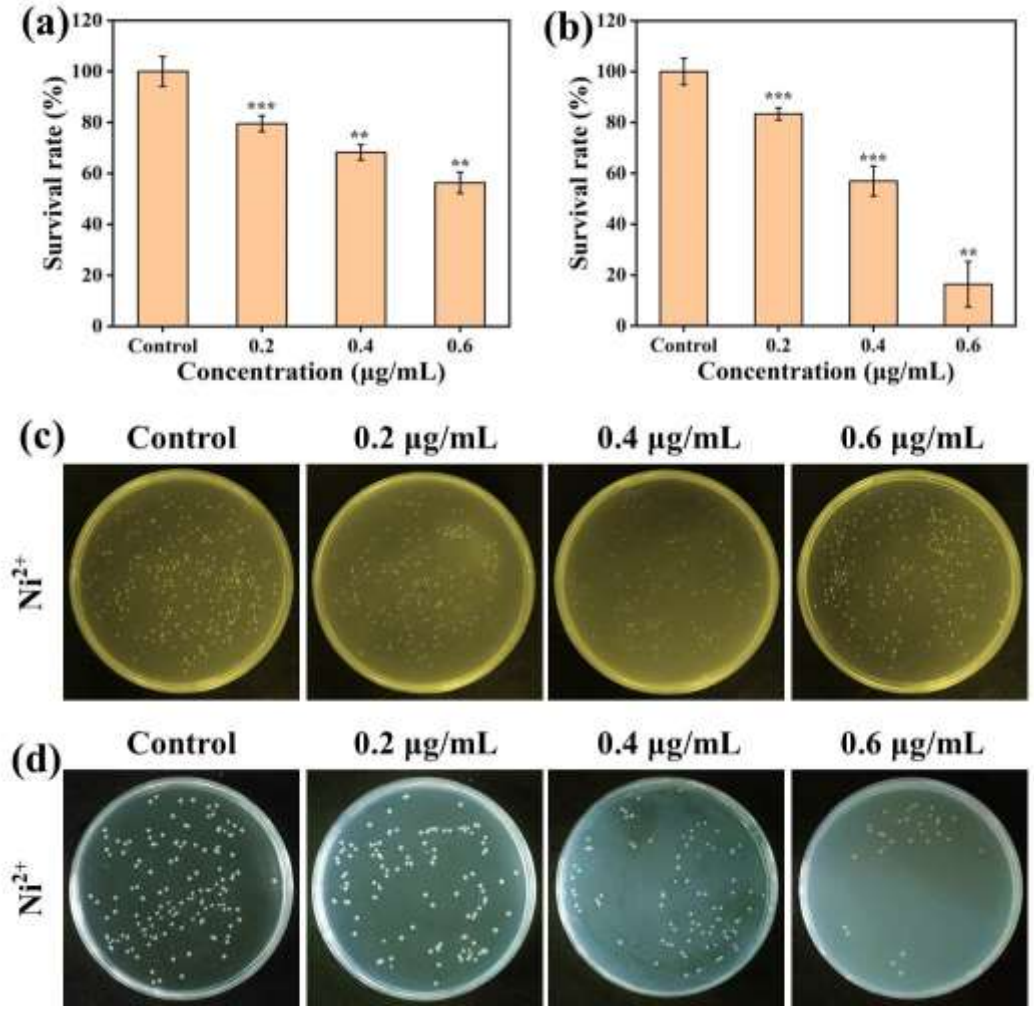

Figure S6. Survival rates of *S. aureus* (a) and *E. coli* (b) treated with different concentrations of Ni^{2+}. The corresponding pictures of colonies formed by *S. aureus* (c) and *E.coli* (d) under the treatment of Ni^{2+} at different concentrations.