Electronic Supplementary Information for

Oxygen-Tuned Nanozyme Polymerization for the Preparation of Hydrogel with Printable and Antibacterial property

Yuemei Ye , Linlin Xiao, Bin He, Qi Zhang, Tao Nie, Xinrui Yang , Dongbei Wu, Heli Cheng, Ping Li*, and Qigang Wang**

Materials and reagents

CuO nanoparticle slurry (10% w/w) was purchased from Xuancheng Jinrui New Material Co., Ltd, glucose oxidase (GOx, 300U/mg) was purchased from Sigma-Aldrich. D-(+)-glucose was purchased from Aladdin. The glucose was dissolved in deionized water (40 mM) and stored for at least 24 h before using. N-dimethylacrylamide (DMAA), hydrogen peroxide (H_2O_2, 30 wt%) and methylene-bis-acrylamide (BIS) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). SYTO-9 Green Fluorescent Nucleic Acid Stain (5 mM solution in DMSO) was purchased from Thermo Fisher Scientific. Propidium iodide fluorescent dye (PI) was purchased from Shanghai treasure biological technology co., LTD. All materials and reagents were used with no further purification.

TEM measurements

Transmission Electron Microscope (TEM) images were obtained by using a JEOL model JEM-1230 microscope at an accelerating voltage of 80 kV. The hydrogel samples for TEM analysis were cut into cubes of about 1×1×1 mm^3. The samples were dehydrated with ethanol/water mixture solution at increasing ethanol concentrations of 50%, 70%, 90% (vol. %) for 15 min in each solution. Then, the samples were immersed sequentially in a mixture of 90% ethanol and 90% acetone (1/1, vol. /vol.) for 30 min, a mixture of 90% acetone and embedding resin (1/1, vol. /vol.) for 12 h, and in the pure resin for 3 h at room temperature. The embedding procedure was conducted in an oven at 37 ^oC for 12 h, at 45 °C for 12 h and at 60 ^oC for 48 h. The ultrathin sample slices of about 70 nm in thickness were prepared by

using a Leica ultramicrotome. The slices were put on copper net and observed.

The TEM images of bacteria before or after treatment with the Nanozyme-Gel and glucose were observed as follow. Firstly, the E. coli bacteria solution before or after hydrogel treatment were dropped onto Cu grid, and the sample on Cu grid were treated with gradient dehydration by a series of ethanol solutions (50%, 70%, 90%, 95%, and 100%) for 20 min with each step. After the gradient dehydration treatment, the samples were naturally dried and observed by TEM.

Figures

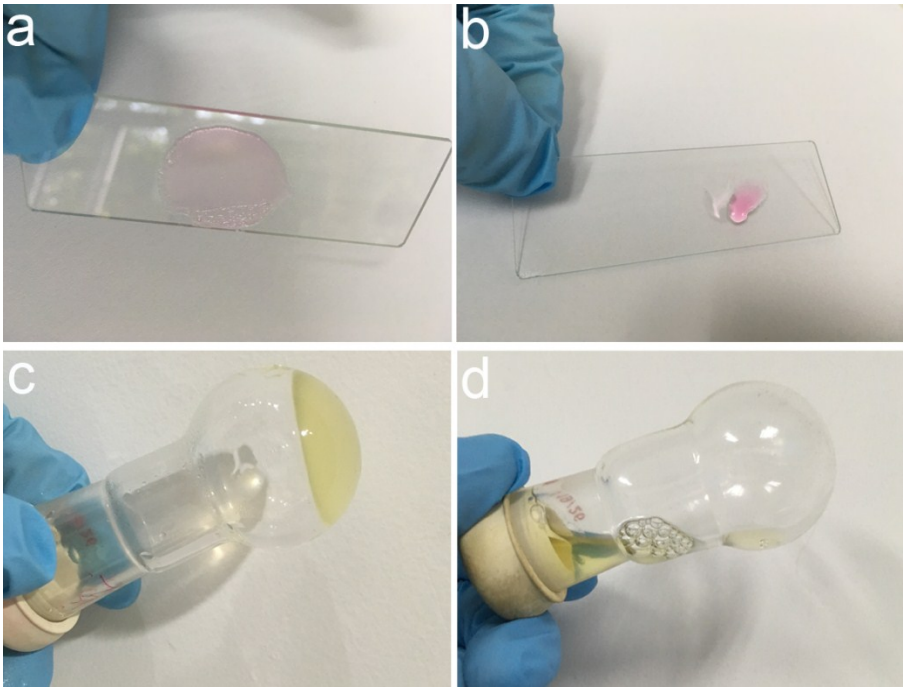

Fig. S1 (a) The picture of 100 μL Nanozyme-Gel precursor with acid fuchsin incubated between two sealed slides at 37 ºC for 1 h. (b) The picture of 100 μL Nanozyme-Gel precursor incubated at the open surface of slide. (c) The picture of 2 mL Nanozyme-Gel precursor incubated within closed flask at 37 ºC for 1 h. (d) The picture of 2 mL completely deoxygenized Nanozyme-Gel precursor within closed flask at 37 ºC for 1 h.

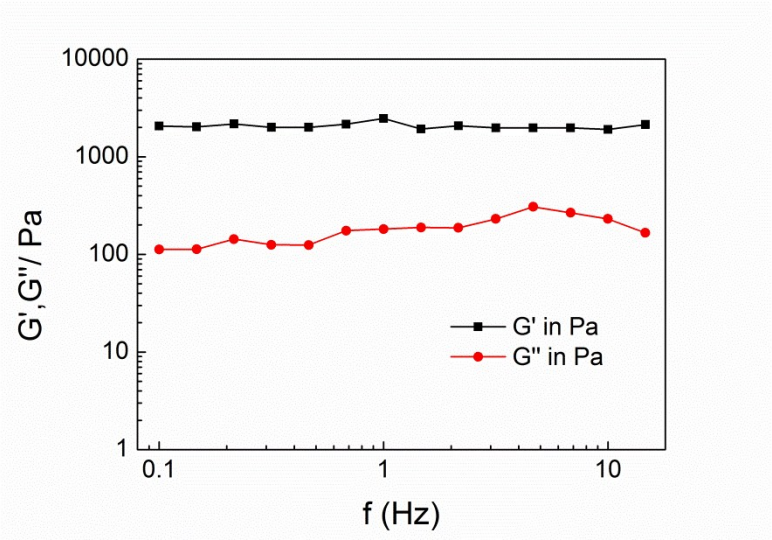

Fig. S2. (a) The frequency sweep curve of Nanozyme-Gel at a strain of 1%.

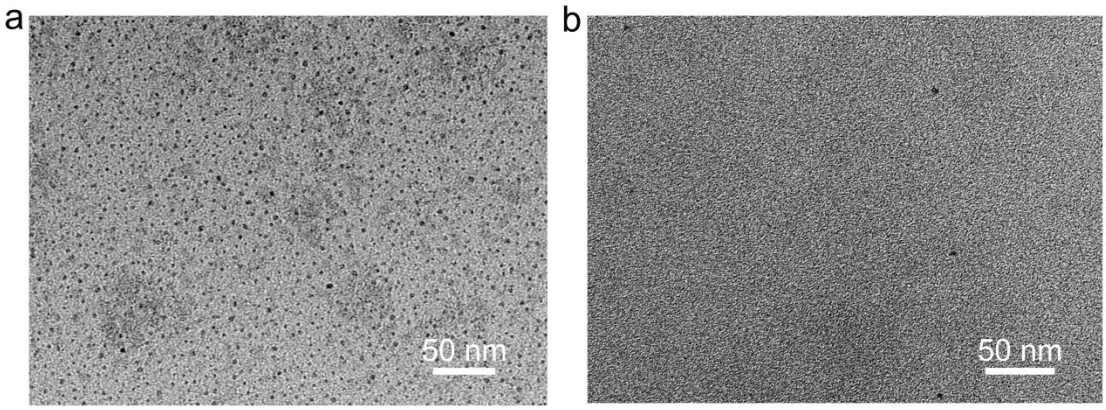

Fig. S3. (a) The TEM image of CuO NPs. (b) TEM image of the Nanozyme-Gel.

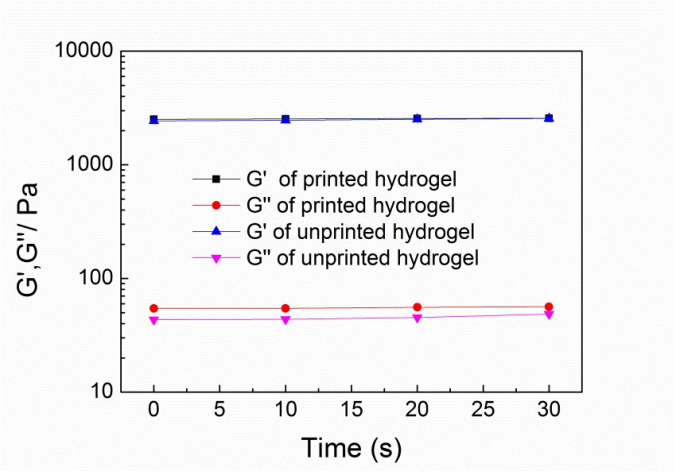

Fig. S4 Comparison of the dynamic time sweeps results of the Nanozyme-Gel that reacted for 2700 s at 37 °C after being printed on the platform of rheometer with the precursor incubated for 900 s and the Nanozyme-Gel that formed on the platform of rheometer at 37 °C for 3600 s at a frequency of 1 rad s^{-1}.

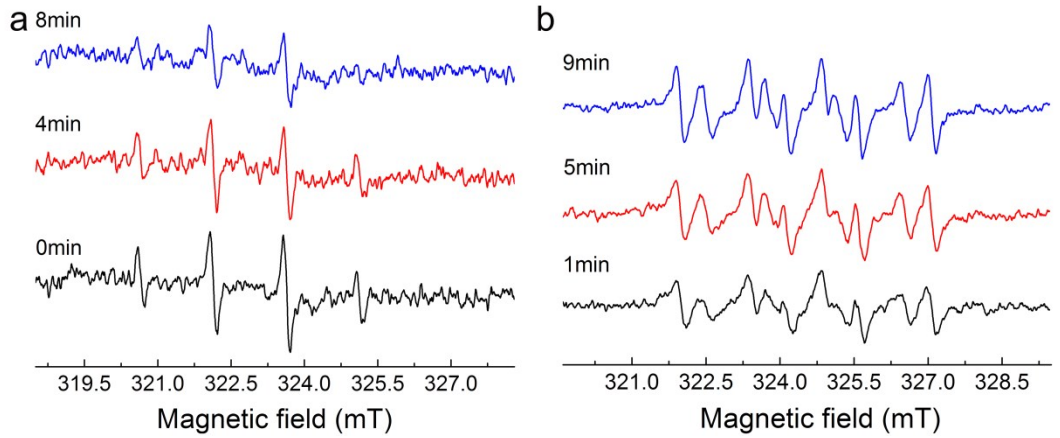

Fig. S5 (a) EPR test of the mixture of CuO and H_2O_2. (b) EPR test of the mixture of CuO, H_2O_2 and DMAA.

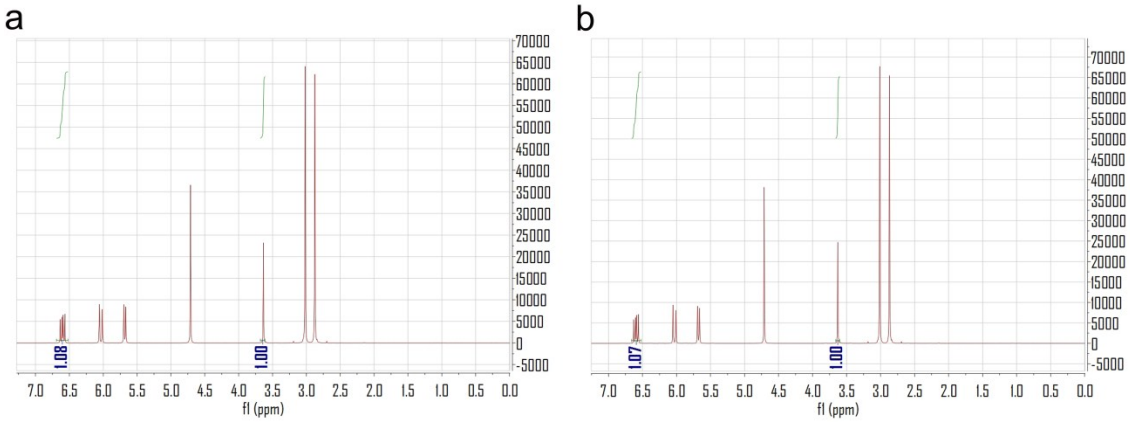

Fig. S6 The ^{1}HNMR spectra of the precursor of 0.05 wt% CuO, 2 mM H_2O_2 and 8% v/v DMAA at 0 h (a) and 1h (b).

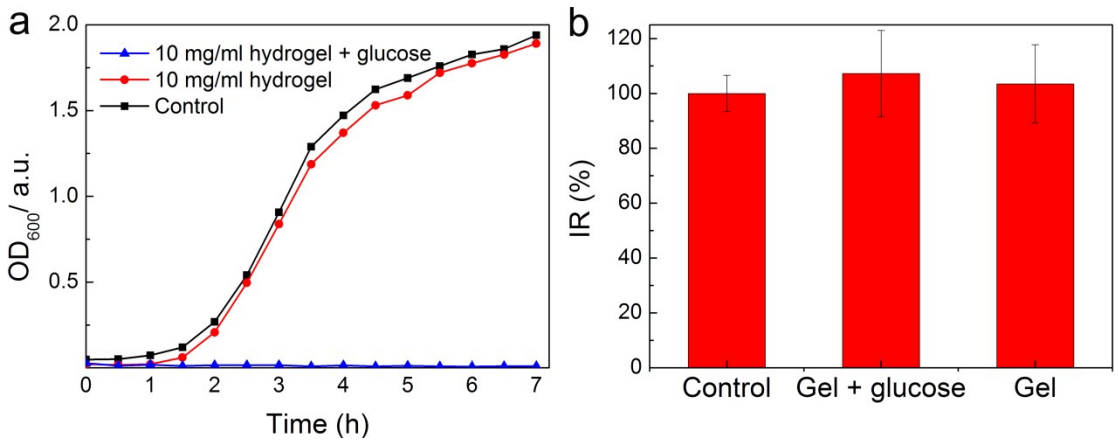

Fig. S7 (a) Time-dependent antimicrobial effects of Nanozyme-Gel/glucose, Nanozyme-Gel, and the blank control at 37 ºC for 7 h. (b) MTT inhibition ratio of endothelial cells after 12h treatment by Nanozyme-Gel.

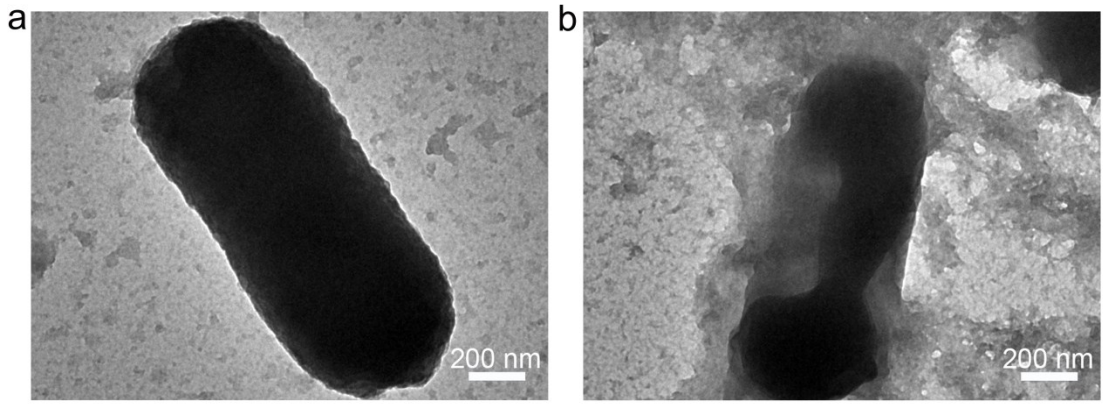

Fig. S8 (a) The TEM image of the undisturbed E. coli. (b) The TEM image of E. coli treated by the Nanozyme-Gel in the presence of glucose.