

Communication

# Will the Bacteria Survive in the CeO<sub>2</sub> Nanozyme-H<sub>2</sub>O<sub>2</sub> System?

Weisheng Zhu<sup>1</sup>, Luyao Wang<sup>2</sup>, Qisi Li<sup>1</sup>, Lizhi Jiao<sup>1</sup>, Xiaokan Yu<sup>1</sup>, Xiangfan Gao<sup>1</sup>, Hao Qiu<sup>1</sup>, Zhijun Zhang<sup>1,\*</sup> and Wei Bing<sup>2,3,\*</sup>

<sup>1</sup> Key Laboratory of Surface & Interface of Polymer Materials of Zhejiang Province, Department of Chemistry, Zhejiang Sci-Tech University, Hangzhou 310018, China; 201930107026@mails.zstu.edu.cn (W.Z.); 202020104127@mails.zstu.edu.cn (Q.L.); 202030107241@mails.zstu.edu.cn (L.J.); xiaokanyu36@gmail.com (X.Y.); g3522166853@gmail.com (X.G.); qiuhao@ciac.ac.cn (H.Q.)

<sup>2</sup> School of Chemistry and Life Science, Changchun University of Technology, 2055 Yanan Street, Changchun 130012, China; wluyao1206@gmail.com

<sup>3</sup> Advanced Institute of Materials Science, Changchun University of Technology, 2055 Yanan Street, Changchun 130012, China

\* Correspondence: zjzhang@zstu.edu.cn (Z.Z.); bingwei@ccut.edu.cn (W.B.)

**Abstract:** As one of the nanostructures with enzyme-like activity, nanozymes have recently attracted extensive attention for their biomedical applications, especially for bacterial disinfection treatment. Nanozymes with high peroxidase activity are considered to be excellent candidates for building bacterial disinfection systems (nanozyme-H<sub>2</sub>O<sub>2</sub>), in which the nanozyme will promote the generation of ROS to kill bacteria based on the decomposition of H<sub>2</sub>O<sub>2</sub>. According to this criterion, a cerium oxide nanoparticle (Nanoceria, CeO<sub>2</sub>, a classical nanozyme with high peroxidase activity)-based nanozyme-H<sub>2</sub>O<sub>2</sub> system would be very efficient for bacterial disinfection. However, CeO<sub>2</sub> is a nanozyme with multiple enzyme-like activities. In addition to high peroxidase activity, CeO<sub>2</sub> nanozymes also possess high superoxide dismutase activity and antioxidant activity, which can act as a ROS scavenger. Considering the fact that CeO<sub>2</sub> nanozymes have both the activity to promote ROS production and the opposite activity for ROS scavenging, it is worth exploring which activity will play the dominating role in the CeO<sub>2</sub>-H<sub>2</sub>O<sub>2</sub> system, as well as whether it will protect bacteria or produce an antibacterial effect. In this work, we focused on this discussion to unveil the role of CeO<sub>2</sub> in the CeO<sub>2</sub>-H<sub>2</sub>O<sub>2</sub> system, so that it can provide valuable knowledge for the design of a nanozyme-H<sub>2</sub>O<sub>2</sub>-based antibacterial system.

**Keywords:** nanozymes; cerium oxide nanoparticles; antibacterial; peroxidase; ROS scavenger



**Citation:** Zhu, W.; Wang, L.; Li, Q.; Jiao, L.; Yu, X.; Gao, X.; Qiu, H.; Zhang, Z.; Bing, W. Will the Bacteria Survive in the CeO<sub>2</sub> Nanozyme-H<sub>2</sub>O<sub>2</sub> System? *Molecules* **2021**, *26*, 3747. <https://doi.org/10.3390/molecules26123747>

Academic Editor: Zhaohui Li

Received: 31 May 2021

Accepted: 16 June 2021

Published: 19 June 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

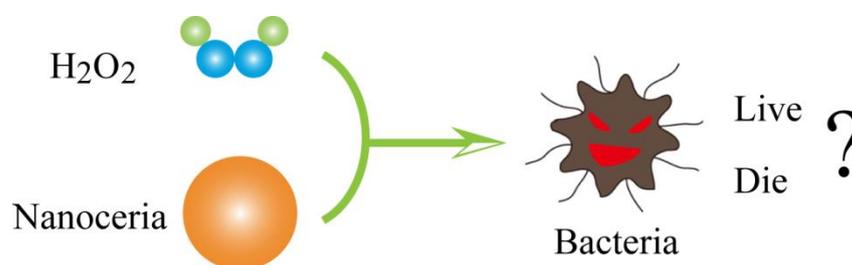
## 1. Introduction

Nanozymes, as a type of nanomaterial with enzyme-like activities, have recently garnered considerable researchers' attention toward their biological and biomedical applications [1–3]. Thus far, various types of nanomaterials have been reported to have catalytic activities similar to those of peroxidase, catalase, superoxide dismutase, oxidase, and other natural enzymes, including different metal and metal oxide nanoparticles, carbon-based nanomaterials, as well as some organic nanostructures [4–7]. Compared with natural enzymes, nanozymes exhibit several advantages such as tunable size and activity, facile preparation, low cost, and high stability against denaturing [8,9]. These superior properties sufficiently meet the demands for diverse biological and biomedical applications. Indeed, nanozymes have recently been frequently used for disease treatment, such as cancer therapy, bacterial disinfection, as well as Alzheimer's disease treatment [10–12].

Bacterial infection has long been a major threat for human health. Antibiotics treatment is currently a golden standard for bacterial disinfection. However, the overuse of antibiotics greatly promotes antibiotic resistance development in bacterial pathogens. Therefore, the exploration of new antimicrobial strategies is urgently demanded [13–16]. Nanozyme-based antibacterial strategies have been recently demonstrated to be a promising new

approach for bacterial infection treatment [17–24]. For example, Qu et al. developed graphene quantum dots (GQDs) that can act as a peroxidase mimic to enhance the antibacterial capacity of  $\text{H}_2\text{O}_2$  and can be used for wound disinfection [25]. Wang and coworkers reported  $\text{Cu}_2\text{WS}_4$  nanocrystals (CWS NCs) with excellent antibacterial activity owing to their high intrinsic peroxidase activity that can catalyze the decomposition of  $\text{H}_2\text{O}_2$  to form  $\cdot\text{OH}$ , so that it inhibits wound bacterial infection [26]. Besides, Qu and his team also proposed that MOF/Ce-based nanozymes with dual enzymatic activities can not only disperse biofilms ascribed to the Ce complex, but also kill bacteria on-site, thereby avoiding the proliferation of bacteria and the recurrence of biofilms [27]. Notably, most of these works were based on the peroxidase activity of nanozymes to promote the reactive oxygen species (ROS) generation of  $\text{H}_2\text{O}_2$  to kill bacteria [28–34]. Thus, the nanozyme with high peroxidase activity can be regarded as an excellent candidate for building a bacterial disinfection nanozyme system.

The cerium oxide nanoparticle (Nanoceria,  $\text{CeO}_2$ ) is a classical nanozyme with high peroxidase activity [35,36]. According to the criterion that high peroxidase activity is more conducive to promoting the production of ROS, the  $\text{CeO}_2$ - $\text{H}_2\text{O}_2$  nanozyme system would be very efficient for bacterial disinfection. However,  $\text{CeO}_2$  could show multiple enzyme-like activities owing to different shapes and sizes [8,37]. In addition to high peroxidase activity,  $\text{CeO}_2$  also possesses high superoxide dismutase activity and antioxidant activity, which can be used as a ROS scavenger. In view of the fact that  $\text{CeO}_2$  has both the activity of promoting ROS production and the opposite activity of ROS scavenging, it is meaningful to discuss which activity will play the dominating role in the  $\text{CeO}_2$ - $\text{H}_2\text{O}_2$  system, and whether it will protect bacteria or produce an antibacterial effect (Scheme 1). Herein, we focus on this discussion to unveil the role of  $\text{CeO}_2$  in the  $\text{CeO}_2$ - $\text{H}_2\text{O}_2$  system, which will offer valuable knowledge for the design of a nanozyme- $\text{H}_2\text{O}_2$  based antibacterial system.



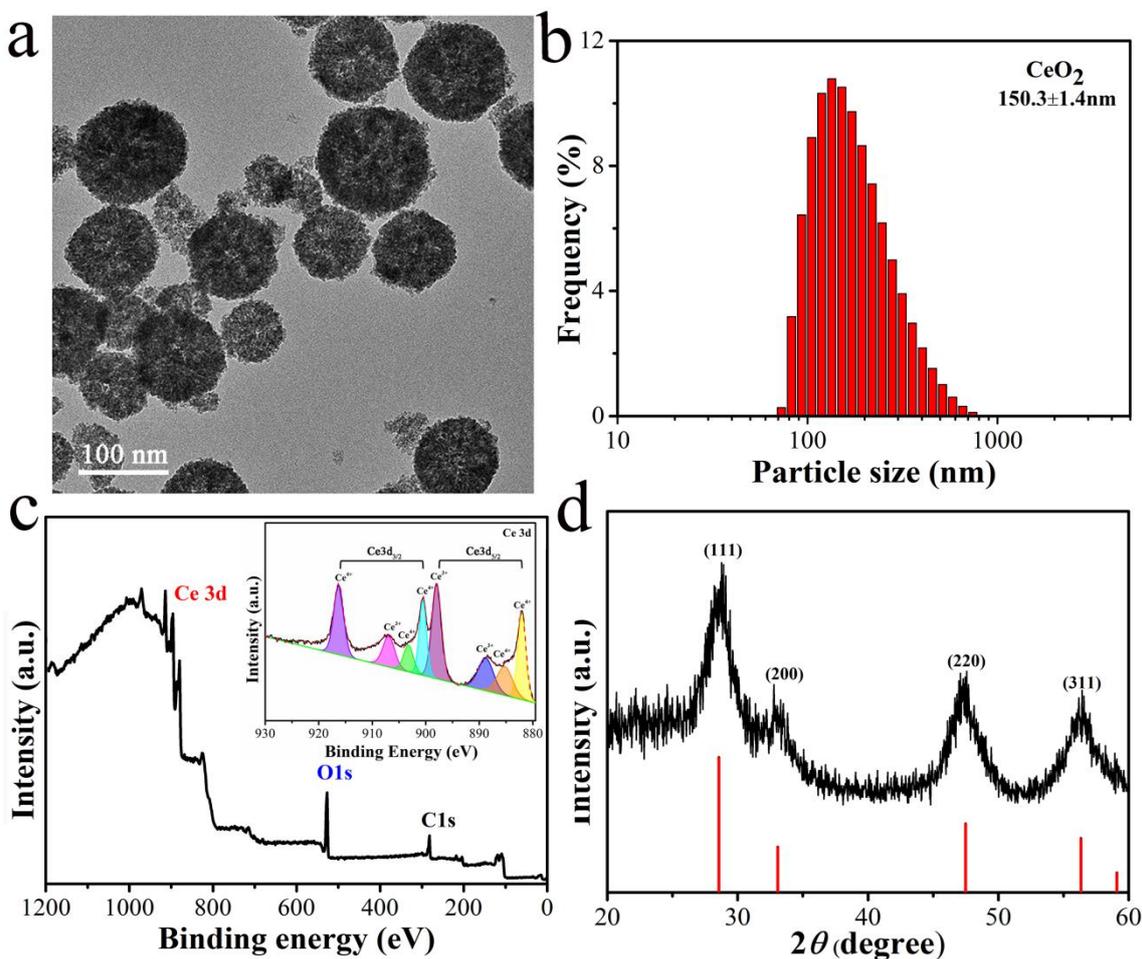
**Scheme 1.** A schematic representation of the question to be answered in this work.

## 2. Results

### 2.1. Preparation and Characterization of $\text{CeO}_2$ Nanozyme

The  $\text{CeO}_2$  nanozyme was first synthesized according to a facile solvothermal method, of which the inorganic salt  $\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$  and organic acid  $\text{C}_2\text{H}_5\text{COOH}$  were selected as the initial materials without any surfactants [27]. As shown in Figure 1a, the transmission electron microscopy (TEM) images demonstrated the formation of  $\text{CeO}_2$  nanospheres with uniform dispersion. Dynamic light scattering (DLS) analysis was performed to determine the distribution of the hydration particle size of  $\text{CeO}_2$ , which is placed at around 150 nm from Figure 1b. Furthermore, the X-ray photoelectron energy spectrum was employed to analyze the element and chemical value state of  $\text{CeO}_2$  nanospheres. We can observe from Figure 1c that there were main peaks such as  $\text{Ce}^{4+} 3d_{3/2}$  and  $\text{Ce}^{4+} 3d_{5/2}$  at around 914.2 and 896.0 eV in the Ce 3d core spectrum, and the  $\text{Ce}^{3+} 3d_{3/2}$  and  $\text{Ce}^{3+} 3d_{5/2}$  peaks are subscribed at 898.4 and 880.0 eV, respectively. In addition, the crystalline and phase information were implied by powder X-ray diffraction (XRD) patterns. As shown in Figure 1d, there were four clearly specific peaks, and the peaks at  $2\theta = 28.5^\circ$ ,  $32.8^\circ$ ,  $47.4^\circ$ , and  $56.4^\circ$  can be subscribed to the characteristic (111), (200), (220), and (311) reflections of the face-centered cubic structure of  $\text{CeO}_2$  nanocrystals (JCPDS No. 43–1002, the red

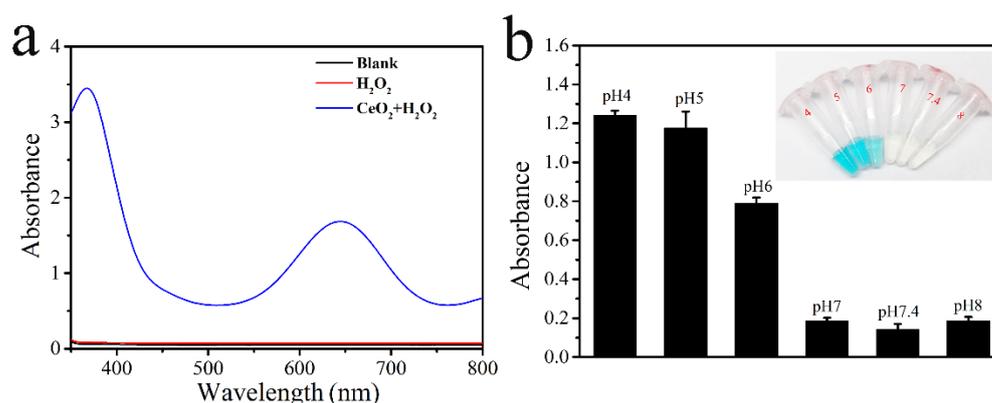
line in Figure 1d). All the above results demonstrate the successful synthesis of the CeO<sub>2</sub> nanoparticles.



**Figure 1.** The characterization of CeO<sub>2</sub> nanozyme. (a) TEM images of CeO<sub>2</sub>; (b) the dynamic light scattering (DLS) analysis of CeO<sub>2</sub>; (c) the XPS spectra of the CeO<sub>2</sub> nanozyme; the inset shows the high-resolution XPS spectra of Ce3d; (d) the XRD pattern of CeO<sub>2</sub> (black curve).

## 2.2. The Peroxidase Activity of CeO<sub>2</sub> Nanozyme

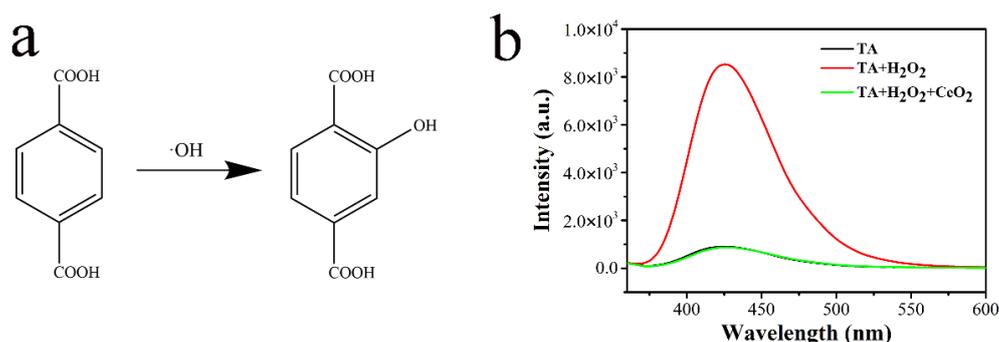
In this work, we utilized a classical colorimetric assay to estimate the peroxidase activity of CeO<sub>2</sub> nanospheres. TMB (3,3',5,5'-tetramethylbenzidine) was used as a substrate. In the presence of peroxidase mimics and H<sub>2</sub>O<sub>2</sub>, colorless TMB can be oxidized to blue oxTMB, which exhibits two specific absorbance peaks at 370 and 652 nm. As shown in Figure 2a, neither the nanozyme nor H<sub>2</sub>O<sub>2</sub> alone could produce any color changes, but there appeared two sharp and strong peaks at 370 and 652 nm with the presence of both CeO<sub>2</sub> and H<sub>2</sub>O<sub>2</sub>, which indicated that CeO<sub>2</sub> nanospheres had intrinsic peroxidase enzymatic activity. Likewise, we investigated the effect of pH on its peroxidase activity, and the nanozyme was demonstrated to possess optimistic catalytic activity at a pH range from 4.0 to 6.0 (Figure 2b).



**Figure 2.** The assay of peroxidase activity of CeO<sub>2</sub>. (a) The absorbance of TMB at 652 nm right in different reaction systems; (b) the effect of different pH on absorbance of TMB at 652 nm.

### 2.3. ROS Generation

Considering that the catalytic capacity of most of the nanozymes with peroxidase activity may originate from the decomposition of H<sub>2</sub>O<sub>2</sub> to generate ROS hydroxyl radicals ( $\cdot$ OH), we further investigated the generation of  $\cdot$ OH in the CeO<sub>2</sub>-H<sub>2</sub>O<sub>2</sub> system to verify the peroxidase activity of the CeO<sub>2</sub> nanozyme. Terephthalic acid (TA), which could capture  $\cdot$ OH to generate fluorescent product 2-hydroxy terephthalic acid (TAOH) (Figure 3a), was used as a fluorescence probe for the tracing of  $\cdot$ OH. As shown in Figure 3b, compared with the fluorescent intensity of TA alone, there was a remarkable increase with the addition of H<sub>2</sub>O<sub>2</sub>. However, when we added the CeO<sub>2</sub> nanozyme to the H<sub>2</sub>O<sub>2</sub>-TA system, the fluorescent intensity did not show any increase but sharply decreased to near the background intensity, which was contrary to what we predicted. This may be because CeO<sub>2</sub> possesses certain antioxidant properties as obstacles to the decomposition of H<sub>2</sub>O<sub>2</sub> to generate  $\cdot$ OH [37]. In other words, CeO<sub>2</sub> shows considerable ROS scavenging capacity in the CeO<sub>2</sub> nanozyme-H<sub>2</sub>O<sub>2</sub> system.

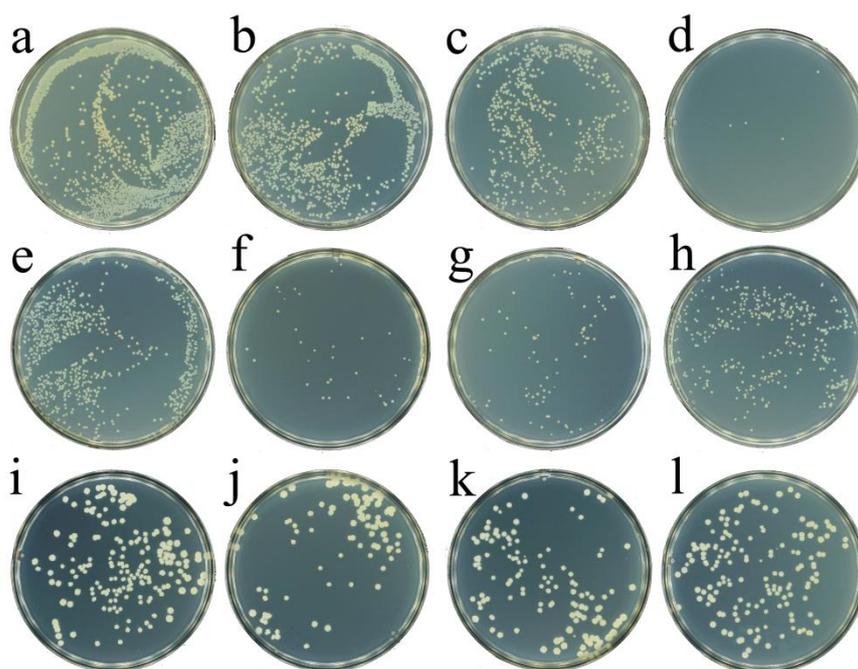


**Figure 3.** (a) Reaction between hydroxyl radical (OH) and terephthalic acid (TA); (b) fluorescence spectra of reaction system including only TA; TA and H<sub>2</sub>O<sub>2</sub>; TA, CeO<sub>2</sub>, and H<sub>2</sub>O<sub>2</sub> after 2 h of reaction. [TA] = 0.5 mM, H<sub>2</sub>O<sub>2</sub> = 1 mM, [CeO<sub>2</sub>] = 0.25 mg/mL.

### 2.4. The Evaluation of Antibacterial Activity of CeO<sub>2</sub>-H<sub>2</sub>O<sub>2</sub> System

Finally, the antibacterial activities were investigated by using a Gram-negative bacterium (*Escherichia coli*, *E. coli*) as a model strain. In view of the effect of the pH value on the nanozyme activity, all the bacterial experiments were performed at pH 6.0 with PBS (phosphate-buffered saline) as the buffer. As shown in Figure 4b, CeO<sub>2</sub> (0.25 mg/mL) alone caused a weak antibacterial effect. H<sub>2</sub>O<sub>2</sub> showed concentration-dependent antibacterial activity. At a low concentration of 0.5 mM, H<sub>2</sub>O<sub>2</sub> showed a weak antibacterial activity (Figure 4c). When the concentration of H<sub>2</sub>O<sub>2</sub> was increased to 1.5 mM, almost all the bacteria were killed, indicating a significant high antibacterial activity (Figure 4d). Thereafter,

the bacterial effect of the CeO<sub>2</sub> nanozyme-H<sub>2</sub>O<sub>2</sub> system was investigated. As shown in Figure 4e, compared with H<sub>2</sub>O<sub>2</sub> (0.5 mM) alone, the presence of CeO<sub>2</sub> (0.25 mg/mL) did not cause an obvious change in the number of bacteria, which demonstrated that the CeO<sub>2</sub> nanozyme did not promote the killing effect of H<sub>2</sub>O<sub>2</sub> against bacteria in this nanozyme-H<sub>2</sub>O<sub>2</sub> system. Interestingly, compared with H<sub>2</sub>O<sub>2</sub> (1.5 mM) alone, the presence of CeO<sub>2</sub> (0.25 mg/mL) caused an obvious increase in the number of bacteria, which indicated that the CeO<sub>2</sub> greatly inhibited the killing effect of H<sub>2</sub>O<sub>2</sub> against bacteria in this nanozyme-H<sub>2</sub>O<sub>2</sub> system (Figure 4g). This role was further found to be in a concentration-dependent manner. When we reduced the CeO<sub>2</sub> concentration to 0.1 mg/mL, the effect of CeO<sub>2</sub> on the bacterial killing activity of H<sub>2</sub>O<sub>2</sub> decreased accordingly (Figure 4f). On the contrary, the effect of CeO<sub>2</sub> on the bacterial killing activity of H<sub>2</sub>O<sub>2</sub> was dramatically increased with the increase in the concentration of CeO<sub>2</sub> to 0.4 mg/mL. The further study demonstrated that CeO<sub>2</sub> played a similar protection role in the nanozyme-H<sub>2</sub>O<sub>2</sub> system toward the Gram-positive bacterium (*Bacillus subtilis*, *B. subtilis*) (Figure 4f–i). All the above results manifested that the peroxidase activity of CeO<sub>2</sub> did not contribute to the antibacterial effect of the nanozyme-H<sub>2</sub>O<sub>2</sub> system. Instead, the ROS scavenging capacity of CeO<sub>2</sub> protected the bacteria from being killed by H<sub>2</sub>O<sub>2</sub>. Although the phenomenon we observed may be different from other enzymes with multiple activities, our work would provide valuable new knowledge for the design of the nanozyme-H<sub>2</sub>O<sub>2</sub>-based antibacterial system.



**Figure 4.** Photographs of bacterial colonies formed by *E. coli* and *B. subtilis* with different treatment at room temperature for 2 h. (a) *E. coli*; (b) *E. coli* + 0.25 mg/mL CeO<sub>2</sub>; (c) *E. coli* + 0.5 mM H<sub>2</sub>O<sub>2</sub>; (d) *E. coli* + 1.5 mM H<sub>2</sub>O<sub>2</sub>; (e) *E. coli* + 0.5 mM H<sub>2</sub>O<sub>2</sub> + 0.25 mg/mL CeO<sub>2</sub>; (f) *E. coli* + 1.5 mM H<sub>2</sub>O<sub>2</sub> + 0.1 mg/mL CeO<sub>2</sub>; (g) *E. coli* + 1.5 mM H<sub>2</sub>O<sub>2</sub> + 0.25 mg/mL CeO<sub>2</sub>; (h) *E. coli* + 1.5 mM H<sub>2</sub>O<sub>2</sub> + 0.4 mg/mL CeO<sub>2</sub>; (i) *B. subtilis*; (j) *B. subtilis* + 1 mM H<sub>2</sub>O<sub>2</sub>; (k) *B. subtilis* + 1 mM H<sub>2</sub>O<sub>2</sub> + 0.25 mg/mL CeO<sub>2</sub>; (l) *B. subtilis* + 1 mM H<sub>2</sub>O<sub>2</sub> + 0.4 mg/mL CeO<sub>2</sub>.

### 3. Materials and Methods

#### 3.1. Chemicals and Instrument

All reagents and solvents were purchased from commercial sources. Hydrogen-peroxide is an AR reagent with a concentration of 30% in water. The degree of purity of other compounds was at least 98%, and they were used without any further treatment.

PL spectra were collected by a FL-970 Fluorescence Spectrometer (slit width of 2.5 nm and PMT voltage of 600 V). UV-vis adsorption spectra were measured by a UV-2600 spectrometer (SHIMADZU, Japan). The size of nanoparticles was monitored with a SZ-100V2 Nano Particle Analyzer. The X-ray diffraction (XRD) patterns of the products were determined on a DX-2700 X-ray diffractometer with Cu K $\alpha$  radiation ( $\lambda = 1.5416 \text{ \AA}$ ), with an operation voltage and current maintained at 35 kV and 25 mA, respectively. X-ray photoelectron spectroscopy (XPS) measurements were conducted on a Kratos AXIS Ultra DLD photoelectron spectrometer with Al K $\alpha$  X-ray radiation as the X-ray source for excitation. The nanostructure of products was analyzed with a 120 kV JEM-1400Flash transmission electron microscope (TEM) with a Gatan Rio16 digital camera. Samples for TEM were prepared by dropping dilute solutions of nanoparticles onto carbon-coated copper grids and then maintained at 40 °C to wait for the solvent to evaporate.

### 3.2. Synthesis of CeO<sub>2</sub> Nanozyme

The CeO<sub>2</sub> nanozyme was synthesized by one simple solvothermal method right in this work. Briefly, 1 g of Ce(NO<sub>3</sub>)<sub>3</sub>·6H<sub>2</sub>O was dissolved in 1 mL of ultrapure water. Then, 1 mL of C<sub>2</sub>H<sub>5</sub>COOH and 30 mL of glycol were added in the above solution with stirring until the formation of a uniform solution. Following that, the mixture solution was transferred to an autoclave and heated to 180 °C for 200 min to obtain the initial product. The product was first centrifuged at 5000 rpm for 10 min to remove large particles; then, the supernatant was centrifuged at 10,000 rpm for 10 min, and the precipitation was washed with ethanol and ultrapure water twice. Ultimately, the product was redispersed into ultrapure water for later use.

### 3.3. Detection of Peroxidase Activity of CeO<sub>2</sub>

The peroxidase activity was investigated by a catalytic reaction of the TMB with the assistance of H<sub>2</sub>O<sub>2</sub>, and the catalytic performance was characterized by a specific peak at 652 nm. Typically, the reaction solution (200  $\mu$ L) containing PBS buffer solution (0.2 M, 20  $\mu$ L), TMB (80 mM, 2  $\mu$ L), H<sub>2</sub>O<sub>2</sub> (10 mM, 40  $\mu$ L), and CeO<sub>2</sub> nanozyme (40  $\mu$ L) was incubated at room temperature for 10 min, and the blue product (oxTMB) was then monitored by a UV-spectrum at 652 nm.

### 3.4. Detection of OH

The detection of  $\cdot$ OH was dependent on the reaction of TA and  $\cdot$ OH. TA has weak fluorescence in the absence of  $\cdot$ OH, but it has unique fluorescence around 425 nm in the presence of  $\cdot$ OH ascribed to the generation of 2-hydroxy terephthalic acid.

The reaction solutions containing TA, TA + H<sub>2</sub>O<sub>2</sub>, and TA + H<sub>2</sub>O<sub>2</sub> + nanozymes were reacted for 2 h and then centrifuged to separate the supernatant. The fluorescent intensity of the supernatant at around 430 nm was determined by a FL-970 fluorescence spectrometer. The concentrations of TA, H<sub>2</sub>O<sub>2</sub>, and CeO<sub>2</sub> nanozyme were 0.5 mM, 1 mM, 0.25 mg/mL, respectively.

### 3.5. Antibacterial Experiment

The spread plate method was utilized to measure the bacterial number under different treatments. *E. coli* was copied with five different groups: (a) without any addition, (b) CeO<sub>2</sub>, (c) H<sub>2</sub>O<sub>2</sub>, and (d) CeO<sub>2</sub> + H<sub>2</sub>O<sub>2</sub> groups. In brief, the mono-colony of *E. coli* on a solid Luria–Bertani (LB) agar plate was diverted to 2 mL of liquid LB culture medium and grown at 37 °C for 5 h under 180 rpm rotation. After finishing this process, we chose 0.3 as the initial optical density of bacteria at OD<sub>600 nm</sub>. Then, 20  $\mu$ L of as-prepared bacteria solution was mixed up with different groups we mentioned above with a 200  $\mu$ L total volume and incubated in 24-cell culture plates at a reaction system (pH = 5, PBS buffer solution 20 mM) for 2 h. Thereafter, the bacteria solution was transferred from the 24-well plate to the solid medium by the spread plate method and was cultured at 37 °C for 12 h. A similar method was used in the *B. subtilis* experiment.

#### 4. Conclusions

In summary, we successfully synthesized a CeO<sub>2</sub> nanozyme with both the peroxidase and ROS scavenging activity. Based on this, we investigated the function between the CeO<sub>2</sub>-H<sub>2</sub>O<sub>2</sub> system and bacteria. The results of the bacterial experiments demonstrated that the peroxidase activity of CeO<sub>2</sub> did not contribute to the antibacterial effect in the CeO<sub>2</sub>-H<sub>2</sub>O<sub>2</sub> system; instead, the ROS scavenging capacity of CeO<sub>2</sub> could protect the bacteria from being killed by H<sub>2</sub>O<sub>2</sub>. Our present work not only unveils the role of CeO<sub>2</sub> in the CeO<sub>2</sub>-H<sub>2</sub>O<sub>2</sub> system toward bacteria but also provides valuable new knowledge for the design of nanozyme-H<sub>2</sub>O<sub>2</sub>-based antibacterial systems.

**Author Contributions:** Conceptualization, W.Z., Z.Z. and W.B.; methodology, W.Z. and Z.Z.; investigation, W.Z., L.W., Q.L., L.J., X.Y., X.G. and H.Q.; writing—original draft preparation, W.Z. and Z.Z.; writing—review and editing, W.Z., Z.Z. and W.B.; supervision, Z.Z.; funding acquisition, Z.Z. and W.B. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research's financial support from the National Natural Science Foundation of China (NSFC) (No. 22007083, No. 52005049), Zhejiang Provincial Natural Science Foundation of China (Grant No. LQ20B010010), Science Foundation of Zhejiang Sci-Tech University (ZSTU) under Grant No. 19062410-Y, and the Key Laboratory Fund of National Defense Science and Technology, China (Grant No. 6142005190201).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data are contained within the article.

**Conflicts of Interest:** The authors declare no conflict of interest.

**Sample Availability:** Samples of the compounds are available from the authors.

#### References

1. Chen, Z.; Wang, Z.; Ren, J.; Qu, X. Enzyme Mimicry for Combating Bacteria and Biofilms. *Acc. Chem. Res.* **2018**, *51*, 789–799. [[CrossRef](#)]
2. Lin, Y.; Ren, J.; Qu, X. Nano-Gold as Artificial Enzymes: Hidden Talents. *Adv. Mater.* **2014**, *26*, 4200–4217. [[CrossRef](#)] [[PubMed](#)]
3. Wu, J.; Wang, X.; Wang, Q.; Lou, Z.; Li, S.; Zhu, Y.; Qin, L.; Wei, H. Nanomaterials with enzyme-like characteristics (nanozymes): Next-generation artificial enzymes (II). *Chem. Soc. Rev.* **2019**, *48*, 1004–1076. [[CrossRef](#)]
4. Huang, Y.; Su, E.; Ren, J.; Qu, X. The recent biological applications of selenium-based nanomaterials. *Nano Today* **2021**, *38*, 101205. [[CrossRef](#)]
5. Jiang, D.; Ni, D.; Rosenkrans, Z.T.; Huang, P.; Yan, X.; Cai, W. Nanozyme: New horizons for responsive biomedical applications. *Chem. Soc. Rev.* **2019**, *48*, 3683–3704. [[CrossRef](#)] [[PubMed](#)]
6. Meng, X.; Zare, I.; Yan, X.; Fan, K. Protein-protected metal nanoclusters: An emerging ultra-small nanozyme. *WIREs Nanomed. Nanobiotechnol.* **2020**, *12*, e1602. [[CrossRef](#)]
7. Sun, H.; Zhou, Y.; Ren, J.; Qu, X. Carbon Nanozymes: Enzymatic Properties, Catalytic Mechanism, and Applications. *Angew. Chem. Int. Ed.* **2018**, *57*, 9224–9237. [[CrossRef](#)] [[PubMed](#)]
8. Huang, Y.; Ren, J.; Qu, X. Nanozymes: Classification, Catalytic Mechanisms, Activity Regulation, and Applications. *Chem. Rev.* **2019**, *119*, 4357–4412. [[CrossRef](#)] [[PubMed](#)]
9. Wang, Q.; Wei, H.; Zhang, Z.; Wang, E.; Dong, S. Nanozyme: An emerging alternative to natural enzyme for biosensing and immunoassay. *TrAC Trends Anal. Chem.* **2018**, *105*, 218–224. [[CrossRef](#)]
10. Dong, H.; Fan, Y.; Zhang, W.; Gu, N.; Zhang, Y. Catalytic Mechanisms of Nanozymes and Their Applications in Biomedicine. *Bioconjugate Chem.* **2019**, *30*, 1273–1296. [[CrossRef](#)]
11. Wang, H.; Wan, K.; Shi, X. Recent Advances in Nanozyme Research. *Adv. Mater.* **2019**, *31*, 1805368. [[CrossRef](#)]
12. Wang, Z.; Zhang, Y.; Ju, E.; Liu, Z.; Cao, F.; Chen, Z.; Ren, J.; Qu, X. Biomimetic nanoflowers by self-assembly of nanozymes to induce intracellular oxidative damage against hypoxic tumors. *Nat. Commun.* **2018**, *9*, 3334. [[CrossRef](#)] [[PubMed](#)]
13. Chen, Z.; Ji, H.; Liu, C.; Bing, W.; Wang, Z.; Qu, X. A Multinuclear Metal Complex Based DNase-Mimetic Artificial Enzyme: Matrix Cleavage for Combating Bacterial Biofilms. *Angew. Chem. Int. Ed.* **2016**, *55*, 10732–10736. [[CrossRef](#)]
14. Han, Q.; Lau, J.W.; Do, T.C.; Zhang, Z.; Xing, B. Near-Infrared Light Brightens Bacterial Disinfection: Recent Progress and Perspectives. *ACS Appl. Bio Mater.* **2020**, *4*, 3937–3961. [[CrossRef](#)]
15. Liu, Y.; Shi, L.; Su, L.; van der Mei, H.C.; Jutte, P.C.; Ren, Y.; Busscher, H.J. Nanotechnology-based antimicrobials and delivery systems for biofilm-infection control. *Chem. Soc. Rev.* **2019**, *48*, 428–446. [[CrossRef](#)] [[PubMed](#)]

16. Zhang, Z.; Han, Q.; Lau, J.W.; Xing, B. Lanthanide-Doped Upconversion Nanoparticles Meet the Needs for Cutting-Edge Bioapplications: Recent Progress and Perspectives. *ACS Mater. Lett.* **2020**, *2*, 1516–1531. [[CrossRef](#)]
17. Cao, F.; Zhang, L.; Wang, H.; You, Y.; Wang, Y.; Gao, N.; Ren, J.; Qu, X. Defect-Rich Adhesive Nanozymes as Efficient Antibiotics for Enhanced Bacterial Inhibition. *Angew. Chem. Int. Ed.* **2019**, *58*, 16236–16242. [[CrossRef](#)]
18. Gao, F.; Shao, T.; Yu, Y.; Xiong, Y.; Yang, L. Surface-bound reactive oxygen species generating nanozymes for selective antibacterial action. *Nat. Commun.* **2021**, *12*. [[CrossRef](#)] [[PubMed](#)]
19. He, X.; Tian, F.; Chang, J.; Bai, X.; Yuan, C.; Wang, C.; Neville, A. Haloperoxidase Mimicry by CeO<sub>2-x</sub> Nanorods of Different Aspect Ratios for Antibacterial Performance. *ACS Sustain. Chem. Eng.* **2020**, *8*, 6744–6752. [[CrossRef](#)]
20. Hu, M.; Korschelt, K.; Viel, M.; Wiesmann, N.; Kappl, M.; Brieger, J.; Landfester, K.; Therien-Aubin, H.; Tremel, W. Nanozymes in Nanofibrous Mats with Haloperoxidase-like Activity to Combat Biofouling. *ACS Appl. Mater. Interfaces* **2018**, *10*, 44722–44730. [[CrossRef](#)] [[PubMed](#)]
21. Liu, X.; Yan, Z.; Zhang, Y.; Liu, Z.; Sun, Y.; Ren, J.; Qu, X. Two-Dimensional Metal-Organic Framework/Enzyme Hybrid Nanocatalyst as a Benign and m Self-Activated Cascade Reagent for in Vivo Wound Healing. *ACS Nano* **2019**, *13*, 5222–5230. [[CrossRef](#)]
22. Liu, Z.; Zhao, X.; Yu, B.; Zhao, N.; Zhang, C.; Xu, F.-J. Rough Carbon-Iron Oxide Nanohybrids for Near-Infrared-II Light-Responsive Synergistic Antibacterial Therapy. *ACS Nano* **2021**, *15*, 7482–7490. [[CrossRef](#)]
23. Wu, R.; Chong, Y.; Fang, G.; Jiang, X.; Pan, Y.; Chen, C.; Yin, J.-J.; Ge, C. Synthesis of Pt Hollow Nanodendrites with Enhanced Peroxidase-Like Activity against Bacterial Infections: Implication for Wound Healing. *Adv. Funct. Mater.* **2018**, *28*. [[CrossRef](#)]
24. Xu, B.; Wang, H.; Wang, W.; Gao, L.; Li, S.; Pan, X.; Wang, H.; Yang, H.; Meng, X.; Wu, Q.; et al. A Single-Atom Nanozyme for Wound Disinfection Applications. *Angew. Chem. Int. Ed.* **2019**, *58*, 4911–4916. [[CrossRef](#)]
25. Sun, H.; Gao, N.; Dong, K.; Ren, J.; Qu, X. Graphene Quantum Dots-Band-Aids Used for Wound Disinfection. *ACS Nano* **2014**, *8*, 6202–6210. [[CrossRef](#)] [[PubMed](#)]
26. Shan, J.; Li, X.; Yang, K.; Xiu, W.; Wen, Q.; Zhang, Y.; Yuwen, L.; Weng, L.; Teng, Z.; Wang, L. Efficient Bacteria Killing by Cu<sub>2</sub>WS<sub>4</sub> Nanocrystals with Enzyme-like Properties and Bacteria-Binding Ability. *ACS Nano* **2019**, *13*, 13797–13808. [[CrossRef](#)]
27. Liang, X.; Xiao, J.; Chen, B.; Li, Y. Catalytically Stable and Active CeO<sub>2</sub> Mesoporous Spheres. *Inorg. Chem.* **2010**, *49*, 8188–8190. [[CrossRef](#)] [[PubMed](#)]
28. Wang, L.; Gao, F.; Wang, A.; Chen, X.; Li, H.; Zhang, X.; Zheng, H.; Ji, R.; Li, B.; Yu, X.; et al. Defect-Rich Adhesive Molybdenum Disulfide/rGO Vertical Heterostructures with Enhanced Nanozyme Activity for Smart Bacterial Killing Application. *Adv. Mater.* **2020**, *32*, 2005423. [[CrossRef](#)] [[PubMed](#)]
29. Yang, Y.; Wu, X.; Ma, L.; He, C.; Cao, S.; Long, Y.; Huang, J.; Rodriguez, R.D.; Cheng, C.; Zhao, C.; et al. Bioinspired Spiky Peroxidase-Mimics for Localized Bacterial Capture and Synergistic Catalytic Sterilization. *Adv. Mater.* **2021**, *33*, 2005477. [[CrossRef](#)]
30. Yim, G.; Kim, C.Y.; Kang, S.; Min, D.-H.; Kang, K.; Jang, H. Intrinsic Peroxidase-Mimicking Ir Nanoplates for Nanozymatic Anticancer and Antibacterial Treatment. *ACS Appl. Mater. Interfaces* **2020**, *12*, 41062–41070. [[CrossRef](#)] [[PubMed](#)]
31. Yu, Z.-H.; Li, X.; Xu, F.; Hu, X.-L.; Yan, J.; Kwon, N.; Chen, G.-R.; Tang, T.; Dong, X.; Mai, Y.; et al. A Supramolecular-Based Dual-Wavelength Phototherapeutic Agent with Broad-Spectrum Antimicrobial Activity Against Drug-Resistant Bacteria. *Angew. Chem. Int. Ed.* **2020**, *59*, 3658–3664. [[CrossRef](#)]
32. Zhang, L.; Zhang, L.; Deng, H.; Li, H.; Tang, W.; Guan, L.; Qiu, Y.; Donovan, M.J.; Chen, Z.; Tan, W. In vivo activation of pH-responsive oxidase-like graphitic nanozymes for selective killing of *Helicobacter pylori*. *Nat. Commun.* **2021**, *12*. [[CrossRef](#)]
33. Zhang, W.; Ren, X.; Shi, S.; Li, M.; Liu, L.; Han, X.; Zhu, W.; Yue, T.; Sun, J.; Wang, J. Ionic silver-infused peroxidase-like metal-organic frameworks as versatile "antibiotic" for enhanced bacterial elimination. *Nanoscale* **2020**, *12*, 16330–16338. [[CrossRef](#)]
34. Zhang, Y.; Li, D.; Tan, J.; Chang, Z.; Liu, X.; Ma, W.; Xu, Y. Near-Infrared Regulated Nanozymatic/Photothermal/Photodynamic Triple-Therapy for Combating Multidrug-Resistant Bacterial Infections via Oxygen-Vacancy Molybdenum Trioxide Nanodots. *Small* **2021**, *17*, 2005739. [[CrossRef](#)] [[PubMed](#)]
35. Liu, Z.; Wang, F.; Ren, J.; Qu, X. A series of MOF/Ce-based nanozymes with dual enzyme-like activity disrupting biofilms and hindering recolonization of bacteria. *Biomaterials* **2019**, *208*, 21–31. [[CrossRef](#)] [[PubMed](#)]
36. Zhao, Y.; Li, H.; Wang, Y.; Wang, Y.; Huang, Z.; Su, H.; Liu, J. CeO<sub>2</sub> Nanoparticle Transformation to Nanorods and Nanoflowers in Acids with Boosted Oxidative Catalytic Activity. *ACS Appl. Nano Mater.* **2021**, *4*, 2098–2107. [[CrossRef](#)]
37. Filippi, A.; Liu, F.; Wilson, J.; Lelieveld, S.; Korschelt, K.; Wang, T.; Wang, Y.; Reich, T.; Poeschl, U.; Tremel, W.; et al. Antioxidant activity of cerium dioxide nanoparticles and nanorods in scavenging hydroxyl radicals. *RSC Adv.* **2019**, *9*, 11077–11081. [[CrossRef](#)]