Intrinsic Enzyme-like Activities of Cerium Oxide Nanocomposite and Its Application for Extracellular H$_2$O$_2$ Detection Using an Electrochemical Microfluidic Device

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ABSTRACT: Artificial enzyme mimics have gained considerable attention for use in sensing applications due to their high stability and outstanding catalytic activity. We show that cerium oxide nanosheets (NSs) exhibit triple-enzyme mimetic activity. The oxidase-, peroxidase-, and catalase-like activities of the proposed nanoparticles are demonstrated using both colorimetric and electron paramagnetic resonance (EPR) spectroscopy. On the basis of the excellent catalytic activity of cerium oxide NSs toward hydrogen peroxide, an electrochemical approach for the high-throughput detection of H$_2$O$_2$ in living cells was established. This report presents an analytical microfluidic chip integrated with a cerium oxide NS mimic enzyme for the fabrication of a simple, sensitive, and low-cost electrochemical sensor. Three Au microelectrodes were fabricated on a glass substrate using photolithography, and the working electrode was functionalized using cerium oxide NSs. The operation of this biosensor is based on cerium oxide NSs and presents a high sensitivity over a wide detection range, between 100 nM and 20 mM, with a low detection limit of 20 nM and a high sensitivity threshold of 226.4 μA·cm$^{-2}$·μM$^{-1}$. This microfluidic sensor shows a strong response to H$_2$O$_2$, suggesting potential applications in monitoring H$_2$O$_2$ directly secreted from living cells. This sensor chip provides a promising platform for applications in the field of diagnostics and sensing.

1. INTRODUCTION

Since the first exciting discovery of ferromagnetic nanoparticles, various efficient nanomaterial-based mimic enzymes (nanozymes) have been developed over the past few decades. Such nanomaterials can catalyze specific redox-like type reactions and exhibit great activity for oxidase-like, peroxidase-like, catalase-like, or superoxide dismutase-like (SOD) reactions. To date, there have been numerous works devoted to exploring nanomaterials’ enzyme mimetics from carbon- and metal-based nanomaterials. Recently, metal nanomaterials have become an area of increasing interest because of unique electronic and a larger variety of enzyme-like characteristics. These nanozymes are widely used in sensing and diagnosis applications. For example, Wang et al. developed a direct electrochemical assay for kanamycin detection based on the peroxidase-like activity of gold nanoparticles. AuNPs could catalyze the reaction between H$_2$O$_2$ and reduced thionine to produce oxidized thionine. This reaction exhibited a distinct reduction peak on gold electrode in differential pulse voltammetry (DPV) and could be utilized to quantify the concentration of kanamycin. Furthermore, Wang and his colleagues fabricated FePt–Au ternary metallic nanoparticles with powerful enzymatic mimic for H$_2$O$_2$ sensing. Among nanozymes, multiactivity nanozymes with two or more catalytic activities have attracted significant attention. Some nanozymes such as Co$_3$O$_4$, Ni–Pd NPs, CoMo hybrids, and V$_2$O$_5$ have been reported with two or more catalytic activities. These kinds of nanozymes can have more effective applications in physiological and pathological processes.

Cerium oxide nanoparticles (nanoceria) have attracted enormous interest in recent years as nanocatalysts due to their unique physical and chemical properties. Nanoceria has been widely applied in various fields, such as catalysis, bioassays, and antioxidant therapy. This rare-earth oxide nanostructure shows high catalytic performance in various applications due to the presence of mixed valence states of Ce$^{3+}$ and Ce$^{4+}$, and the presence of oxygen vacancies. The key to this catalytic activity is that the redox couple can switch...
between each state in a CeO$_2$ ↔ CeO$_2$−x + x/2O$_2$ (Ce$^{4+}$ ↔ Ce$^{3+}$) recycle process$^{26,27}$. The catalytic activity of nanoceria originates from the surface oxygen; thus, the active oxygen content on the surface must be increased to improve catalytic activities. As a result, increasing the Ce$^{3+}$/Ce$^{3+}$ + Ce$^{4+}$ ratio (shorted as “Ce$^{3+}$ ratio”) enhances the surface oxygen defect in the structure, leading to improvement in catalytic properties$^{28,29}$.

It is worth noting that H$_2$O$_2$ has a considerable impact on food production, textile industry, paper bleaching, pharmaceutical research, and environment pollution$^{30,31}$. It is a byproduct of various enzymatic reactions including glucose oxidase, cholesterol oxidase, glutamate oxidase, urate oxidase, lactate oxidase, alcohol oxidase, lysine oxidase, oxalate oxidase, and horseradish peroxidase$^{32}$. In living organisms, H$_2$O$_2$ regulates diverse biological processes such as immune cell activation, vascular remodeling, apoptosis, and root growth. The presence of excess H$_2$O$_2$ in living organisms also causes severe diseases like cancer and Parkinson’s disease$^{33,34}$. The determination of H$_2$O$_2$ in biological environments is of critical importance. Electrochemical methods have attracted great interest over competing H$_2$O$_2$ detection techniques such as chromatography$^{35}$, chemiluminescence$^{36}$, colorimetry$^{37}$, and fluorescence$^{38}$, due to their high sensitivity, fast response, low cost, and convenient operation$^{39,40}$.

The microfluidic lab-on-a-chip (LOC) technology is recognized as one of the most promising tools to develop novel diagnostic platforms$^{41}$. Microfluidic chips can be applied as point-of-care (POC) devices for clinical diagnostics because of their inherent small size, portability, low cost, easy operation, and low amount of biological sample required$^{42,43}$. These devices include a set of microfluidic channels to control fluid flow throughout the chip, in which various procedures such as reagent mixing, affinity-based binding, and signal transduction can be implemented side-by-side$^{44}$. Sensors can be integrated within microfluidic devices to enable continuous monitoring of the target analyte$^{45}$. It is worth noting that H$_2$O$_2$ has a considerable impact on food production, textile industry, paper bleaching, pharmaceutical research, and environment pollution$^{30,31}$. It is a byproduct of various enzymatic reactions including glucose oxidase, cholesterol oxidase, glutamate oxidase, urate oxidase, lactate oxidase, alcohol oxidase, lysine oxidase, oxalate oxidase, and horseradish peroxidase$^{32}$. In living organisms, H$_2$O$_2$ regulates diverse biological processes such as immune cell activation, vascular remodeling, apoptosis, and root growth. The presence of excess H$_2$O$_2$ in living organisms also causes severe diseases like cancer and Parkinson’s disease$^{33,34}$. The determination of H$_2$O$_2$ in biological environments is of critical importance. Electrochemical methods have attracted great interest over competing H$_2$O$_2$ detection techniques such as chromatography$^{35}$, chemiluminescence$^{36}$, colorimetry$^{37}$, and fluorescence$^{38}$, due to their high sensitivity, fast response, low cost, and convenient operation$^{39,40}$.

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measurement of single or multiple analytes in small sample volumes.\textsuperscript{45}

In this work, we present a microfluidic electrochemical LOC for the real-time detection of H\textsubscript{2}O\textsubscript{2} using a cerium oxide nanosheet (NS)-modified Au working electrode. Cerium oxide NSs were synthesized via a simple hydrothermal route, and they simultaneously displayed oxidase-, peroxidase-, and catalase enzyme-like activities (Scheme 1). Cerium oxide NSs were integrated with a microfluidic platform for the effective detection of H\textsubscript{2}O\textsubscript{2}. This sensor is found to be highly selective and specific toward H\textsubscript{2}O\textsubscript{2} with negligible interference from analytes such as glucose, dopamine, uric acid, glutathione, and ascorbic acid. Furthermore, cerium oxide NS-based LOC devices can find practical use in monitoring H\textsubscript{2}O\textsubscript{2} inside living cells, which is indicative of their viability in real-world analysis applications.

2. RESULTS AND DISCUSSION

2.1. Structure Characterization of Prepared Cerium Oxide. The morphology of cerium oxide was investigated by transmission electron microscopy (TEM). Figure 1A shows the wrinkled nanosheet structure of the prepared cerium oxide, which shows a large surface area for the reaction with H\textsubscript{2}O\textsubscript{2}. The energy-dispersive spectrometry (EDS) analysis of cerium oxide NSs revealed their elemental composition and corroborated the presence of Ce, C, and O in these nanostructures (Figure 1B).

Figure 2A shows the X-ray diffraction (XRD) patterns of cerium oxide NSs. The XRD peaks are located at angles (2\theta) of 28.6, 32.84, 47.27, 55.83, 59.09, 69.02, 76.74, and 78.64\textdegree, corresponding to the (111), (200), (220), (311), (222), (400), (331), and (420) planes of CeO\textsubscript{2} in the face-centered cubic phase (JCPDS data card no: 34-0394).\textsuperscript{46} Peaks at angles 2\theta = 44.12 and 64.42\textdegree can be assigned to Ce\textsubscript{2}O\textsubscript{2}CO\textsubscript{3}\textsubscript{2}H\textsubscript{2}O.\textsuperscript{47} The formation of Ce\textsubscript{2}O\textsubscript{2}CO\textsubscript{3}\textsubscript{2}H\textsubscript{2}O is due to the reaction of the Ce\textsuperscript{3+} ions from cerium nitrate hexahydrate with the CO\textsubscript{3}\textsuperscript{2−} and OH\textsuperscript{−} ions from the hydrolysis of urea and terephthalic acid.\textsuperscript{48} Ce\textsubscript{3}O\textsubscript{2}CO\textsubscript{3}\textsubscript{2}H\textsubscript{2}O improves the Ce\textsuperscript{3+}/(Ce\textsuperscript{3+} + Ce\textsuperscript{4+}) ratio, which is representative of the surface Ce\textsuperscript{3+}/(Ce\textsuperscript{3+} + Ce\textsuperscript{4+}) ratio; and (3) minimum weight loss above 470 °C, which can be attributed to the removal of captured CO\textsubscript{2}.\textsuperscript{49} Figure 2B shows the Fourier transform infrared (FTIR) spectra of urea, terephthalic acid, and cerium oxide NSs. Urea has characteristic vibrational peaks at 3348 and 3443 cm\textsuperscript{-1} (NH\textsubscript{2} group stretching) and at 1680 cm\textsuperscript{-1} (−C−N group stretching) (curve a).\textsuperscript{50} Peaks from terephthalic acid, at 2542, 1682, and 1570−1424 cm\textsuperscript{-1}, are assigned to −COOH, −C==O, and an aromatic ring of the terephthalic acid, respectively. Concerning the IR spectrum of terephthalic acid (curve b) peaks in the 1285−1000 cm\textsuperscript{-1} region are fingerprints of −C==O, −C≡O, −C−CH, and −C−H bending modes, while the 700−800 cm\textsuperscript{-1} region contains the terephthalic acid aromatic ring bending mode.\textsuperscript{51} The IR spectrum of cerium oxide NSs (curve c) is different from its counterpart from reagents, in which most of the IR peaks are not present. Specifically, the broad IR absorption band 3422 cm\textsuperscript{-1} in the spectra of cerium oxide NSs is assigned to O−H stretching modes from residual water and Ce−OH, which is present in nanostructured cerium oxide because of its higher surface-to-volume ratio. Likewise, the same effect may be responsible for the absorption peak observed at 1019 cm\textsuperscript{-1}, which can be attributed to C−O−Ce and is also not present in bulk cerium oxide.

2.2. Triple-Enzyme Catalytic Activity of Cerium Oxide NSs. To investigate the triple-enzyme catalytic activity of cerium oxide NSs and their oxidase-, peroxidase-, and catalase-like activities, a series of experiments were carried out as indicated in the following subsections.

2.2.1. Peroxidase-like Catalytic Activity of Cerium Oxide NSs. The peroxidase-like catalytic activity of cerium oxide NSs was investigated through thermogravimetric analysis (TGA). As can be seen from Figure 2B, weight loss occurs by the following three steps: (1) release of physically adsorbed water, with 7.9% weight loss from room temperature to 160 °C; (2) decomposition of cerium carbonate to form ceria, with 12.0% weight loss from 160 to 470 °C, which is representative of the surface Ce\textsuperscript{3+}/(Ce\textsuperscript{3+} + Ce\textsuperscript{4+}) ratio; and (3) minimum weight loss above 470 °C, which can be attributed to the removal of captured CO\textsubscript{2}.\textsuperscript{49} Figure 2C shows the Fourier transform infrared (FTIR) spectra of urea, terephthalic acid, and cerium oxide NSs. Urea has characteristic vibrational peaks at 3348 and 3443 cm\textsuperscript{-1} (NH\textsubscript{2} group stretching) and at 1680 cm\textsuperscript{-1} (−C−N group stretching) (curve a).\textsuperscript{50} Peaks from terephthalic acid, at 2542, 1682, and 1570−1424 cm\textsuperscript{-1}, are assigned to −COOH, −C==O, and an aromatic ring of the terephthalic acid, respectively. Concerning the IR spectrum of terephthalic acid (curve b) peaks in the 1285−1000 cm\textsuperscript{-1} region are fingerprints of −C==O, −C≡O, −C−CH, and −C−H bending modes, while the 700−800 cm\textsuperscript{-1} region contains the terephthalic acid aromatic ring bending mode.\textsuperscript{51} The IR spectrum of cerium oxide NSs (curve c) is different from its counterpart from reagents, in which most of the IR peaks are not present. Specifically, the broad IR absorption band 3422 cm\textsuperscript{-1} in the spectra of cerium oxide NSs is assigned to O−H stretching modes from residual water and Ce−OH, which is present in nanostructured cerium oxide because of its higher surface-to-volume ratio. Likewise, the same effect may be responsible for the absorption peak observed at 1019 cm\textsuperscript{-1}, which can be attributed to C−O−Ce and is also not present in bulk cerium oxide.
oxide NSs to an aqueous solution of TMB + H₂O₂, an additional strong adsorption peak at 652 nm is observed, and the color of the solution turned blue (Figure 3A, curve b). After adding H₂SO₄, the adsorption peak at 652 nm disappears with the appearance of a peak at 450 nm and the color of the solution changed from blue to yellow (Figure 3A, curve c).

The oxidation reaction was catalyzed by peroxidase, but the catalytic activity of cerium oxide could be stopped by H₂SO₄, a parametric study was performed, as demonstrated in Figure 4. The EPR spectrum of DMPO−OH corresponds to line a in Figure 5A. The signal increases at decreasing pH, with increasing amounts of DMPO−•OH adducts being observed at lower pH when the reaction was performed in the presence of a constant amount of cerium oxide NSs. Conversely, no pH dependence of the EPR signal of DMPO was observed when the reaction was performed in the absence of the nanocomposite (Figure 5A, line a), which thus acts as a catalyst for the H₂O₂ decomposition. This model is in agreement with increasing enzyme activity at lower pH because more •OH radicals are produced under acidic conditions. These results confirmed the production of •OH radicals catalyzed by cerium oxide NSs. Furthermore, not only the concentration of •OH radicals but also the concentration of the cerium oxide NS catalyst is affected by the pH of the solution. The g-values were 2.00553, 2.00548, and 2.00557 at pH values of 11, 7, and 3, respectively, which are close to the values reported in previous studies. The EPR spectrum of DMPO−OH was contaminated by a triplet signal due to the nitrosyl adduct of DMPO (DMPO−NO) reported in previous studies. The EPR spectrum of DMPO−•OH is unstable (lifetime is 30–90 s) and it spontaneously decays into the DMPO−OOH adduct. Furthermore, the reaction rates of DMPO with •O₂− and •O₂H are extremely smaller compared to that with OH radical. The rate constants of DMPO with •O₂− and •O₂H are 2−170 and 6.6 × 10⁹ M⁻¹s⁻¹, respectively, while that with •OH is reported to be 1.9−4.3 × 10⁸ M⁻¹s⁻¹. Therefore, the detection of •O₂− with DMPO is not feasible. Figure 5B shows that when the nanoparticle concentration is varied from 0 (line a) to 12 mg·mL⁻¹ (line d), the catalytic activity also increases, consistent with an increase of the •OH signal up to 8 mg·mL⁻¹ cerium oxide (line c) NP and a saturation of the
effect at higher concentrations. The variation of H$_2$O$_2$ concentration from 0% (Figure 5C, line a) to 12% (line d) was also observed to improve the process effectiveness through the addition of more reagents, with an increase in the EPR signal intensity, indicating the generation of more DMPO−•OH(aq) adducts.

2.2.2. Oxidase-like Catalytic Activity of Cerium Oxide NSs.

The prepared cerium oxide NSs could directly oxidize TMB, leading to blue color products even in the absence of H$_2$O$_2$ (Figure 3B). This indicates that cerium oxide NSs also exhibit oxidase-like catalytic activity. To further study the oxidation of the TMB chromogenic substrate by cerium oxide NSs, the effect of the oxidizing agent (dissolved oxygen) in the reaction system was investigated. Compared to bubbling an inert gas into the system of N$_2$, the absorbance of oxidized TMB at 652 nm was significantly increased after saturation with O$_2$. It is therefore concluded that increasing the concentration of oxygen as the electron acceptor in the oxidation of TMB can enhance the oxidase-like activity of cerium oxide NSs.

2.2.3. Catalase-like Catalytic Activity of Cerium Oxide NSs.

To investigate the catalase-like activity of cerium oxide NSs, the concentration of dissolved oxygen in the system, consisting of cerium oxide NSs and H$_2$O$_2$, was recorded using a portable meter. The value of the dissolved oxygen concentration was monitored for 10 min as a function of cerium oxide NS concentration. As shown in Figure 3C, the concentration of dissolved oxygen increased proportionally to the concentration. This indicated that cerium oxide NSs can decompose H$_2$O$_2$ into O$_2$ and provided strong evidence of the catalase-like activity. The production of O$_2$ was also monitored by EPR by incorporating a spin probe (15N-PDT) in the system. The bimolecular combination of paramagnetic O$_2$ and N-PDT results in shorter spin−spin relaxation times, broadening the EPR line widths with respect to the pristine EPR signal of the pure spin probe. As shown in Figure 6A, where the pH increased to 11 in the presence of cerium oxide NSs and H$_2$O$_2$, the EPR signal line width increased as well, with a consistent signal intensity decrease to account for a constant N-PDT concentration. The EPR line width also broadens with
increasing cerium oxide NS concentration (Figure 6B). This also indicates increased oxygen formation due to the stronger catalase-like activity of more concentrated cerium oxide NSs.

### 2.3. Electrocatalytic Activity of Cerium Oxide NSs

The electrocatalytic activity of cerium oxide NSs was tested by modifying the Au working electrode with cerium oxide NSs. A three-electrode microfluidic chip is used as the electrochemical cell for recording cyclic voltammograms (CV) and determining sensor performance. Cyclic voltammograms (CVs) were recorded for (a) bare Au electrode, (b) cerium oxide NS-modified Au electrode, (c) Au electrode + H₂O₂, and (d) cerium oxide NS-modified Au electrode + H₂O₂ in 0.1 M phosphate-buffered saline (PBS) (pH = 7.4) with a scan rate of 50 mV s⁻¹ (Figure 7A). The bare Au electrode and cerium oxide NS-modified Au electrode did not show any voltammetric response (curves a and b), and upon addition of H₂O₂, a redox process was detected on the Au bare electrode, indicating reduction of H₂O₂ (curve c). However, a large enhancement in the H₂O₂ redox response was observed for the nanoparticle-modified Au electrode (curve d). The oxidation and reduction responses of the electrode-modified cerium oxide NSs when reacting with H₂O₂ clearly show the catalase and peroxidase activities of cerium oxide NSs. The cerium oxide/Au electrode therefore can act as a mimetic catalase, where it significantly electrocatalyzes the decomposition of hydrogen peroxide (H₂O₂) to water (H₂O) and molecular oxygen (O₂) and also acts as a mimetic peroxidase to generate OH⁻ radicals via decomposition of H₂O₂.³⁰,⁶²

### 2.4. Investigation of the Effect of pH on Cerium Oxide/Au Electrode Response

To understand the effect of pH on the electrochemical properties of the cerium oxide/Au electrode in the presence of H₂O₂, electrocatalytic studies were performed at three different pH values: 5.0, 7.0, and 9.0 in the presence of H₂O₂ (10 mM) using a N₂-purged PBS (0.1 M) with a scan rate of 50 mV s⁻¹, and the results are shown in Figure 7B. At pH = 5.0, both oxidation and reduction were observed; however, the cathodic peak current is higher than the anodic peak, indicating that the peroxidase activity is dominant in cerium oxide NSs at lower pH (curve a). By increasing the buffer solution pH, the cathodic peak current decreases while the anodic peak current is increased relative to curve a, showing dominant catalase activity at higher pH values (curves b and c). These results demonstrate the pH switchability of the catalytic properties of cerium oxide NSs: at acidic pH (pH < 7), the peroxidase catalytic activity is dominant, while at basic pH (pH > 7), the catalase activity is dominant.

### 2.5. Analytical Performance of Cerium Oxide NS-Modified Au Electrode for Detection of H₂O₂

Figure 7C shows the CVs of cerium oxide NS-modified Au electrode in the presence of different concentrations of H₂O₂. As can be seen, with increasing H₂O₂ concentration, the reduction current increased, demonstrating the excellent catalytic activity of cerium oxide NSs toward the reduction of H₂O₂. Thereupon, the detection sensitivity of cerium oxide NS-modified electrodes to aqueous H₂O₂ was explored through a chronocoulometric study shown in Figure 8. The response for the different amounts of H₂O₂ is shown by the i-t curves collected at −0.5 V in Figure 8A, which indicates that the reduction currents increase gradually with higher concentrations of H₂O₂. The calibration plot indicates good linearity for the reduction current versus H₂O₂ concentrations in the range from 100 to 20 mM (Figure 8A). The linear regression equation generated for cerium oxide NS-modified electrodes was $i (\mu A) = 1.03 \log C (\mu M) + 1.72 \mu A$ with a correlation coefficient of $R^2 = 0.992$ (Figure 8B). The lowest concentration of H₂O₂ that could be estimated by this microfluidic electrochemical sensor was 20 nM (S/N = 3), and the sensitivity was calculated to be 226.4 μA·cm⁻²·μM⁻¹ based on this result. Compared to previously reported H₂O₂ sensors based on other nanomaterials or enzymes, this detection method based on microfluidic device outperforms the sensitivity and detection limit of other sensors, as shown in Table 1. The stability of the microfluidic device was examined after 2 weeks, with the result showing that the current through the device with 1 mM H₂O₂ exhibited only a small deviation over time with a relative standard deviation (RSD) of 3.04%. Six repeated measurements of 1 mM H₂O₂ led to a relative standard deviation of 2.9%, further showing good reproducibility of the biosensing interface. The selectivity of cerium oxide/Au electrode for H₂O₂ detection in PBS was also studied by evaluation of the interference effect of coexisting
compounds such as ascorbic acid (AA), dopamine (DA), uric acid (UA) glutathione (GSH), and glucose on the electrode response. As illustrated in Figure 8C, there are negligible current responses when interfering molecules were used, confirming the good anti-interference ability of the biomimetic sensor for H\textsubscript{2}O\textsubscript{2} detection.

2.6. Real-Time Detection of H\textsubscript{2}O\textsubscript{2} Released from Living Cells. To investigate the capability of the proposed system for real-time detection of H\textsubscript{2}O\textsubscript{2}, we chose the PC 12 cell as a model because it can release a trace amount of H\textsubscript{2}O\textsubscript{2} under the stimulation of ascorbic acid (AA).\textsuperscript{63} The as-prepared 10\textsuperscript{6} cells were suspended in 500 μL of PBS (pH = 7.4) for further use. As shown in Figure 8D, in the presence of PC 12 cells, the cathodic current increases to a higher platform after the addition of 4 μM AA (curve c), which corresponds to about 0.1 μM H\textsubscript{2}O\textsubscript{2} released from the living cells, confirming that the trace amounts of H\textsubscript{2}O\textsubscript{2} released from a living cell can be detected rapidly by the cerium oxide NSs. However, no changes in current are observed in the absence of either cells (curve b) or AA (curve a) under the same conditions, indicating that H\textsubscript{2}O\textsubscript{2} is released from cells under the stimulation of AA. These results suggest that the fabricated microfluidic device is highly sensitive and reliable for the detection of H\textsubscript{2}O\textsubscript{2} in living cells. So, compared to the advantages such as high activity, low detection limit, wide concentration range, and applicability of the presented sensor for measuring H\textsubscript{2}O\textsubscript{2} release from cells, the limitation of the purposed sensor is negligible.

Figure 5. Experimental EPR spectra recorded at room temperature after the reaction of cerium oxide NSs with H\textsubscript{2}O\textsubscript{2} in the presence of DMPO spin trap. (The nitroxide degradation product of the spin trap is indicated by black dots.) EPR spectrum of the liquid phase separated from the (A) cerium oxide NSs at varying pH: (a) control (without cerium oxide NSs), (b) pH = 11, (c) pH = 7.0, and (d) pH = 3; (B) various cerium oxide NS concentrations: (a) 0 mg·mL\textsuperscript{-1}, (b) 4 mg·mL\textsuperscript{-1}, (c) 8 mg·mL\textsuperscript{-1}, and (d) 12 mg·mL\textsuperscript{-1}; and (C) various H\textsubscript{2}O\textsubscript{2} concentrations: (a) 0%, (b) 4%, (c) 8%, and (d) 12%.

Figure 6. EPR spectra of \textsuperscript{15}N-PDT (A) in the presence of 2 mg of cerium oxide NSs and H\textsubscript{2}O\textsubscript{2} (5%) at different pH values, and (B) in the presence of H\textsubscript{2}O\textsubscript{2} (5%) and different concentration of cerium oxide NSs.
3. CONCLUSIONS

In summary, cerium oxide NSs were synthesized by a facile hydrothermal route and exhibited triple-enzyme mimetic activity: oxidase-, peroxidase-, and catalase-like activities. The enzyme mimic properties of cerium oxide NSs can be modulated by adjusting the pH. The peroxidase-like activity is predominant under acidic pH, while the catalase-like activity is prevalent under alkaline conditions. The underlying mechanisms of the catalytic processes involving cerium oxide NSs were investigated by means of EPR spectroscopy, which revealed that the peroxidase-like activity originates from the ability to produce hydroxyl (•OH) radicals. The catalase-like activity causes the decomposition of H₂O₂ to O₂. The as-prepared nanocomposite was used for the electrochemical detection of H₂O₂. The linear range of this method was found to be between 100 nM and 20 mM, with a detection limit of 20 nM. The methods we developed have decisive advantages in terms of wide linear range, low detection limit, high sensitivity, easiness of operation, and good practicability. The developed methods were applied to the detection of H₂O₂ in living cells, which may be competitive with existing methods because of their low cost, simplicity, and reproducibility. We believe that our microfluidic sensors, along with electrochemical detection, may contribute to the growth of biosensing technologies toward practical applications in bioanalysis, food safety, and environmental diagnostics.

4. EXPERIMENTAL SECTION

4.1. Materials and Instruments. Cerium nitrate hexahydrate Ce(NO₃)₃·6H₂O, terephthalic acid C₆H₄(CO₂H)₂, urea (CO(NH₂)₂), H₂O₂, 3,3′,5,5-tetramethylbenzidine (TMB), glucose, dopamine, uric acid, glutathione, and ascorbic acid and all other reagents were purchased from Merck or Fluka. All chemicals and reagents were of analytical grade and directly used without further purification. Deionized water produced from a Milli-Q Plus system (Millipore) was used in all experiments.

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images were obtained with a MIRA3 TESCAN HV:20.0 kV instrument and a Philips EM280 microscope, respectively. XRD patterns were recorded on a Bruker D8 Advance diffractometer equipped with a copper source and a general area detector diffraction system (GADDS). The FTIR and UV−vis spectra were recorded by a Vector-22 Bruker spectrophotometer and a SPECTROD 250-Analytik Jena spectrophotometer, respectively. Dissolved oxygen was monitored after the addition of H₂O₂ by a handheld meter Oxi 330i/340i (WTW GmbH & Co. KG).

Electron paramagnetic resonance measurements were performed using a Jeol FA-200 EPR spectrometer operating in the X-band at 9.1 MHz and equipped with a cylindrical resonator. The hydroxyl radicals generated in the liquid phase were detected by applying the spin trapping technique. 5,5-Dimethyl-1-pyrroline N-oxide (DMPO) with a concentration of 20 mM was chosen as a suitable spin trap because of its high trapping ability and selectivity toward oxygen-centered radicals. In the spin trapping experiments, a predetermined amount of cerium oxide NSs was contacted with a mixture of 1 mL of H₂O₂/H₂O and 10 μL of DMPO. The liquid sample (5 μL) was inserted into a quartz capillary tube with 1.0 mm inner diameter using a micropipette. The filled capillary was then sealed with parafilm and placed in a quartz glass EPR tube of 5 mm inner diameter (Wilmad LabGlass, 710-SQ-250M) and inserted in the microwave cavity, with all measurements recorded at room temperature unless otherwise stated.56

![Figure 7](https://dx.doi.org/10.1021/acsomega.9b03252)
4.2. Synthesis of Cerium Oxide NSs. Cerium oxide NSs were prepared by a wet-chemical deposition precipitation method. Briefly, 0.25 g of Ce(NO$_3$)$_3$·6H$_2$O was dissolved in 10 mL of distilled water. Subsequently, 0.02 g of urea was added to the solution under vigorous stirring. Then, 10 mL of terephthalic acid solution (60 μM) was added to the reaction solution. After stirring for 15 min, the precipitate product was transferred to a 40 mL Teflon-lined stainless steel autoclave and kept in an electric oven at 150 °C for 6 h. The autoclave was then taken out from the oven and left to cool to room temperature. The produced precipitate was collected via centrifugation, washed thoroughly with water and ethanol, and dried at 60 °C overnight.

4.3. Fabrication of Electrochemical Microfluidic Devices. All experiments were carried out in microfluidic chips made of polydimethylsiloxane (PDMS). The fabrication process can be broken down into three major steps: (1) fabrication of a three-electrode setup, (2) casting of PDMS, and (3) plasma bonding of PDMS over the prepared electrodes on the glass substrate. The electrochemical cell for detection comprised a set of three electrodes: a counter electrode (CE), a working electrode (WE), and a reference electrode (RE). The WE was a gold electrode modified with cerium oxide NSs. The CE was a platinum electrode, and the RE was a saturated calomel electrode (SCE).

![Figure 8](https://dx.doi.org/10.1021/acsomega.9b03252)

**Table 1. Comparison of the Performance of Various Hydrogen Peroxide Sensors**

| Electrode Materials         | Linear Range (μM) | Detection Limit (μM) | Refs |
|-----------------------------|-------------------|----------------------|------|
| MnO$_2$ nanosheets          | up to 454         | 0.005                | 63   |
| Graphene/Pt nanocomposite   | 0.5–3475          | 0.2                  | 64   |
| Se/Pt nanocomposites        | 10–15 000         | 3.1                  | 65   |
| RGO–Au–PTBO                 | 5.0–25 362        | 0.2                  | 66   |
| rGO@CeO$_2$–AgNPs           | 0.3–12 000        | 0.21                 | 67   |
| TiO$_2$@Cu$_2$O             | 1–15 mM           | 0.15                 | 68   |
| Au/GS/HRP/CS                | 5–5130            | 1.7                  | 69   |
| Cerium oxide NSs            | 0.1–20 000        | 0.01                 | this work |

Figure 8. (A) Chronoamperometric responses of Au electrode/cerium oxide upon addition of different H$_2$O$_2$ concentrations. Applied potential: −0.5 V. (B) Logarithmic relationship between the concentrations of H$_2$O$_2$ (0.1, 0.5, 10, 100, 1000, 2000, and 20 000 μM). (C) Interference studies of cerium oxide-based lab-on-a-chip device on addition of 1 mM UA, DA, AA, GLU, GSH and 0.2 mM H$_2$O$_2$. (D) Chronoamperometric responses of Au electrode/cerium oxide for the reduction of H$_2$O$_2$ released from 10^6 PC 12 cells in 1 mL of 1 × PBS (pH = 7.4): (a) PC12 cells, (b) AA (4 μM), and (c) PC12 cells upon injection of 4 μM AA.
were subjected to baseline correction before further analysis. Potential of cultured in Dulbecco with sterile buffer, followed by suspension in fresh DMEM.

Upon the addition of AA (4 μM), the chronoamperometric current response of hydrogen peroxide was performed at a scanning rate of 50 mV s⁻¹. Chronoamperometry was performed at a constant applied potential of −0.5 V, and the resulting chronoamperograms were subjected to baseline correction before further analysis.

5.4. Detection of H₂O₂ in Real Sample. PC 12 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) solution containing 1% penicillin, 1% streptomycin, and 10% fetal bovine serum in a humidified atmosphere of 5% CO₂ for 24 h at 37 °C in culture dishes. Then, the cells were removed from the Petri dish by trypsinization and washed three times with sterile buffer, followed by suspension in fresh DMEM. Upon the addition of AA (4 μM), the chronoamperometric current response flux of H₂O₂ in about 10⁶ cells was recorded.

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Notes
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