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Novel hierarchical NiO nanoflowers exhibiting intrinsic superoxide dismutase-like activity

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Experimental details

1. Synthesis of NiO nanomaterials:

NiO nanoflowers (NiO NFs): 1 mmol Ni(NO₃)· $6H_2O$ and 1 mmol urea were dissolved in 40 mL ethanol and stirred for 10 min. The solution was transferred into a Teflon-lined stainless steel autoclave and then heated at 120 °C for 6 h. The synthesized products were obtained by centrifugation at 8000 rpm for 10 min, washed with water and ethanol several times, and dried at 60 °C overnight. Finally, the NiO NFs were obtained by calcination in air at 300 °C for 2 h under ambient conditions.

NiO hexagonal nanoplates (NiO NPs): The NiO NPs were synthesized by the reported method.¹ 0.4 M NaOH solution was heated at 60 °C, and 0.6 M Ni(NO₃)₂ solution was added drop wise to the above solution under stirring. The resulted mixture was stirred again for 2 h. Subsequently, the precipitate Ni(OH)₂ was separated, and washed with water and ethanol for several times. Then, it was dried at 60 °C, and calcinated in 350 °C for 2 h for the synthesis of NiO NPs.

NiO nanorods (NiO NRs): The NiO NRs were prepared with the reported procedure.² Firstly, Ni foam was cleaned with HCl solution (37%) for 5 min with ultrasound, and washed with water. $1.45 \text{ g Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and 0.6 g urea were dissolved in 35 ml water to form a solution. Then, the solution and cleaned NiO foam were transferred to a 40 mL Teflon-lined stainless-steel autoclave, sealed, and maintained at 100 °C for 12 h. The NiO foam was washed several times with distilled water and ethanol. Finally the sample was annealed at 300 °C for 3 h in an N₂ atmosphere.

2. Characterization: X-ray diffraction (XRD) patterns were obtained by a D8 ADVANCE X-ray diffractometer (Bruker, Germany) using Cu K α radiation ($\lambda = 1.5418$ A). The morphology of the synthesized products were measured by a SU-8000 scanning electron microscopy (Hitachi, Japan) and a Tecnai G2 F30 transmission electron microscope (FEI, USA). N₂ adsorption–desorption isotherms were performed on an ASAP 2020 Physisorption Analyzer (Micromeritics, USA), and the specific surface areas were obtained by the Brunauer–Emmett–Teller (BET) method. X-ray photoelectron spectroscopy (XPS) measurement was carried out on an AXIS Ultra DLD X-ray photoelectron spectroscopy (Shimadzu, Japan).

3. Scavenging of O_2^{-r} by NiO NFs: O_2^{-r} was relatively stable in aqueous condition with high pH. Efforts of investigating the decomposition of KO₂ at neutral condition was not realized in the present study, because complete self-dismutation of O_2^{-r} occur during the time of KO₂ addition at neutral condition. Therefore, to achieve sufficient and precise concentrations of O_2^{-r} in aqueous solution, the catalytic decomposition of O_2^{-r} by NiO NFs are carried out in 50 mM NaOH buffers (~pH 13). O_2^{-r} could be generated from KO₂ in crown ether/DMSO, and an amount of KO₂ in DMSO was then dissolved in aqueous buffers. 50 mM KO₂ stock solution was prepared as follows: 35.6 mg KO₂ and 300 mg 18-crown-6 were dissolved in 10 mL DMSO (extra dry solvent), and stirred for about 30 min until a clear pale yellow solution was obtained. The scavenging of O_2^{-r} by NiO NFs was examined in 3 mL 50 mM NaOH buffer containing 1 µg mL⁻¹ NiO NFs in the presence of O_2^{-r} (40 µL 50 mM KO₂). The above solution was monitored in time-drive mode at 250 nm using a Cary 300 UV-Vis spectrophotometer (Varian, USA). And the variation of O_2^{-r} concentration was calculated by its molar absorption coefficient ($\varepsilon_{250} = 2000 \text{ M}^{-1} \text{ cm}^{-1}$).

4. Detection of O₂: Generation of O₂ during the decomposition of O₂⁻⁻ by NiO NFs was assayed with a NeoFox Sport oxygen sensing system (Ocean Optics, USA). The reaction mixture (3.0 mL) contained 50 mM Na₂HPO₄ buffer, 1 mM KO₂, and 100 μ g mL⁻¹ NiO NFs. The reaction was started by addition of KO₂.

5. Detection of H_2O_2 : The reaction mixture (3.0 mL) containing 50 mM NaOH buffer and 1 mM KO₂ in absence and presence of 10 µg mL⁻¹ NiO NFs was firstly incubated for 3 min or 8 min. And 2 mL of the above mixture was added to 2 mL pH 6.0 NaH₂PO₄-Na₂HPO₄ buffer containing 0.75 mM 3,3',5,5'- tetramethylbenzidine (TMB) and 1 U/mL horseradish peroxidase (HRP). Finally, the catalytic reaction of TMB with hydrogen peroxide by HRP was measured using UV-visible spectrophotometer in time-drive mode at 652 nm and scanning mode.

6. Catalytic activity with the method of pyrogallol autoxidation: The product of pyrogallol autoxidation has the absorption peak at 320 nm. 6 mM pyrogallol stock solution was prepared in 10 mM HCl solution. And the reaction systems (3 mL) containing 0.2 mM pyrogallol (100 μ L 6 mM pyrogallol) and different concentration of NiO NFs or NiO NPs in 100 mM pH 8.2 Tris-HCl buffer were measured by UV-Vis spectrophotometer in time-drive mode at 320 nm. Using the different concentration of SOD (\geq 3000 units/mg from bovine erythrocytes, purchased from sigma), the measurement of SOD was same to that of NiO NFs.

7. Steady-state kinetic of NiO NFs: The catalyzed decomposition of O_2^{\bullet} by NiO NFs was examined in 3 mL 50 mM NaOH buffer containing 8 µg mL⁻¹ NiO NFs with different concentration of O_2^{\bullet} . These reaction mixtures were monitored by UV-Vis spectrophotometer in time-drive mode at 250 nm. The velocity was calculated from the initial straight sections of these kinetic curves and then fitted to the Michaelis–Menten model:

$$v = \frac{V_{max} \cdot [S]}{K_m + [S]}$$
[1]

where *v* is the initial velocity, V_{max} is the maximal reaction velocity, [S] is the concentration of substrate O_2^{\bullet} and K_m is the Michaelis constant. The V_{max} and K_m were obtained by the Lineweaver–Burk double reciprocal plot:

$$\frac{1}{v} = \frac{k_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}} \quad [2]$$

A nanoflower of NiO NFs was defined as an enzyme molecule,³ and the mole concentration of NiO NFs could be obtained by their molecular weight and mass concentration. The thickness of nanoflakes was ~7 nm and the distance between the nanoflakes was ~350 nm, therefore 1/50 volume of a nanoflower contained NiO. The molecular weight of NiO NFs was 6.04×10^{12} g mol⁻¹. 8 µg mL⁻¹ NiO NFs was calculated as 1.33×10^{-15} M. Turnover number (TON or K_{cat}) = $V_{max}/[E]$, where [E] is the mole concentration of NiO NFs (1.33×10^{-15} M).

8. Electrochemical studies: The platinum disk working electrode (CHI102, 2.0 mm in diameter) were firstly polished with 0.3 and 0.05 mm alumina slurry, and then cleaned ultrasonically in nitric acid, ethanol and distilled water successively. NiO NFs were dispersed into distilled water by ultrasonic dispersion to obtain the suspension solution (3 mg mL⁻¹). And 5 μ L suspension was then dropped on the pretreated CHI102 electrode and allowed to dry at 70 °C. Then, 2 μ L nafion solution (0.5 wt%) was dropped on the layer of NiO NFs and dried at 70 °C. Cyclic voltammetry (CV) measurements were performed on Epsilon Electrochemical Workstation (BAS, USA). A three-electrode system comprising a platinum wire counter electrode as auxiliary, a Ag/AgCl electrode as reference and the NiO NFs-modified Pt electrode as working electrodes was used for the above electrochemical experiments.

Electrochemical impedance spectroscopy (EIS) was measured using an Autolab (Netherlands) in 0.1 M KCl solution containing 5 mM K₃[Fe(CN)₆] which served as a redox probe, in the frequency range from 100 KHz to 0.1 Hz at an open circuit potential, with the amplitude of sine voltage of 10mV. 8 μ L NiO NFs suspension (3 mg mL⁻¹) or 8 μ L SOD solution (0.33 mg mL⁻¹) was then dropped on the pretreated glass carbon electrode and allowed to dry to obtain the modified electrode. A three-electrode system comprising a

platinum wire counter electrode as auxiliary, a Ag/AgCl electrode as reference and the NiO NFs or SODmodified glass carbon electrode as working electrodes was used for the ESI experiments.

9. Recyclability of NiO NFs: The SOD-like activity of NiO NFs was carried out in 1 M NaOH buffer with addition of 10 μ g mL⁻¹ NiO NFs and 0.33 mM O₂⁻⁻. After finishing the catalytic reaction, the NiO NFs were separated by centrifugation, washed by water and ethanol for three times, and dried at 70 °C for 2 h. Then, the obtained NiO NFs was used for the next catalytic reaction with the same procedures as before.



Fig. S1 XRD patterns of NiO NFs before (a) and after (b) scavenging the O_2 .



Fig. S2 XPS spectra of the Ni 2p for NiO NFs before (a) and after (b) scavenging the O_2 .



Fig. S3 The N₂ adsorption-desorption isotherms (a) and pore size distribution (b) of the NiO NFs.



Fig. S4 The generation of H_2O_2 in presence and absence of NiO NFs. a, the UV-Vis spectra of different reaction systems. b, the catalytic kinetic curves of different reaction systems.



Fig. S5 The catalytic activity of SOD with the method of pyrogallol autoxidation without and with different concentrations of SOD (a) and IC_{50} curve of SOD using the percentage of inhibition of pyrogallol autoxidation (b). The IC_{50} value of SOD was about 1.5 µg mL⁻¹.



Fig. S6 TEM of NiO NPs (a and b), SEM images of NiO NRs (c), XRD patterns of the NiO NPs and NiO NRs (d). 1, NiO NPs; 2, NiO NRs.



Fig. S7 N_2 adsorption-desorption isotherms of the NiO NPs (1) and NiO NRs (2).



Fig. S8 The SOD-like activity of NiO NPs (a) and NiO NRs (b), IC₅₀ curves of NiO NPs (c) and NiO NRs (d) with the method of pyrogallol autoxidation.



Fig. S9 Steady-state kinetic analysis of NiO NFs using Michaelis–Menten (a) and Linewaver–Burk models (b).

Catalysts	K _m /mM	$V_{\rm max}/{ m mM~s^{-1}}$	TON/s ⁻¹
NiO NFs	0.043	0.035	2.6×10 ¹⁰
PEG-HCCs ⁴	0.750	0.088	1.97×10 ⁵
SOD ⁵	0.080	_	2.6×10 ⁴

Table S1 Kinetic parameters of NiO NFs and other catalysts. Turnover number (TON or K_{cat})= $V_{max}/[E]$, where [E] is the mole concentration of NiO NFs (1.33×10⁻¹⁵ M).



Fig. S10 Cyclic voltammogram of NiO NFs. a, bare electrode. b, NiO NFs modified electrode. 5 mV/s.



Fig. S11 Electrochemical impedance spectroscopy of bared GCE, SOD and NiO NFs modified GCE. The insert shows the equivalent circuit. R_s: electrolyte resistance between working and reference electrode, R_{ct}: charge-transfer resistance, Z_w: Warburg impedance, CPE: constant phase element representing the double-layer capacitance because of the non-ideal behavior of the double layer.

Electrode	R_s/Ω	$R_{ct}/k\Omega$	$W/\mu\Omega$	CPE/µF
BGC	96.2	9.79	52.9	1.25
SOD-modified	100	1.09	295	3.76
NiO NFs-modified	102	1.46	467	40.3

Table S2 The fitted equivalent circuit elements for bare glassy carbon and the different materials modified electrodes.



Fig. S12 Catalytic curves (a) and change of activity (b) of NiO NFs after recycling.

References

- H. Karimi-Maleh, A. L. Sanati, V. K. Gupta, M. Yoosefian, M. Asif and A. Bahari, *Sens. Actuators,* B, 2014, 204, 647-654.
- 2. Z. Lu, Z. Chang, J. Liu and X. Sun, *Nano Res.*, 2011, 4, 658-665.
- L. Gao, J. Zhuang, L. Nie, J. Zhang, Y. Zhang, N. Gu, T. Wang, J. Feng, D. Yang, S. Perrett and X. Yan, *Nat. Nanotechnol.*, 2007, 2, 577-583.
- E. L. G. Samuel, D. C. Marcano, V. Berka, B. R. Bitner, G. Wu, A. Potter, R. H. Fabian, R. G. Pautler,
 T. A. Kent, A.-L. Tsai and J. M. Tour, *Proc. Natl. Acad. Sci. U. S. A.*, 2015, 112, 2343-2348.
- 5. C. Bull and J. A. Fee, J. Am. Chem. Soc., 1985, 107, 3295-3304.