Rhodium Nanoparticle-mesoporous Silicon Nanowire Nanohybrids for Hydrogen Peroxide Detection with High Selectivity

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Developing nanostructured electrocatalysts, with low overpotential, high selectivity and activity has fundamental and technical importance in many fields. We report here rhodium nanoparticle and mesoporous silicon nanowire (RhNP@mSiNW) hybrids for hydrogen peroxide (H$_2$O$_2$) detection with high electrocatalytic activity and selectivity. By employing electrodes that loaded with RhNP@mSiNW nanohybrids, interference caused from both many electroactive substances and dissolved oxygen were eliminated by electrochemical assaying at an optimal potential of 175 mV. Furthermore, the electrodes exhibited a high detection sensitivity of 0.53 μA/mM and fast response (< 5 s). This high-performance nanohybrid electrocatalyst has great potential for future practical application in various oxidase-base biosensors.

Results

RhNP@mSiNW nanohybrids were prepared via a two-step approach. Firstly, mpSiNWs were prepared via a metal-assisted chemical etching approach. Figure 1a is a typical field emission scanning electron microscopy (FE-SEM) cross-sectional view of the as-synthesized mpSiNW arrays. The NWs have homogenously distributed nano-pores with an average size of about 15 nm as revealed from the transmission electron microscopy (TEM) shown in Supplementary Fig. S1a. The continuous crystal lattices across these nano-pores shown in Supplementary Fig. S1b indicate a single crystal feature of the as-prepared mpSiNWs. After the as-prepared NWs were scratched off from the silicon substrate they were loaded with RhNPs via chemical reduction approach using NaBH$_4$ as a reducing agent. As revealed from Figure 1b, a typical SEM image of RhNP@mSiNW nanohybrids, small size of RhNPs are distributed on the side surface of mpSiNW. TEM image shown in
Figure 1c indicates that the large RhNP is an aggregation of small particles with a size of 4–6 nm. Each of these small particles is a single crystal as can be seen from the high-resolution TEM (HR-TEM) image shown in Figure 1d.

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The as-prepared nanohybrids were loaded onto glassy carbon (GC) electrode for \( \text{H}_2\text{O}_2 \) measurement. Figure 2a shows the linear sweep voltammetry (LSV) scan of the RhNP@mpSiNW/GC electrode in an oxygen-free buffer (solid line), air-saturated buffer (dash line), and oxygen-free buffer containing 1 mM \( \text{H}_2\text{O}_2 \) (dash dot line). In comparison to electrode response in oxygen-free buffer, only a slightly higher cathodic current was observed in air-saturated buffer which can be attributed to reduction of dissolved oxygen. The cathodic reduction starts at the potential of around +60 mV. In marked contrast, the electrode gives a significantly higher cathodic reduction current in oxygen-free buffer containing 1 mM \( \text{H}_2\text{O}_2 \). This could be attributed to the high electrocatalytic activity of the RhNPs towards \( \text{H}_2\text{O}_2 \) reduction. From these results, it can be seen that the dissolved oxygen will have little effect on the cathodic reduction of \( \text{H}_2\text{O}_2 \) if electrode potential of more positive than +60 mV is used. Figure 2b depicts two almost identical calibrated lines of the RhNP@mpSiNW/GC electrodes at +75 mV for the reduction of \( \text{H}_2\text{O}_2 \) with different concentrations in the presence and absence of dissolved oxygen (their original electrode LSV responses in oxygen-free solutions to various concentrations of \( \text{H}_2\text{O}_2 \) are shown in Supplementary Fig. S2). The sensitivity can be calculated to be 0.53 \( \mu \text{A/mM} \) from the linear part of the calibration curve. Inset in Figure 2b is electrode responses in low concentration ranges. These results show that almost no influence caused from oxygen was observed over a broad range of \( \text{H}_2\text{O}_2 \) concentrations, which can be attributed to the high electroactivity of nanohybrids to \( \text{H}_2\text{O}_2 \) reduction and the considerably suppressed cathodic reduction activity to oxygen.

Figure 3 shows a dynamic amperometric current-time response of RhNP@mpSiNW/GC electrode at +75 mV with a successive addition of \( \text{H}_2\text{O}_2 \) from 1 \( \mu \text{M} \) to 0.5 \( \mu \text{M} \). The currents increase immediately and reached their equilibrium states rapidly after the addition of \( \text{H}_2\text{O}_2 \). The average rising time is less than 5 s, indicating a fast amperometric response towards \( \text{H}_2\text{O}_2 \) cathodic reduction. From the inset in Figure 3 we can see that the detection limit can be down to micro-mole range even in the presence of soluble oxygen. These results further reveal that the as-prepared RhNP@mpSiNW nanohybrids have good electrocatalytic activity towards \( \text{H}_2\text{O}_2 \) reduction and high selectivity against dissolved oxygen.

Figure 4a shows the current response of electroactive compounds of AA, urea, and uric acid on RhNP@mpSiNW/GC electrode with different amounts of RhNPs loading at a potential of +75 mV during the measurement of 1 mM \( \text{H}_2\text{O}_2 \). The electrode amperometric response to \( \text{H}_2\text{O}_2 \) is not influenced by the addition of urea and uric acid in all three cases. The relative current changes of AA to that of \( \text{H}_2\text{O}_2 \) were as low as 4.5%, 2.2%, and 1.0% with RhNP loading of 3.7, 7.4, and 11.1 \( \mu \text{g/mm}^2 \), respectively. Such behavior indicates that the activity towards \( \text{H}_2\text{O}_2 \) reduction increases with the increase of RhNP loading, while the response of AA is not sensitive to the RhNP loading variation. As a result, the relative current response of AA to that of...
In recent years, a large range of electrocatalysts have been developed in order to lower the $H_2O_2$ electro-oxidation potential so that interferences caused from electroactive species could be eliminated. Despite these efforts, detection error still exists because substances, such as AA are electroactive even at low anodic oxidation potential. Cathodic reduction is a promising approach that can avoid such interference during $H_2O_2$ detection. But, cathodic reduction lacks of reliability because the oxygen, whose concentrations are fluctuant in solution, can be reduced at similar potential. Mesoporous silicon nanowires are attractive for developing high-performance biosensors owing to their excellent semiconducting properties, mechanical, optical properties and favorable biocompatibility. In this work, the RhNP-mpSiNWs composite structure resulted in excellent selectivity against both many electroactive substrates and dissolved oxygen at an optimal potential of $+75$ mV. The remarkable selectivity against electroactive species of RhNP@mpSiNW can be attributed to the unfavorable electrostatic interaction between the mesoporous silicon and the probe molecule. In a buffered solution with pH value of 7.2, the SiNW matrix covered with a large density of hydroxyl may bear a negatively charged surface and work as a perm-selective barrier that likely to prevent the negatively charged AA from reaching the electrode surface easily.

**Conclusion**

In this paper, RhNP@mpSiNW nanohybrids were prepared and their electro-catalytic properties were demonstrated. The amperometric detection of $H_2O_2$ was achieved by cathodic reduction at low potential of $+75$ mV, where acceptable low interferences caused from both dissolved oxygen and regular electroactive substances were achieved. Therefore, such nontoxic RhNP@mpSiNW nanohybrids are promising and have great potential for future practical artificial mediator free biosensor applications.

**Methods**

**Chemicals.** Hydroxyfluor acid (HF, ≥40%), silver nitrate (AgNO₃, ≥99.8%), $H_2O_2$ (≥30%), sulfuric acid (H₂SO₄, 98%), nitric acid (HNO₃, 65% to 68%), sodium hydroxide (NaOH, ≥96%), sodium borohydride (NaBH₄, ≥98%), acetone, ethanol, potassium chloride (KCl, ≥99.8%), urea, uric acid and AA were purchased from Sinopharm Chemical Reagent Co., Ltd. Silicon (100) wafers (p-type, boron-doped, 100 mm, 0.1 mΩ cm) were purchased from Nanohydrotech Co., Ltd. The resistivity of the silicon wafers is 18.5 ± 2 mΩ cm according to square resistance measurement. Sodium and rhodium chloride (RhCl₃, 98%) were purchased from Sigma and used as received. All solutions were prepared with Milli-Q water.

**Instrumentation and characterization.** All electrochemical experiments were carried out with a CHI 660E (CHI Instruments Inc., Austin, USA). A conventional three-electrode system was used in this work. GC electrode, platinum wire and Ag/AgCl (3 M KCl) electrode were used as working, counter and reference electrode, respectively. Phosphate buffer with a concentration of 0.2 M (pH 7.2) was employed as the supporting electrolyte. Electrolyte solutions were bubbled with argon for at least 40 min and argon atmosphere was maintained over the solution during the deoxygenated electrochemical measurements. The microstructures of the samples were characterized using FE-SEM (%8000, Hitachi, Tokyo, Japan) and TEM Tecnai F20 (FEI, Hillsboro, OR, USA) electronicroscope at an accelerating voltage of 200 kV.

**Preparation of mpSiNWs.** Silicon (100) wafers (1 cm × 1 cm) were washed in a mixed solution of ethanol, acetone and water for 15 min and then immersed in a mixed solution of $H_2SO_4$ and $H_2O_2$ (v/v = 1 : 1) for 10 min to remove surface organic materials. The wafers were then immersed in 8% HF solution for 10 min to form H-terminated surface and then dipped into a solution containing 8% HF and 8.3 mM AgNO₃ to coat a uniform layer of Ag nanoparticles. Subsequently the wafers were transferred to an ethan salt solution composed of 8% HF and 0.27 M $H_2O_2$. After being etched for 30 min at room temperature, the wafers were washed using 1 M diluted HNO₃ to remove the Ag catalyst and then washed with 0.1 M NaOH solution for 10 min to remove the oxide sheath. After being thoroughly washed with water, the as-prepared mpSiNWs were detached from the Si substrate by sonicating treatment, collected and re-dispersed in 5 mL water.

**Preparation of RhNP@mpSiNW hybrid.** 90 μL of 1 wt% RhCl₃ solution was added to the as-prepared mpSiNWs solution (adding 45, 90 and 135 μL of the RhCl₃ solution resulted in RhNP@mpSiNW with RhNP loading of 3.7, 7.4 and 11.1 μg/mm², respectively). Then excess amount of 0.1 wt% NaBH₄ solution was added to the solution slowly at 4°C during stirring in order to prepare RhNP@mpSiNW nanohybrid. The resultant solution was stirred for another 30 min to ensure full loading of the RhNPs.
obtained RhNP@mpSiNW nanohybrid was collected, washed and re-dispersed in 1 mL water.

**Preparation of RhNP@mpSiNW/GC electrode.** GC electrodes were mechanically polished with 1, 0.3 and 0.05 mm alumina slurry and then washed in water, ethanol and water for 5 min sequentially. GC electrodes were then dried with nitrogen stream. The RhNP@mpSiNW solution was thoroughly mixed with Nafion solution (0.5 wt%) at 1:1 ratio (v/v). The RhNP@mpSiNW hybrid modified GC electrodes were obtained by drop coating 3 µL of the mixed solution onto GC electrode.

**Preparation of the RhNP loading GC electrode.** Various amounts (45, 90 and 135 µL) of 1 wt% of RhCl₃ were added to 5 mL water. Then excess amount of 0.1 wt% NaBH₄ solution was added slowly at 4 °C under stirring to form RhNPs. The resultant solution was stirred for another 30 min, washed and re-dispersed in 1 mL water. RhNP loading is similar to the process of RhNP@mpSiNW hybrid loading.

1. Chen, W., Cai, S., Ren, Q., Wen, W. & Zhao, Y. Recent advances in electrochemical sensing for hydrogen peroxide: a review. *Analyst* 137, 49–58 (2012).
2. Wang, J. Electrochemical biosensors: Towards point-of-care cancer diagnostics. *Biosens. Bioelectron.* 21, 1887–1892 (2006).
3. Wang, J. Electrochemical glucose biosensors. *Chem. Rev.* 108, 814–825 (2008).
4. Frew, J. & Hill, H. A. Electrochemical biosensors. *Anal. Chem.* 59, 933A–944A (1987).
5. Rodriguez, M. C. & Rivas, G. A. An enzymatic glucose biosensor based on the codeposition of rhodium, iridium, and glucose oxidase onto a glassy carbon transducer. *Anal. Lett.* 34, 1829–1840 (2001).
6. Mischiora, S. A., Barrera, G. D. & Rivas, G. A. Glucose biosensors based on the immobilization of glucose oxidase and polytyramine on rhodiniyzed glassy carbon and screen printed electrodes. *Sens. Actuators B* 115, 205–211 (2006).
7. Mischiora, S. A., Barrera, G. D. & Rivas, G. A. Analytical performance of a glucose biosensor prepared by immobilization of glucose oxidase and different metals into a carbon paste electrode. *Electroanalysis.* 14, 981–987 (2002).
8. Chang, H. et al. Strongly coupled rhodium/graphene hybrids for H₂O₂ oxidation with ultra-low potential and enhanced activity. *ChemElectroChem* 1, 1–4 (2014).
9. Wang, K., Xu, J.-J. & Chen, H.-Y. A novel glucose biosensor based on the nanoscaled cobalt phthalocyanine-glucose oxidase composite. *Biosens. Bioelectron.* 20, 1388–1396 (2005).
10. Otozenma, K. I., Mashazi, P. N. & Nyokong, T. Tetracarboxylic acid cobalt phthalocyanine SAM on gold: potential applications as amperometric sensor for H₂O₂ and fabrication of glucose biosensor. *Electrochim. Acta.* 52, 177–186 (2006).
11. Rodriguez, M. C. & Rivas, G. A. Highly selective first generation glucose biosensor based on carbon paste containing copper and glucose oxidase. *Electroanalysis.* 13, 1179–1184 (2001).
12. Hua, M. Y. et al. The intrinsic redox reactions of polyamidic acid derivatives and their application in hydrogen peroxide sensor. *Biomaterials.* 32, 4885–4895 (2011).
13. Dong, S. J., Zhou, M. & Zhai, Y. M. Electrochemical sensing and biosensing platform based on chemically reduced graphene oxide. *Anal. Chem.* 81, 5603–5613 (2009).
14. Shan, C. S. et al. Direct electrochemistry of glucose oxidase and biosensing for glucose based on graphene. *Anal. Chem.* 81, 2378–2382 (2009).
15. Wang, J., Musamh, M. & Lin, Y. H. Solubilization of carbon nanotubes by nafion toward the preparation of amperometric biosensors. *J. Am. Chem. Soc.* 125, 2408–2409 (2003).
16. Wang, J. & Musamh, M. Carbon nanotube/tellur composite electrochemical sensors and biosensors. *Anal. Chem.* 75, 2075–2079 (2003).
17. Lee, K. P., Manesh, K. M., Kim, H. T., Santhosh, P. & Gopalani, A. I. A novel glucose biosensor based on immobilization of glucose oxidase into multi-wall carbon nanotubes–polyelectrolyte-loaded electrosion nanofluidic membrane. *Biosens. Bioelectron.* 23, 771–779 (2008).
18. Wang, J. & Chen, Q. Enzyme microelectrode array strips for glucose and lactate. *Anal. Chem.* 66, 1007–1011 (1994).
19. Wang, J., Liu, J., Chen, L. & Lu, F. Highly selective membrane-free, mediator-free glucose biosensor. *Anal. Chem.* 66, 3600–3603 (1994).

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**Author contributions**

X.F. developed the concept and coordinated the project. Z.S. and H.C. performed the research, analyzed the data and wrote the paper. C.X. and W.Z. contributed to the SEM and TEM characterizations.

**Additional information**

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