

Electronic Supplementary Information:

Disposable indium-tin-oxide sensor modified by gold nanorod-chitosan nanocomposite for the detection of H₂O₂ in cancer cells

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1. Experimental details

1.1. Materials

Hydrogen tetrachloroaurate trihydrate (HAuCl₄, 99%), sodium borohydride (NaBH₄, 99%), hexadecyltrimethylammonium bromide (CTAB), L-ascorbic acid (AA), silver nitrate (AgNO₃, 99%), 5-bromosalicylic acid (>98.0%), hydrochloric acid (HCl, 37 wt % in water), poly (sodium-p-styrenesulfonate) (PSS, MW: 70,000), hydrogen peroxide solution (H₂O₂, 30%), uric acid (UA), glucose (Glu) and dopamine (DA) were obtained from Sigma-Aldrich (St. Louis, MO). Chitosan (90% deacetylation) was from Sinopharm Chemical Reagent Co., Ltd. The Indium tin oxide (ITO) conductive glass (355.6×406.4×1.1 mm STN, 10 Ω/cm²) was purchased from Nanbo Display Technology Co. LTD (Shenzhen, China). The qualitative filter papers (Whatman No.1) were from Whatman International Ltd. (Maidstone, United Kingdom). Phosphate buffer saline (PBS, pH 7.4) containing 137 mM NaCl, 2.7 mM KCl, 87.2 mM Na₂HPO₄ and 14.1 mM KH₂PO₄ was used as the electrolyte. All the solutions were prepared with doubly distilled water.

1.2 Preparation of gold nanorods (GNRs)

GNRs were chemically synthesized using seed mediated growth method¹. Seed

solution was prepared by mixing 5 mL HAuCl₄ (0.5mM) with 5 mL (0.2 M) CTAB for 1 min and adding 0.6 mL ice-cold NaBH₄ (0.01M) under vigorous stirring. After 2 min of mixing, the prepared seeds were stored at room temperature for 30 min before use.

The growth solution was prepared by mixing 9.0 g CTAB and 1.1 g 5-bromosalicylic acid in 250 mL of warm water. Then 24 mL AgNO₃ (4 mM) solution was added. The mixture was kept undisturbed at 30 °C for 15 min, after which 250 mL of HAuCl₄ (1 mM) solution and 0.5 mL HCl (12.1 M) was added. After 15 min of stirring (400 rpm), 2.0 mL ascorbic acid (0.064 M) was added, and the solution was vigorously stirred for 30 s until it became colorless. Finally, 0.8 mL of seed solution was quickly added to the solution. The resultant mixture was stirred for 30 s and left undisturbed at 30 °C for 12 h for NR growth. The reaction products were centrifuged three times at 9000 rpm for 15 min to remove excess CTAB surfactant. The precipitates were redispersed in 10 mL of deionized water.

1.3. Preparation of PSS-GNRs and PSS-GNRs-chitosan nanocomposite

Physical adsorption of negatively charged PSS polymers to wrap over positively charged CTAB bilayer on the GNRs surface was achieved by mixing GNRs with PSS solution (10 mg/mL in 1 mM NaCl solution) and allowed to react for 1 day. The nanorods were then collected by centrifugation at 7000 rpm for 15 min, and resuspended in deionized water.

To prepare the PSS-GNRs-chitosan nanocomposites, chitosan was dissolved in 0.05 M acetate buffer to form 0.2 wt% solution and then filtered using a 0.45 mm syringe filter unit. The pH value was adjusted to 5.0 with NaOH solution. PSS-GNRs-chitosan nanocomposite with various concentrations was obtained by dispersing different amount of PSS-GNRs in the 1 mL chitosan solution. The suspension was mixed by ultrasonic irradiation for 30 min. Finally, a viscous solution of chitosan with uniformly dispersed PSS-GNRs was obtained.

1.4. Design and fabrication of the disposable electrode

A sheet of ITO plate was cut into strips of 20 mm×7 mm. The ITO electrodes were ultrasonically cleaned sequentially with acetone, absolute ethanol, and doubly ultrapure water for 5 min, respectively, and dried with nitrogen gas. Each ITO chip was treated uniformly and the electrical conductivity was tested by a typical four-point probe (RTS-8) technique to ensure the consistency between the electrodes (The resistance value was between 9.6-10.2 Ω/cm^2).

For the fabrication of the disposable electrode, a section of plastic adhesive tape was punched with a hole (4 mm diameter) and then attached on the ITO in order to provide an identical area for electrochemical experiment. The hole is prepared as the electrochemical cell for the accommodation of living cells. For the modification of the electrode, 6 μL of PSS-GNRs-chitosan nanocomposite was dropped on the electrode region on ITO chip and were allowed to dry at room temperature.

1.5. Characterization and electrochemical studies

The UV-vis spectra were recorded on an UV-2450 spectrophotometer spectrophotometer (Shimadzu, Japan). The morphology of GNRs was observed by a JEM-1230 transmission electron microscope (TEM). The zeta potential was measured using the Malvern particle size analyzer (Malvern Instruments Ltd, UK).

The electrochemical experiments were performed with a CHI1230B workstation (CH Instrumentation, Shanghai, China). A three-electrode system comprised the nanocomposite modified ITO chip as the working electrode, a platinum wire as the auxiliary electrode, and Ag/AgCl as the reference electrode. The three-electrode system was integrated in a cover made of elastomeric material, PDMS (polydimethylsiloxane). Prior to each experiment, a stream of highly pure nitrogen was blown gently inside the detection device for at least 15 min and a nitrogen atmosphere was maintained during the measurements.

1.6. Cell culture

SH-SY5Y (human neuroblastoma), HepG2 (human hepatocarcinoma), PC12 (rat adrenal medulla pheochromocytoma) and K562 (human leukemia) cells were

obtained as a gift from the Affiliated Hospital of Nantong University (Nantong, China). They were maintained in DMEM (high glucose, Gibco) medium supplemented with 10 % heat-inactivated fetal calf serum (Sigma, USA), 0.1 % penicillin/streptomycin (Sigma, USA) at 37 °C in a humidified atmosphere of 5 % CO₂ and 95 % air. Cells were fed every 3 day and sub-cultured once they reached 80–90 % confluency in the 100 mm² cell culture dish. The cells were counted by using a Petroff–Hausser counter (USA).

1.7. Electrochemical detection of H₂O₂ released by cells

The cells were separated from the culture medium by performing centrifugation at 1000 rpm for 5 min and washed twice with sterile pH 7.4 PBS. The sediment was suspended in deoxygenated PBS to obtain a homogeneous cell suspension for use in electrochemical measurements with the final concentration of 5×10⁶ cell mL⁻¹. Before measurements, a stream of nitrogen was blown gently above the surface of the working electrode in order to maintain the nitrogen atmosphere throughout the experiments.

Firstly, the cell suspension of 8 uL was dropped on the hole of the nanocomposite modified ITO chip. Then a piece of the Whatman filter paper with 6 mm long and 6 mm wide was put on the surface of the electrode. The paper used here could store the reagents for electrochemical detection and protect the living cells. In addition, three electrodes were separated in our approach since the working electrode is disposable while the counter and reference electrodes could be reusable. In this way, the cost of the detection device could be further decreased although integration of three electrodes directly in the paper would be more convenient². By coupling the three-electrode system in the PDMS cover, the paper-based analytical device was developed for voltammetric analysis. After a steady state background was attained, AA was injected into the electrode region, and the response current corresponding to the electrocatalytic reduction of H₂O₂ released from the cells was recorded at –0.3 V (vs. Ag/AgCl).

2. Results section

2.1. Characterization of the GNRs and PSS-GNRs

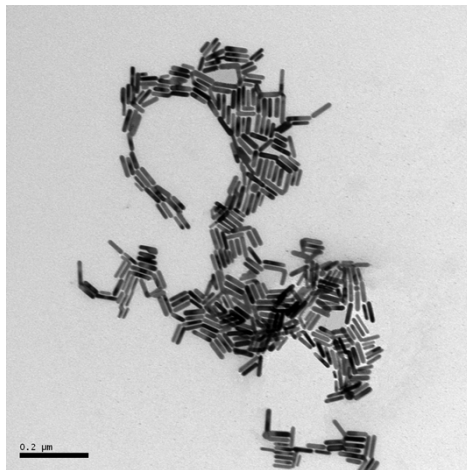


Fig. S1 TEM micrograph of GNRs.

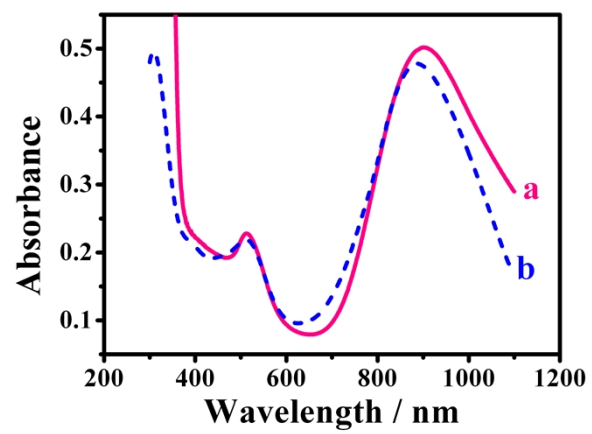


Fig. S2 Absorption spectroscopy of (a) GNRs and (b) PSS-GNRs.

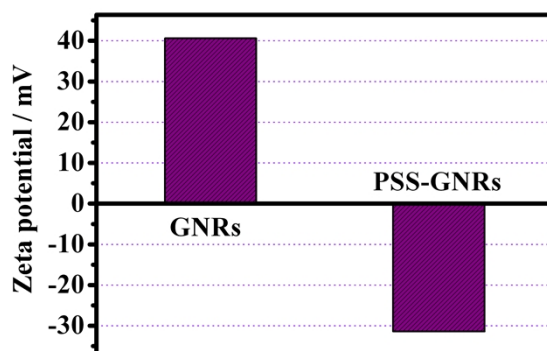
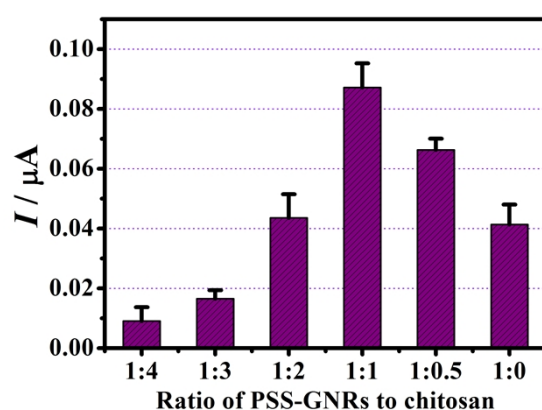


Fig. S3 Zeta potentials of GNRs and PSS-GNRs.

2.2. Dependence of the H_2O_2 reduction on the ratio of PSS-GNRs to chitosan

We further studied PSS-GNRs/chitosan composition-dependent H_2O_2 reduction activity (Fig.S4). This can be controlled by using the same volume (6 μ L) of the suspensions casted on the ITO electrode surface with the different volumetric ratio of PSS-GNRs to chitosan. The reduction current for the detection of 10 μ M H_2O_2 increased by increasing the ratio of PSS-GNRs to chitosan from 1:4 to 1:1. The further increase caused a gradual decrease in the reduction peak, suggesting that overhigh

would lead
the response



PSS-GNRs
concentration
to the instability of
and leakage of

PSS-GNRs out of the film. Thus, 1:1 was selected as the optimum ratio for further study.

Fig. S4 Dependence of current responses for 10 μM H_2O_2 reduction on the ratio of PSS-GNRs to chitosan.

2.3. The electrocatalytic reduction of H_2O_2 of the disposable electrode

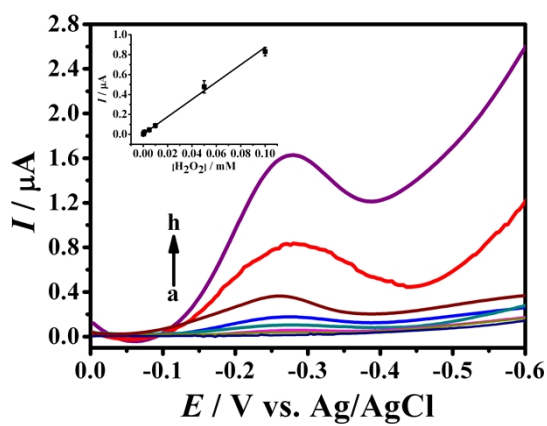


Fig. S5 DPV curves of H₂O₂ in pH 7.4 PBS with different concentrations (a-h): 0, 0.1, 0.5, 1.0, 5.0, 10, 50 and 100 μ M. Inset: Plot of the peak current against the concentration of H₂O₂.

Table S1 Comparison of the proposed H₂O₂ sensor with other electrochemical sensors.

H ₂ O ₂ sensors	Linear range (μ M)	Detection limit (μ M)	Response time	References
Cyt c/AuNP/ITO	800–6000	0.5	in 10s	2
Hb-Bi ₂ Se ₃ /GCE	2.0–100	0.63	in 15s	3
HRP-TiO ₂ nanotube electrode	0.5–35	0.18	-	4
CuS/RGO/GCE	5–1500	0.27	in 2s	5
PtM/MWCNTs/GCE	2.5–125	1.2	in 5s	6
Fe ^{III} /MPPA/Au electrode	1.5–270	0.4	in 0.7s	7
GNRs-chitosan/ITO	0.1–100	0.06	in 0.5s	This work

2.4. The selectivity of the disposable electrode toward the electrocatalytic reduction of H₂O₂

Table S2 Interference experiments with the proposed H₂O₂ biosensor.

Interferent	$\Delta I(\text{H}_2\text{O}_2 + \text{Interferent}) / \Delta I(\text{H}_2\text{O}_2)$
Dopamine	1.025
Uric acid	1.043
Glucose	1.082
Ascorbic acid	1.103

2.5. The control experiment of electrode response in the cell-free solution

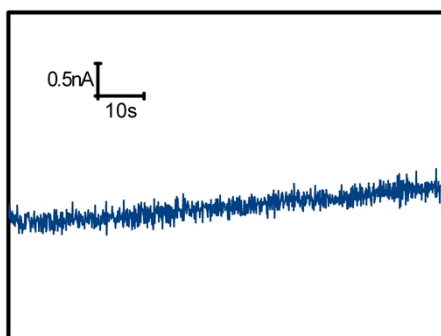


Fig. S6 Measurements of electrode response upon the addition of 1.0 μM AA in the cell-free detection solution.

2.6. The time course of H_2O_2 released from four kinds of cancer cells

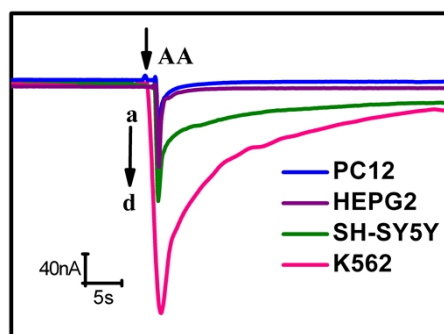


Fig. S7 The time course of H_2O_2 released from four kinds of cells induced by 1.0 μM AA: (a) PC12, (b) HepG2, (c) SH-SY5Y and (d) K562 cells.

References

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