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Electronic Supplementary Information

Pt nanospheres grown paper working electrode based electrochemical device for in-situ and real-time determination of the flux of H_2O_2 releasing from SK-BR-3 cancer cells

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Materials and methods

Reagents

All chemicals and solvents used here were aseptic after heat sterilization treatment. Chloroplatinic acid (H_2PtCl_6) was obtained from Shanghai Chemical Reagent Company (Shanghai, China). Catalase (from bovine liver, lyophilized powder, 2000-5000 U/ mg, Sigma), phorbol myristate acetate (PMA, ~99%, Sigma), N-formylmethionyl-leucylphenylalanine (fMLP, ⩾97%, Sigma), adenosine 5'-diphosphate (ADP, ⩾95%, Sigma), ascorbic acid (AA, ⩾99.0%, Sigma), Solutions of H_2O_2 were freshly diluted from the 30% solution, and their concentrations were determined using a standard $KMnO_4$ solution. The S6 aptamer with high specificity for SK-BR-3 cells was synthesized and purified by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China).

The human breast cancer cells SK-BR-3 were provided by Shandong Tumor Hospital. The sequence of S6 aptamer was 5'-NH_2-TGGATGGGGAGATCCGTTGAGTAAGCGGGCGTGTCTCTCTGCCGCCTTGCTATGGGG-3'. Calcein

acetoxymethyl ester (AM) and fluorescein isothiocyanate labeled annexin-V (FITC-annexin-V) were purchased from Beijing Biosynthesis Biotechnology Co. Ltd. (Beijing, China).

0.1 M phosphate buffer solution (PBS, pH 7.4) was employed as the supporting electrolyte. Carbon ink (ED423ss) and Ag/AgCl ink (CNC-01) were purchased from Acheson. Whatman chromatography paper #1 (58.0 cm × 68.0 cm) (pure cellulose paper) was obtained from GE Healthcare Worldwide (Pudong, Shanghai, China) and used with further adjustment of size (A4 size).

Apparatus

Scanning electron microscope (SEM) analyses were performed using QUANTA FEG 250 thermal field emission scanning electron microscopy (FEI Co., USA). Electrochemical impedance spectra (EIS) were performed on a CHI 604D electrochemical workstation (Shanghai CH Instruments Inc., China). Electrochemical measurements were carried out with a homemade EC system. The current was measured on a CHI 660D electrochemical workstation (Shanghai Chenhua Apparatus Corporation, China) with a three-electrode system, whereas the modified PWE with a diameter of 6.0 mm was used as the working electrode, screen-printed carbon electrode and Ag/AgCl electrode were used as the counter electrode and the reference electrode, respectively.

Preparation of Pt nanoparticles

Pt naoparticles were prepared according to a facile method described as follows. H_2PtCl_6 and trisodium citrate solutions were filtered through a 0.22-μm microporous

membrane filter prior to use, and then 3.0 mL of 1% trisodium citrate was added to 20 mL of boiling 0.05% H_2PtCl_6 solution and stirred for 30 min at the boiling point. The final Pt naoparticles prepared by this method have an average diameter of ~5 nm.

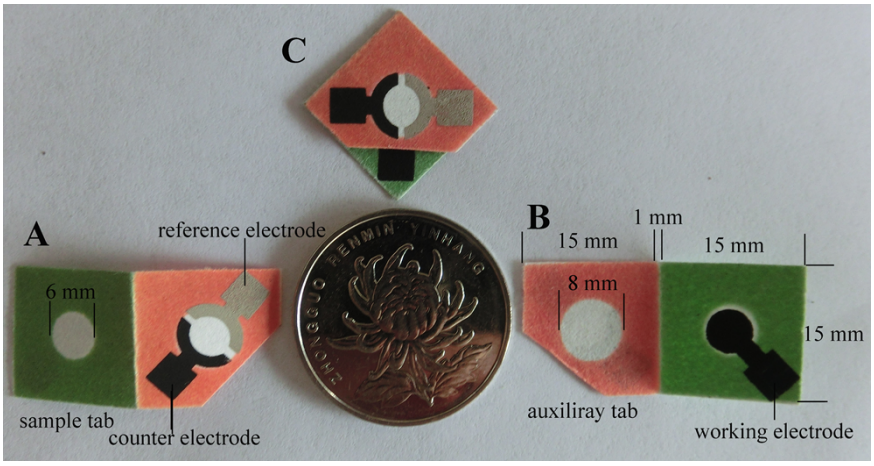
Fabrication of Pt-PWE

Prior to the S6 aptamer immobilization, growth of Pt layer on the surfaces of cellulose fibers in the paper working zone of PWE was implemented to fabricate a novel Pt-PWE with enlarged effective surface area and enhanced electrocatalytic reduction property for sensitive H_2O_2 sensing. This novel Pt-PWE was fabricated on this 3D origami electrochemical device through a direct chemical reduction of H_2PtCl_6 by AA. Fist, as-prepared Pt nanoparticle seeds solution (15.0 μL) was dropped into the bare PWE. Then the origami device was equilibrated at room temperature for 1 h to optimize the surface immobilization of Pt nanoparticle seeds on cellulose fibers. After rinsing with water thoroughly to remove loosely bound Pt nanoparticle seeds, freshly prepared silver-growth solution (15.0 μL) containing 0.50 mM H_2PtCl_6 and 0.25 mM AA was applied into the Pt nanoparticles seeded PWE, respectively, and incubated at room temperature for 4 min. During the growth process, the Pt nanoparticle seeds acted as catalysts for the reduction of H_2PtCl_6 by AA, resulting in the enhancement of the Pt nanoparticle seeds. Subsequently, the resulting Pt-PWEs were washed with water thoroughly. Thus a layer of interconnected Pt nanoparticle on cellulose fibers with good conductivity was obtained (Scheme 1B), which was dried at room temperature for 20 min.

Cell culture

The SK-BR-3 human breast cancer cells were obtained from Shandong Tumor Hospital. Breast cancer cells were maintained in dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cultures were maintained at 37 °C and in 5% CO_2 atmosphere. The culture medium was changed every other day and the cells were passaged when they reached 80-90% confluency. For cancer cell electrochemical measurement, cells were placed inside a 12-well plate. When cultured for two days, cancer cells were digested by pancreatic enzymes and dispersed in PBS. After being blown well, cancer cells were counted by a Petroff- Hausser cell counter (U.S.A.).

Fabrication and characterization of this 3D origami EC device



Scheme S1. (A) The schematic representation, size, and shape of this μ-PAD. (B) One side of the μ-PAD with the screen-printed reference and counter electrode; (C) The reverse side of (B) with the screen-printed working electrode.

The preparation of this μ-PAD was similarly to our previous work [1] with large modifications and a detailed procedure was described below. This origami device was

comprised of a square sample tab (Scheme S1A, 15.0 mm × 15.0 mm) and a square auxiliary tab (Scheme S1B, 15.0 mm × 15.0 mm). An angle of the square auxiliary tab was cut off for exposure of the contact pad of screen-printed carbon working electrode (Scheme S1B). The entire origami device could be produced in bulk on an A4 paper sheet by a commercially available solid-wax printer (Xerox Phaser 8560N color printer). Owing to the porous structure of paper, the melted wax can penetrate into the paper network to decrease the hydrophilicity of paper remarkably while the unprinted area (paper auxiliary zone and paper sample zone) still maintained good hydrophilicity, flexibility, and porous structure and will not affect the further screen-printing of electrodes and modifications [2].

Between the sample tab and auxiliary tab, the unprinted line (1 mm in width) was defined as fold line (Scheme S1B). The unprinted hydrophilic area (paper auxiliary zone and paper sample zone) constituted the reservoir of the paper electrochemical cell (~40 μL) after being folded at the predefined fold line. Then, the wax-penetrated paper sheet was ready for screen-printing of electrode containing the wire and contact pad on its corresponding paper zone (Scheme S1B, C). The electrode array consisted of a screen-printed Ag/AgCl reference electrode and carbon counter electrode on the auxiliary zone (Scheme S1A) and screen-printed carbon working electrode (6 mm in diameter) on the reverse side of paper sample zone (Scheme S1B), respectively. After folding (Scheme S1C), the three screen-printed electrodes will be connected once the paper electrochemical cell was filled with solution.

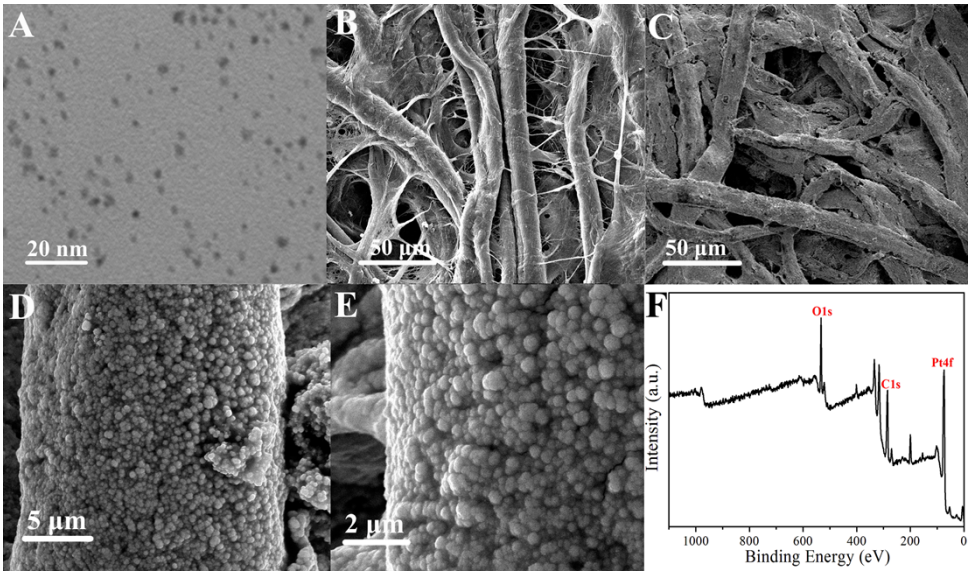
Electrical resistivity of Pt nanospheres grown paper sample zone

To measure the resistivity of Pt nanospheres grown paper sample zone exactly, rectangular paper zones (1.0 mm×20.0 mm) were fabricated by wax-printing and then were modified through the growth of dense Pt nanospheres conducting layer on the surfaces of cellulose fibers in these rectangular paper zones using the same method described in experimental section under the same experimental conditions in each case, the average electrical resistivity of which revealed the electrical resistivity of Pt nanospheres grown paper sample zone in this work. Prior to the measurements, both the bare and Pt nanospheres grown rectangular paper zones were dried in a drying oven for 3 h. Then the measurements were performed with a four-point measurement setup using a digital multi-meter (Agilent, U1251B).

Detection of H_2O_2 standard solution at our cancer cell immobilized Pt-PWE

Our work aimed at developing a biosensor for monitoring the release process and measuring the flux of H_2O_2 from cells. To evaluate the possibility of the biosensor used in this purpose, the current responses of our modified Pt-PWEs toward the various concentrations of H_2O_2 were studied. And the potential swept from 0 to –0.6 V with scan rate of 100 mV s^{-1} after 10 μL of PBS buffer solution (pH 7.4) was added. PBS buffer containing different concentrations of H_2O_2 standard solution (early determined using a standard $KMnO_4$ solution) were used in this experiment as detection solution. At the absence of H_2O_2, the current response of the modified porous Pt-PWE is weak. Upon addition of H_2O_2 with increasing concentrations, the intensity of the cathodic current of the modified porous Pt-PWE increases gradually.

Characterizations of Pt-PWE



127 Fig. S1 TEM image of Fig. S1 TEM image of the as-prepared Pt nanoparticle seeds (A); SEM
128 images of bare paper sample zone of PWE (B); Growth of Pt nanospheres layer on the surfaces
129 of cellulose fibers in paper sample zone of PWE under different magnification: (C, D, E); XPS of
130 Pt nanospheres modified paper sample zone (F).

131

132 The PWE was modified through the growth of a Pt nanospheres layer on the
133 surfaces of cellulose fibers in paper sample zone from Pt nanoparticle seeds to
134 fabricate this novel Pt-PWE. Fig. 1SA showed the TEM image of the as-prepared Pt
135 nanoparticle seeds with the average diameter of about 5 nm. As shown in Fig. 1SB,
136 the porous bare paper sample possessed high ratio of surface area to weight with
137 rough cellulose fibers, which could offer an excellent adsorption microenvironment
138 for the Pt nanoparticles. After Pt nanoparticles were successfully seeded and grown
139 on the cellulose fiber, a continuous and dense Pt nanospheres conducting layer on the
140 cellulose fiber surfaces was observed (Fig. 1SC, D). The enlarged image of Pt
141 nanospheres grown cellulose fiber was shown in Fig. 1SE, showing the uniform

growth of Pt nanospheres. This indicated that a good coverage of Pt nanospheres on the surfaces of the cellulose fibers was obtained using our simple growth method. The Pt nanospheres grown paper sample zone maintained good 3D interwoven and incompact cellulose fibers networks structure after the growth process, which would facilitate the area-to-volume ratio as well as increase the catalytic active sites to obtain better electrochemical performance. In addition, the successful modification of Pt nanospheres on cellulose fibers was confirmed by X-ray photoelectron spectroscopy (XPS) (Fig. 1SF) and the peaks observed at 73 eV were ascribed to metallic Pt.

Optimization of experiment conditions

To achieve the optimal analytical properties of the sensing system, pH and ionic strength of assay solution on the sensitivity of the sensor were investigated (Fig. 2S). Fig. 2SA shows the dependence of currents on pH of PBS. An optimal current was obtained at pH 7.4 PBS. A higher or lower pH resulted in the decrease of catalytic currents. In addition, as shown in Fig. 2SB, the current value increased with the increment of ionic strength of assay solution and tended to level off after 10 mM PBS. Thus, a pH 7.4 PBS (10 mM) was chosen as the supporting electrolyte.

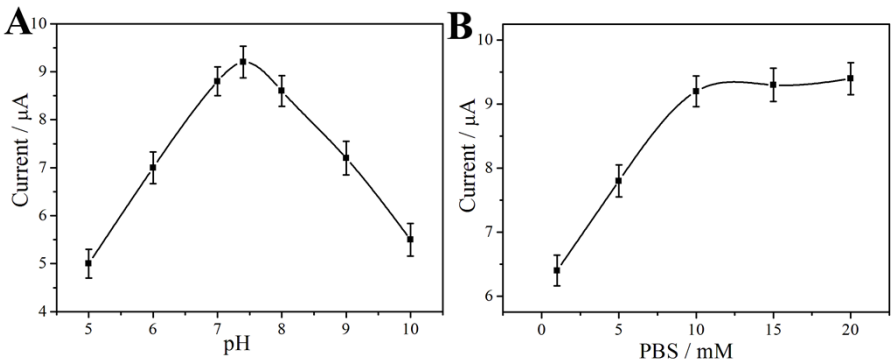


Fig. 2S Current signal dependence of the Pt-PWE based μ-PADs electrochemical H_2O_2 sensor on (A) different pH of PBS from 5.0 to 10.0; (B) different ionic strength of the assay solution in 1mM, 5 mM, 10 mM, 15 mM and 20 mM PBS solution, respectively.

The H_2O_2 standard solution concentration-dependent electrocatalytic current

According to the selectivity of results, 10 mM PBS (pH 7.4) was chosen as the supporting electrolyte. Then, under the optimal conditions, our constructed cancer cells immobilized Pt-PWEs were employed to obtain the varied current values towards various concentrations of H_2O_2 (Fig. S3A). The current increased gradually with an increasing concentration of H_2O_2. When the concentration of H_2O_2 exceeded 1.0 mM, the current response became steady (Fig. S3B). The current displays a good linear increase with H_2O_2 in the range 0.10 nM – 1.0 mM, and the calibration equation between the current (I) and the H_2O_2 concentration (c) is expressed as follows: I (μA)=2.2591+1.3189 lg$c$ (nM), R^2= 0.9989.

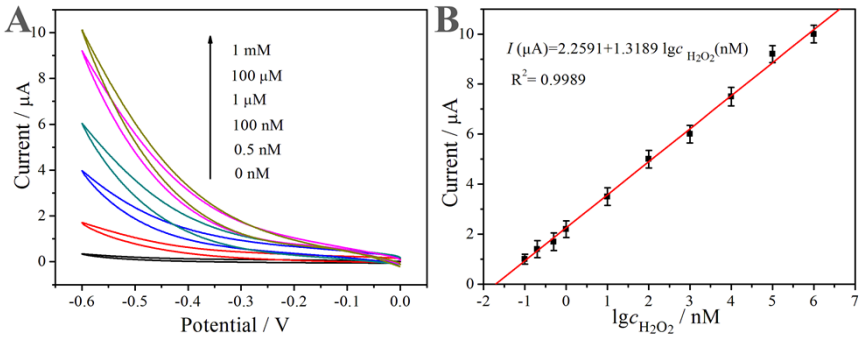


Fig. S3 The current responses at -0.6 V of the cancer cells immobilized Pt-PWE towards various concentrations of H_2O_2 in 10 mM PBS (pH 7.4) (A); calibration curve for H_2O_2 determination (B).

The cell concentration-dependent electrocatalytic current

Under the optimal conditions, we also have investigated the influence of the cell concentration on the electrode to the electrical signal of the releasing H_2O_2. And our modified Pt-PWEs were incubated with various concentrations of SK-BR-3 cells. Subsequently, cells were stimulated by PMA (100 mg mL^{-1}), and the varied current responses were recorded in Fig. S4. The current increases gradually with an increasing concentration of SK-BR-3 cells (Fig. S4A) and the current displays a good linear increase with the logarithm value of cell concentration cells H_2O_2 in the range of 1×10^2 to 5×10^6 cells mL^{-1} (Fig. S4B). The linear relation is not applicative when the cell concentration exceed 5×10^6 cells mL^{-1}. As cell is a nonconductive biomacromolecule and may obstruct electron transfer and mass trans-port of the electrochemical probe. The calibration equation between the current (I) and the H_2O_2 concentration (c) is expressed as follows: I (μA)= -1.3935+ 1.2795 $\lg c_{SK\text{-}BR\text{-}3}$ (cells mL^{-1}), R^2= 0.9987.

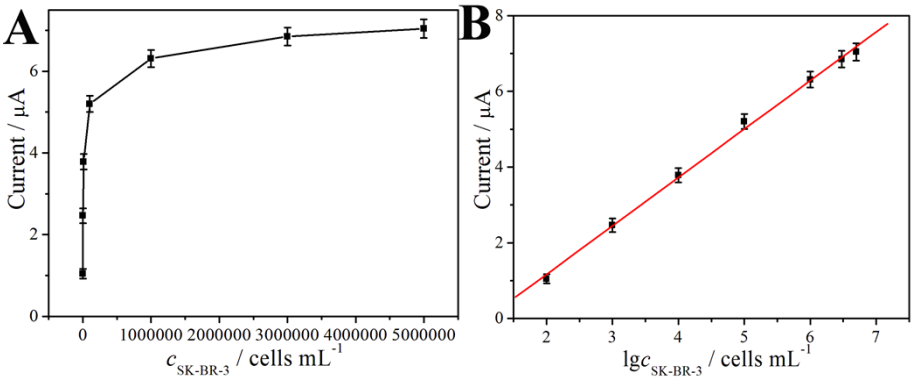


Fig S4 Relationship between current and SK-BR-3 cells concentration, each point is the average of five measurements (A); logarithmic calibration curve for SK-BR-3 cells (B).

Current analysis of the flux of H_2O_2 releasing from SK-BR-3 cells

Information on the mechanism of H_2O_2 release from cells can be inferred from the time-current response. The H_2O_2 determination was measured at different time after the PMA buffer was added to the cancer cells immobilized Pt-PWE. As shown in Fig. S5A, the current increased with the time varing from t_0 to t_1, demonstrating that a short period is needed for cancer cells to be stimulated completely. Then a stable current reached featuring the end of the event. So our H_2O_2 determination was measured after t_1 (8 s), so as to obtain the stable and accurate current value.

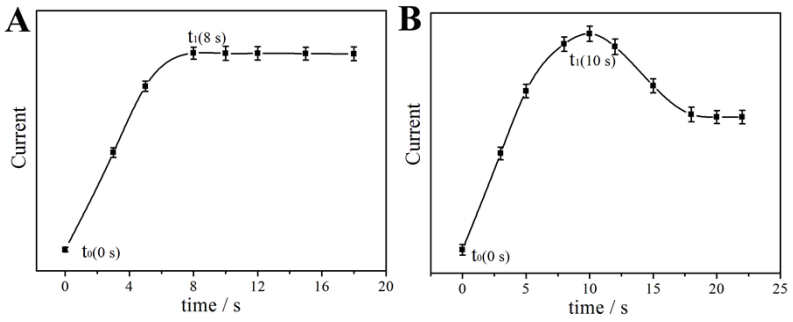


Fig. S5 The time-current response of H_2O_2 released from cancer cells induced by 100 ng mL^{-1} PMA based on our cancer cells immobilized Pt-PWE (A) and Pt nanospheres modified ordinary glassy carbon electrode (B).

As a comparation, Pt nanospheres modified ordinary glassy carbon electrode was applied for the determination of the flux of H_2O_2 releasing from SK-BR-3 cells. To achieve the electrochemical measurements, a conventional three electrode system was constructed: a modified glassy carbon electrode as the working electrode, Pt and Ag/AgCl electrode were used as the counter electrode and reference electrode, respectively. 2.0 mL of PBS solution containing PMA (100 mg mL^{-1}) was used as determination and stimulation buffer and time-current course was recorded in Fig.

S5B. It can be observed that the trend was similar to that of the curve in Fig. S5A with the time varying from t_0 to t_1. The current reduces gradually to a lower value beyond the time t_1 (10 s) due to the diffusion process of H_2O_2 released from SK-BR-3 cancer cells. The diffusion process of H_2O_2 lower the determination accuracy, and this can be largely improved by using our cancer cells immobilized Pt-PWE. It can be explained that our Pt nanospheres grown paper sample zone maintained good 3D interwoven and incompact cellulose fibers networks structure, allowing cells to adhere (highly affinitive binding with aptamers) to the cellulose fibers to achieve the in-situ and real-time determination of extracellular H_2O_2 (as shown in Scheme 1D). Only 10 μL of buffer is needed to the Pt-PWE, so H_2O_2 can be electrocatalyzed adequately when releasing from cancer cell and rarely diffuse away. Based on the above, our designed cancer cell immobilized Pt-PWE demonstrates some advantages in accuracy and sensitivity of H_2O_2 determination. To further confirm the superiority of our H_2O_2 biosensors, analytical performance of some other H_2O_2 biosensors has been collected in Table S1. As shown in Table S1, our strategy shows shorter response time and lower detection limit than other H_2O_2 assays as reported previously.

Table S1. Comparison analytical performance of some H_2O_2 biosensors

H_2O_2 biosensor	Response time /s	Detection limit /μM	Reference
HRP-Au colloid-cysteamine	15	0.58	[3]
nitrogen-doped graphene	20	0.05	[4]
HRP-Nafion-silica-sonogel-carbon	35	1.6	[5]

Pt grown paper working electrode	8	0.0001	This work

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