SUPPORTING INFORMATION

Paper Electrode-Based Flexible Pressure Sensor for Point-of-Care Immunoassay with Digital Multimeter

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TABLE OF CONTENTS

EXPERIMENTAL SECTION

Material and Reagent. Hexachloroplatinic (IV) acid hexahydrate ($H_2PtCl_6 \cdot 6H_2O$), L-ascorbic acid (AA), sodium carbonate (Na_2CO_3), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$), potassium phosphate monobasic (KH_2PO_4), sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide (NaN_3), hydrogen peroxide (H_2O_2, 30%) and Tween 20 were purchased from Sinopharm Chem. Re. Co., Ltd. (Shanghai, China). Pluronic F-127 was obtained from Sigma-Aldrich (St. Louis, MO, USA). Multi-walled carbon nanotubes (MWCNTs, diameter 10-20 nm, and length 1-2 μm) were the product of Shenzhen Nanotech Port Co., Ltd (Shenzhen, China). Filter paper acquired from Hangzhou Special Paper Co., Ltd (Hangzhou, China). Polydimethylsiloxane (PDMS, Sylgard 184) was supplied by Dow Corning (USA). Carcinoembryonic antigen standards (CEA), monoclonal anti-CEA antibody (Ab_1) and polyclonal anti-CEA antibody (Ab_2) were achieved from BiosPacific, Inc. (CA, USA). Human CEA enzyme-linked immunosorbent assay (ELISA) kit was purchased from Biocell Biotechnol. Inc. (Zhengzhou, China). Ultrapure water was obtained from a Milli-Q purification system (18.2 MΩ/cm, Millipore) and used in all runs. Phosphate-buffered saline (PBS, 0.1 M) solution was prepared by Na_2HPO_4 and KH_2PO_4 with 0.1 M NaCl.

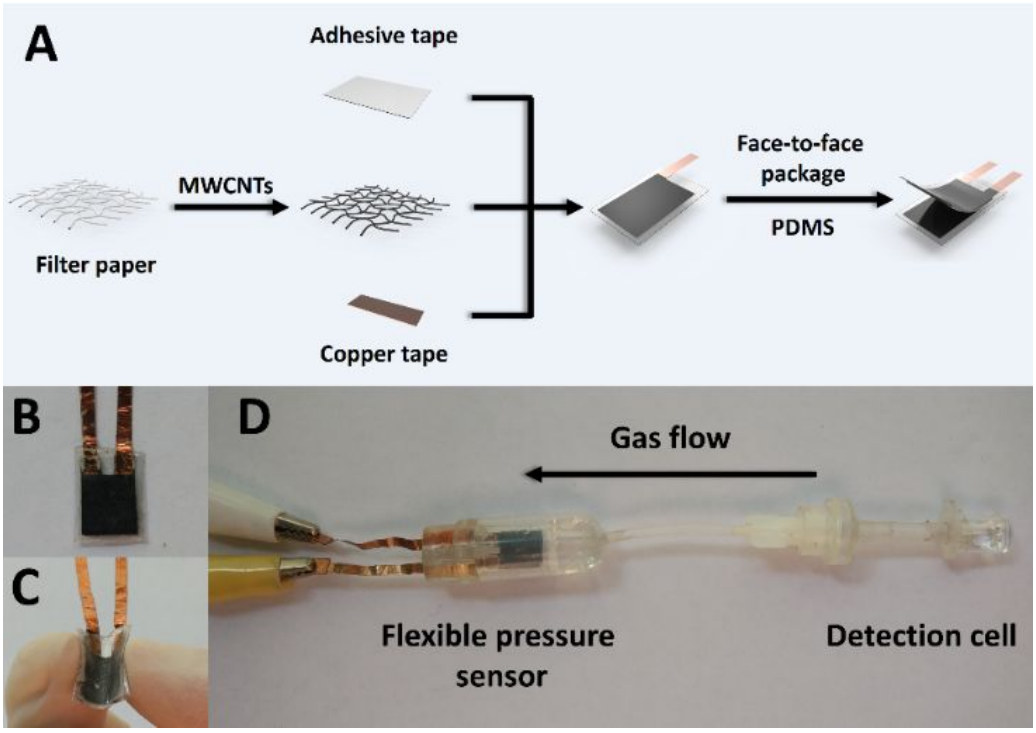
Preparation of Platinum Nanoparticles (PtNPs). PtNPs were synthesized following a previous report with a minor modification.[1] Initially, 10 mL of $H_2PtCl_6 \cdot 6H_2O$ solution (1 mM) and 1 mL of AA solution (0.4 M) were mixed in a vial. Then, the mixture was heated at 80 °C for 30 min. Finally, the synthesized PtNPs were cooled down and stored at 4 °C before use.

Preparation of PtNPs-Labeled Detection Antibody (Ab_2-PtNPs). The Ab_2-PtNPs were prepared according to the previously reported method with minor modifications.[2] Briefly, the pH of prepared PtNPs (5.0 mL) was adjusted to 9.0 using Na_2CO_3 solution (0.1 M). Then, 50 μL of Ab_2 (0.5 mg/mL) were added into the PtNPs solution. After shaking for 30 min at room temperature, the mixture was incubated at 4 °C overnight. Finally, the prepared Ab_2-PtNPs were collected by centrifugation (14000 g, 15 min, 4 °C), and redispersed in 3 mL of PBS solution (pH 7.4) containing BSA (1.0 wt %), Tween 20 (0.5 wt %) and NaN_3 (0.05 wt %). The Ab_2-PtNPs were stored at 4 °C for future usage.

Fabrication of Paper Electrode-Based Flexible Pressure Sensor. Briefly, MWCNTs (0.1

g) was added to 40 mL water containing Pluronic F-127 (2 wt %) and sonicated for 6 h. Then, the mixture was centrifuged (1000 g, 10 min) to remove the undispersed MWCNTs. Finally, a homogeneous MWCNTs solution was obtained for subsequent use.

The fabrication process of the flexible pressure sensor is shown in Scheme S1-A. Firstly, the filter paper was cut into a rectangle (0.8 × 1 cm^2) with a square (0.3 × 0.3 cm^2) on the side. Then the sheared filter paper was dipped into the above mentioned MWCNTs solution for 10 min and dried at 60 °C. The dipping and drying processes were repeated for several times until the electrical resistance of paper reached to ~60 kΩ (note: In this device, the contact resistance between the copper and the paper electrode was ~8.0 kΩ). Following that, the copper tape was attached to the square part of the filter paper, which was assisted with silver paste to reduce the electrical resistance. After it was dried at 60 °C, a piece of adhesive tape (1 × 1.5 cm^2) was covered on one side of the filter paper, and a paper electrode was complete. Finally, two pieces of prepared paper electrodes were face-to-face packaged and permanently sealed by a thin layer of PDMS after dried at 70 °C for 2 h. The completed flexible pressure sensor is shown in Scheme S1-B, and the bendability of the sensor is displayed in Scheme S1-C.



Scheme S1. (A) Schematic illustration of the fabrication process of the flexible pressure sensor; and photographs of (B) the prepared flexible pressure sensor; (C) the bendability of the flexible pressure sensor;

and (D) the fabricated detection system.

To measure the pressure of generated gas sensitively, the fabricated pressure sensor was sealed in a plastic tube by PDMS with the copper tapes exposed outside. Meanwhile, at the bottom of the tube, a catheter was combined to link the detection cell (high-binding microplate) with a small cap. In the airway, a leach was introduced to avoid the liquid overflowing from the detection cell. All of the gaps were sealed by PDMS except the detection cell. At last, the pressure sensor was linked with a digital multimeter (DMM) to measure the pressure change. The finished detection system was shown in Scheme S1-D.

Immunoreaction Protocol and Digital Multimeter Measurement. In a standard pressure-based immunoassay, 50 μL of CEA monoclonal antibody (10 μg/mL, Ab_1) was first added into a 96-well microtiter plate and incubated overnight at 4 °C. After being washed three times by washing buffer (0.01 M PBS containing 0.05 wt % Tween 20, pH 7.4), each well was blocked by 300 μL blocking buffer (0.01M PBS containing 0.05 wt % Tween 20 and 1.0 wt % BSA, pH = 7.4) for 1 hour at room temperature, and washed with washing buffer for three times before the target addition. Following that, 50 μL of CEA standards with different concentrations were added to the well and shaken on a shaker for 40 min at room temperature. After repeated washing, 100 μL of the Ab_2-PtNPs was added and incubated for 40 min at room temperature. The washing process was repeated three times and 100 μL of saturated H_2O_2 (30%) was added to react for 270 s. Finally, the pressure change was read from the DMM. All measurements were carried out at room temperature (25 ± 1.0 °C).

PARTIAL RESULTS AND DISCUSSION

Optimization of experimental conditions. In order to optimize the analytical performance of the proposed POC testing, several possible experimental parameters influencing the detection result should be investigated, including incubation time, catalytic reaction time of PtNPs, and the concentration of H_2O_2. In this case, 5 ng/mL CEA was used as an example and the conductivity change of the pressure sensor was directly monitored by a DMM (note: resistance change was used to indicate its conductivity change). As an immunoassay, the

incubation time was first optimized. As shown in Figure S1-A, along with the extending incubation time, the resistance change increased gradually and remained to a steady value after 40 min. Thus, 40 min was utilized as the incubation time. Obviously, the catalytic time of PtNPs was another crucial factor. As indicated in Figure S1-B, the resistance change initially increased with the increasing time and finally reached a plateau after 270 s. To shorten the detection time, 270 s of catalytic time was chosen as the optimal time. Moreover, the concentration of H_2O_2 was also studied. As depicted in Figure S1-C, with the concentration of increasing, the resistance change presented an upward trend until the H_2O_2 was saturated (20%) and then a plateau appeared. However, to simplify the preparation of solution, the saturated H_2O_2 (30%) was chosen as the suitable concentration.

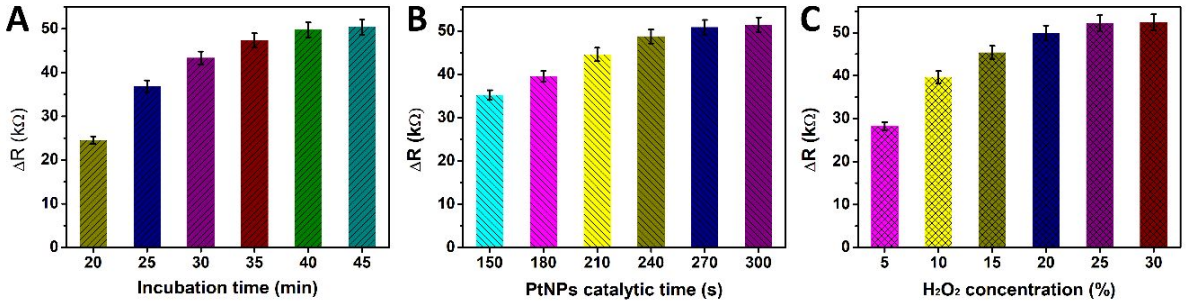


Figure S1. Effects of (A) incubation time; (B) PtNPs catalytic time; and (C) H_2O_2 concentration on the response of the pressure-based POC testing (5 ng/mL CEA used in this case).

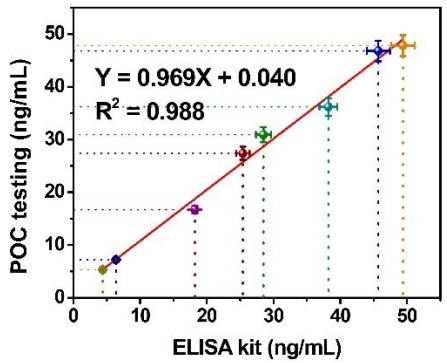


Figure S2. Comparison of the results for human serum specimens obtained by the pressure-based POC testing and human CEA ELISA kit, respectively.

References

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