SUPPORTING INFORMATION

Saw-toothed microstructure-based flexible pressure sensor as the signal readout for point-of-care immunoassay

Zhenzhong Yu, Guoneng Cai, Ping Tong and Dianping Tang*

Key Laboratory of Analytical Science for Food Safety and Biology (MOE & Fujian Province), Department of Chemistry, Testing Center, Fuzhou University, Fuzhou 350116, People's Republic of China

CORRESPONDING AUTHOR INFORMATION

Phone: +86-591-2286 6125; fax: +86-591-2286 6135; e-mail: dianping.tang@fzu.edu.cn (D. Tang)

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EXPERIMENTAL SECTION

Material and Reagent. Iron chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), pyrrole, methanol, octadecyltrichlorosilane (OTS), methylbenzene, 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), potassium chloride (KCl), bovine serum albumin (BSA), hydrogen peroxide (H_2O_2, 30%) and Tween 20 were purchased from Sinopharm Chem. Re. Co., Ltd. (Shanghai, China). Polydimethylsiloxane (PDMS, Sylgard 184) was supplied by Dow Corning (USA). Carcinoembryonic antigen standards (CEA), monoclonal anti-CEA antibody (as the capture antibody, cAb) and polyclonal anti-CEA antibody (as the detection antibody, dAb) 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. The pH 7.4 phosphate-buffered saline (PBS, 0.01 M) solution was prepared by adding $Na_2HPO_4 \cdot 12H_2O$ (2.9 g), KH_2PO_4 (0.24 g), KCl (0.2 g), and NaCl (8.0 g) into 1000 mL water.

Fabrication of the Flexible Pressure Sensor. To prepared the patterned PDMS film, a mould with saw-toothed array was directly printed by a 3D printer (Figure S1). After the mould was treated with 2% OTS solution in methylbenzene for 30 min and washed with methylbenzene, the PDMS mixture of base and cross-linker in a 10:1 ratio (w/w) was coated on the mould surface and degassed in vacuum for 20 min to remove bubbles. After incubated at 70 ^{0}C for 2 h, a patterned PDMS thin film with saw-toothed array could be peeled off from the mould.

The polypyrrole/PDMS (PPy/PDMS) conductive film was prepared by conventional chemical polymerization. First, the PDMS film was washed with ethanol and water. Then, a piece of PDMS (2 × 2 cm^2) was inserted into the methanol/H_2O solution (18 mL, v/v = 1:1), which contained 0.324 g of $FeCl_3 \cdot 6H_2O$. After the temperature of the solution was dropped to about 4 ^{0}C in a refrigerator, 120 μL of pyrrole dissolved in 2 mL of methanol/H_2O solution (v/v = 1:1, 4 ^{0}C) was added. After reacting at 4 ^{0}C for 6 h, the obtained PPy/PDMS conductive film

was washed with ethanol and water for several times. Finally, the film was dried at 60 ℃ for subsequent use.

After the PDMS film was modified with PPy, the conductive film was wired out using copper wires and silver glue. Two pieces of such PPy/PDMS film were assembled face-to-face, whose orientations were perpendicular to each other. Subsequently, the surface of the assembled films was permanently sealed by a thin layer of PDMS to isolate the air. And then, the prepared flexible pressure sensor was placed into a homemade vessel printed by a 3D printer, which was linked with the detection cell (*i.e.*, separable high-binding polystyrene microwell). In the same way, the device was sealed by resin (obtained from the 3D printer) to form an airtight environment. Finally, a digital multimeter was employed to monitor the signal of the pressure sensor. This completed device was shown in Figure 2B.

Preparation of Platinum Nanoparticles (PtNPs). PtNPs were synthesized following a previous report with a minor modification.[1] Initially, 5 mL of $H_2PtCl_6 \cdot 6H_2O$ solution (1 mM) was heated at 80 °C for 20 min. Then 0.5 mL of AA solution (0.4 M) was added and continue incubated at 80 °C for 30 min. Finally, the synthesized PtNPs were cooled down and stored at 4 °C before use.

Preparation of PtNP-Labeled Detection Antibody (dAb-PtNP). The dAb-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 dAb (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 dAb-PtNPs were collected by centrifugation (14000 g, 15 min, 4 °C), and redispersed in 3 mL of PBS solution (0.01M, pH 7.4) containing BSA (1.0 wt %) and Tween 20 (0.05 wt %). The dAb-PtNPs solution was stored at 4 °C for future usage.

Immunoreaction Protocol. In a standard pressure-based immunoassay, 50 μL of cAb (10 μg/mL,) was first added into a separable high-binding polystyrene microwell 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 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 wells and shaken on a shaker for 1 h at room temperature. After repeated washing, 100 μL of the dAb-PtNPs was added and incubated for 30 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 8 min. Finally, the pressure change was read from the digital multimeter (DDM).

APPARATUS

The vessel and mould were printed by a 3D printer (Formlabs2, USA). The scanning electron microscopy (SEM) images were acquired from the FEI Quanta 250 (Field Electron and Ion Company, USA). The Raman spectra were characterized by a Raman spectrometer (Renishaw, UK). The transmission electron microscope (TEM) image was obtained from HT-7700 (Hitachi, Japan). The dynamic light scattering (ZEN5600, Malvern, UK) was used to estimate the size of PtNPs. All the electrochemical measurements were performed by CHI850D electrochemical workstation (Shanghai Chenhua Instrument Corporation, China) with an applied potential of 1.0 V. In addition, a digital multimeter VICTOR 86E (Victor, China) was used to measure to resistance change in immunoassay.

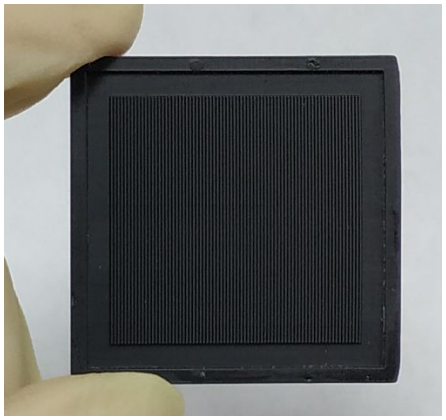

Figure S1. The photograph of the mould with saw-toothed microstructure.

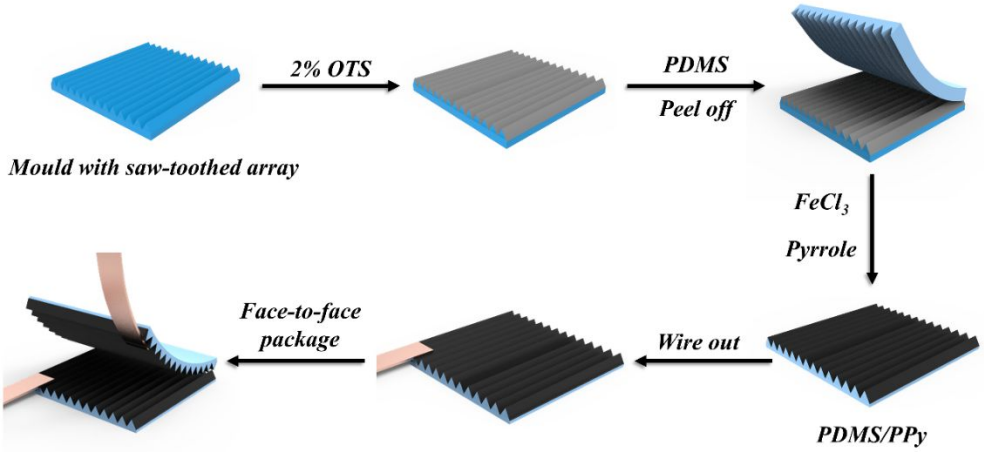

Figure S2. Schematic illustration of the fabrication of the flexible sensor with the saw-toothed array.

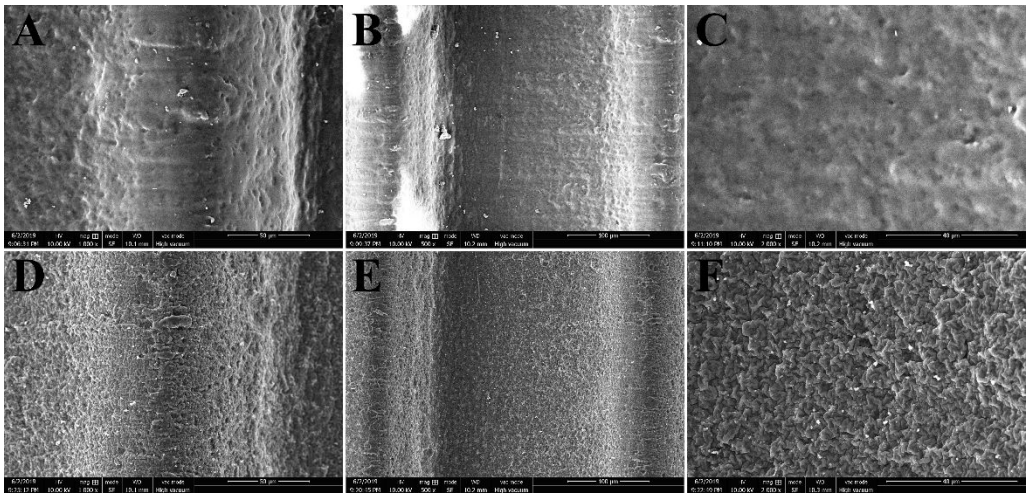

Figure S3. The SEM images of different parts of the PDMS microstructure and PPy/PDMS microstructure:

(A and D) peak, (B and E) groove, and (C and F) bevel, respectively.

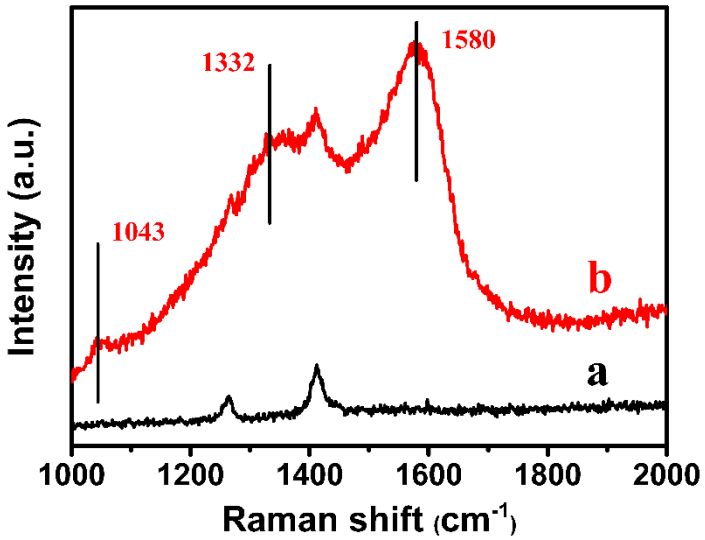

Figure S4. The Raman spectra of (a) PDMS and (b) PPy/PDMS.

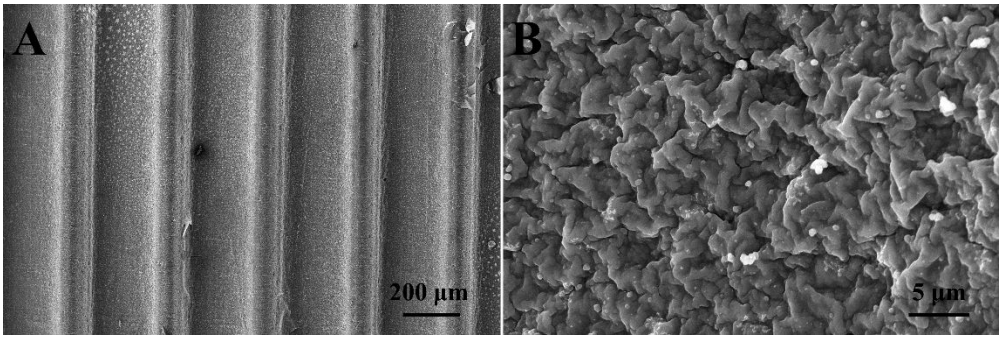

Figure S5. (A) The SEM images of PPy/PDMS film; (B) the magnification image of PPy/PDMS film.

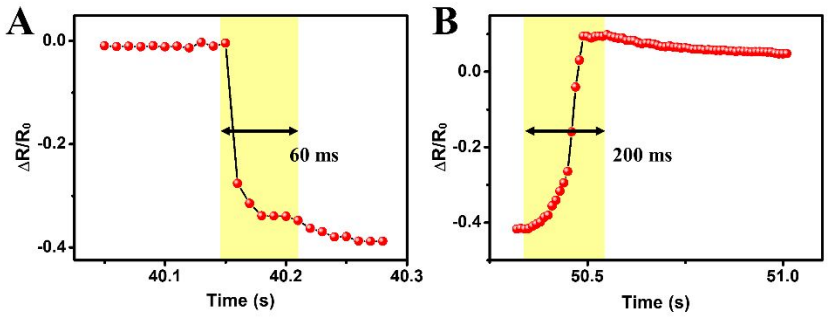

Figure S6. (A) The response time and (B) releasing time of the pressure sensor with an applied pressure of 1 kPa.

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, the 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 resistance change of the pressure sensor was directly monitored by a DMM. As an immunoassay, the incubation time was first optimized. As shown in Figure S7A, along with the extending incubation time, the resistance change increased gradually and remained to a steady value after 30 min. Thus, 30 min was utilized as the incubation time. Obviously, the catalytic time of PtNPs was another crucial factor. As indicated in Figure S7B, the resistance change initially increased with the increasing time and finally reached a plateau after 8 min. To shorten the detection time, 8 min of catalytic time was chosen as the optimal time. Moreover, the concentration of H_2O_2 was also studied. As depicted in Figure S7C, 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 the solution, the saturated H_2O_2 (30%) was chosen as the suitable concentration.

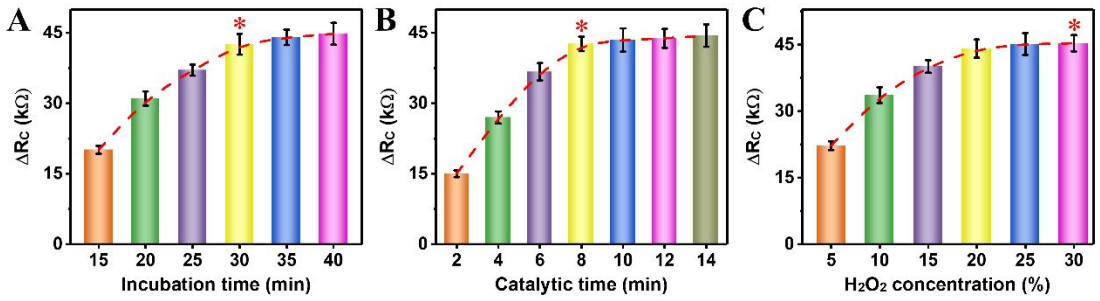

Figure S7. Effects of (A) incubation time; (B) PtNPs catalytic time; and (C) H_2O_2 concentration on the response of the pressure-based POC testing (5 ng/mL CEA used in this case).

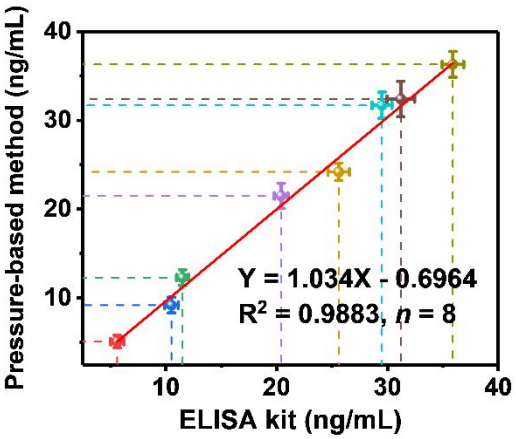

Figure S8. Comparison of the results for human serum samples obtained between the pressure-based immunoassay and the referenced CEA ELISA kit.

SUPPORTING VIDEO CAPTION

Video S1. The response of the pressure sensor for air pressure.

References

(1) Ji, T. H.; Liu, D.; Liu, F.; Li, J. X.; Ruan, Q. Y.; Song, Y. L.; Tian, T.; Zhu, Z.; Zhou, L. J.; Lin, H.; Yang, C. Y.; Wang, D. A pressure-based bioassay for the rapid, portable and quantitative detection of C-reactive protein. *Chem. Commun.* **2016**, *52*, 8452-8454..

(2) Gao, Z. Q.; Ye, H. H.; Tang, D. Y.; Tao, J.; Habibi, S.; Minerick, A.; Tang, D. P.; Xia, X. H. Platinum-Decorated Gold Nanoparticles with Dual Functionalities for Ultrasensitive Colorimetric in Vitro Diagnostics. *Nano Lett.* **2017**, *17*, 5572-5579.