Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Detection of The Effect of Polydopamine (PDA)-Coated Polydimethylsiloxane (PDMS) Substrates on The Release of H_2O_2 from Single Hela Cell

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Experimental

Chemicals

Potassium ferricyanide ($K_3[Fe(CN)_6]$), ferric chloride ($FeCl_3$), hydrogen peroxide(30%) (H_2O_2), and potassium chloride were purchased from Aladdin-Reagent Co. Ltd. (China). Dopamine, ascorbic acid (AA), lactic acid (LA), uric acid (UA), and glucose were provided by Aladdin-Reagent Co. Ltd. (China). Phorbol myristate acetate (PMA) was purchased from Beijing Solarbio Science & Technology Co., Ltd. (China). Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), trypsin digestion solution, and phosphate buffer saline (PBS) were provided by GIBCO Invitrogen Corp. Mitochondrial Membrane Potential Detection Kit (JC-1) were obtained from Beyotime Institute of Biotechnology (China).

Cell culture Human cervical cancer (HeLa) cells were cultured in high glucose-Dulbecco's modified eagle medium (DMEM) containing 15% fetal bovine serum (FBS), 1% penicillin and streptomycin at 37 °C in a humidified incubator (95% air with 5% CO_2).

Preparation of polydimethylsiloxane (PDMS) substrate modified by polydopamine

A mixture of poly(dimethylsiloxane) (PDMS) prepolymers and curing agent was prepared at ratio of 10:1, which was then placed in a vacuum chamber to remove air bubbles, followed by pouring onto a petri dish and then baking at 70 ^oC for 2h to form the PDMS membrane. Then, the as-prepared PDMS membrane was putted in 40 mL Tris-HCl buffer solution (pH 8.5) containing dopamine (160 mg) for 3 hours for modification with polydopamine via self-polymerization of dopamine.

Isolation and culture of single cells

A microhole chip with an array of microhole in a diameter of 40 μm was designed to sort individual HeLa cell. Prior to use, the microhole chip was sterilized by ultraviolet irradiation for 30 min. Then, it was placed on the bottom of a petri dish containing culture medium overnight. Afterwards, a certain volume of single cells suspension was added. After natural cells sedimentation and low-speed centrifugation or natural settlement inside the microholes, single cell inside the microhole adhered on the bottom of the dish. The adherent single cells were cultured for 24 h, then the microhole chip was gently removed.

Preparation of Prussian blue (PB)-modified gold microelectrode and detection of H_2O_2

Gold microelectrode (1 μm in the tip diameter, Rui Ming, Jiangsu, China) was placed in 0.1 M H_2SO_4 for electrochemical pretreatment. Afterwards, PB nanoparticles were modified on the the pretreated gold microelectrode by chemical modification. In brief, the gold microelectrode was placed in a mixed solution (pH 3.0) containing 25 mM $K_3[Fe(CN)_6]$ and 25 mM $FeCl_3$ for three minutes. After the surfaces of the gold microelectrode were modified with PB, it was rinsed twice with dilute HCl, and the PB-modified microelectrode was placed in an oven at 85^oC for 2 h, and then taken out for use as the working electrode.

Detection of extracellular H_2O_2 and MMP of single Hela cell attached on different substrates under high glucose culture conditions

The single HeLa cells were attached on the PDA-coated PDMS and the PDMS substrate,

respectively. Electrochemical detection of H_2O_2 released by single Hela cell was performed in a solution of 0.1 M PBS (pH 7.4) via the *I-t* curve method. After the baseline was stabilized, the single cell on different substrates was stimulated with 0.3 μM PMA to produce endogenous H_2O_2. For MMP imaging, the cell was firstly incubated with JC-1 staining solution for 20 minutes, and washed twice with buffer solution. Then, 2 mL 0.1 M pH 7.4 PBS buffer solution (treated with nitrogen gas deoxygenation) was added, and a certain amount of 0.3 μM PMA was pushed around the cells through a micro-injection tube. The changes of cell MMP were observed under the fluorescent microscope.

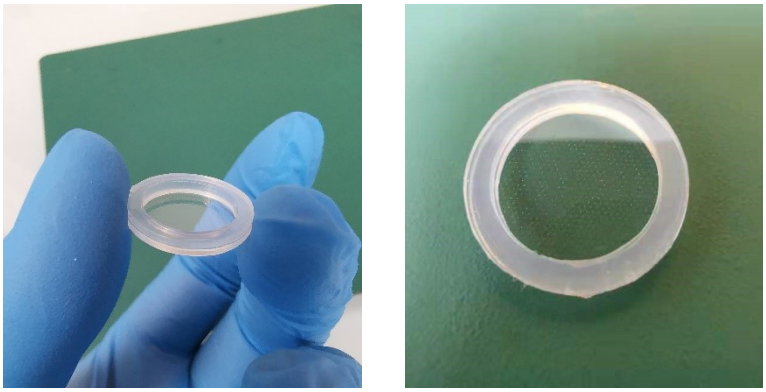

Figure S1. The photographs of the microfluidic-based microhole chip.

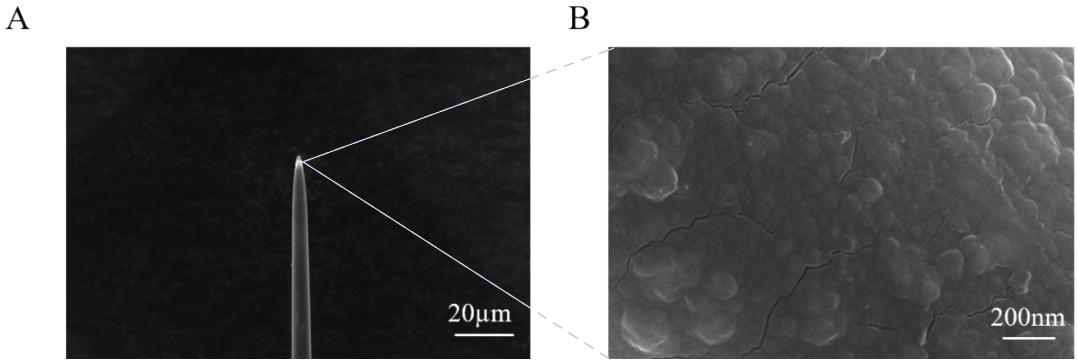

Figure S2. SEM images of the PB-modified Au microelectrode.

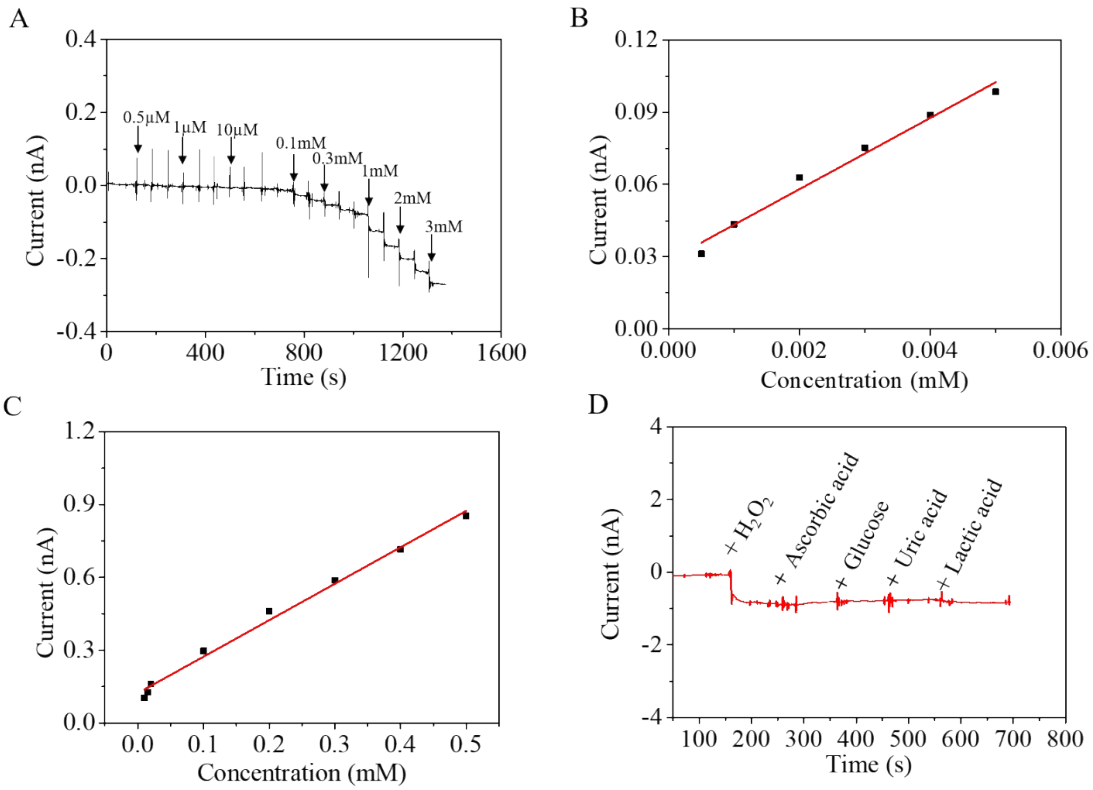

Figure S3. Time-current response of a PB-modified gold microelectrode in 0.1 M PBS (pH 7.4) with continuous dropwise addition of H_2O_2 (arrows indicate cumulative concentrations). E = 0.1 V vs Ag/AgCl. (B) Linear plot of response current versus H_2O_2 in the concentration range of 0.0005 mM-0.005 mM. (C) Linear plot of response current versus H_2O_2 in the concentration range of 0.1 mM-0.5 mM. (D) Time-current response of a PB-modified gold microelectrode in 0.1 M PBS (pH 7.4) with continuous dropwise addition of 0.5 mM H_2O_2, 0.5 mM ascorbic acid, 0.5 mM glucose, 0.5 mM uric acid and 0.5 mM lactic acid. E = 0.1 V vs Ag/AgCl.

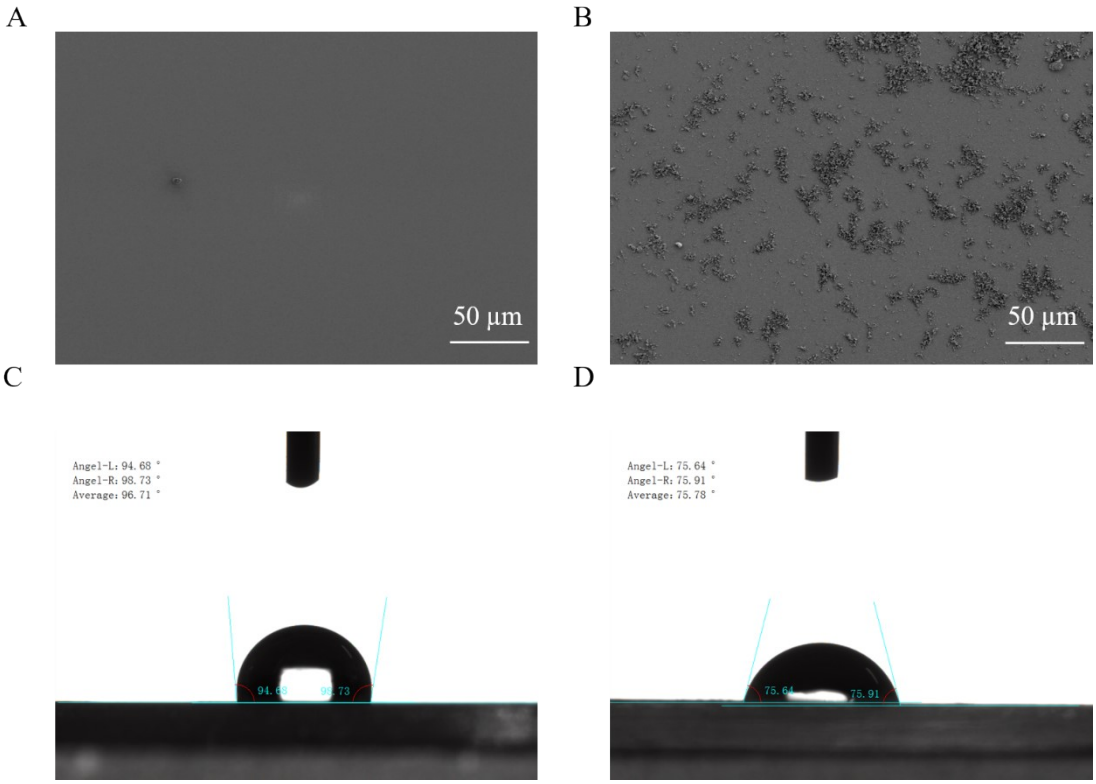

Figure S4. SEM images of blank PDMS (A) and polydopamine-modified PDMS (B); water contact angle images of blank PDMS surface (C) and polydopamine-modified PDMS surface (D).

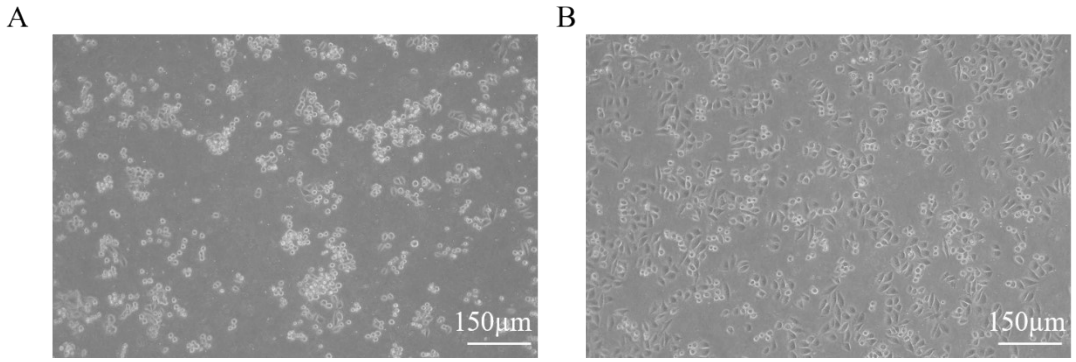

Figure S5. (A) Shapes of HeLa cells to blank PDMS. (B) Shapes of HeLa cells to the surface of polydopamine-modified PDMS. Microscope image (x100) taken after HeLa cells were cultured for 24 hours.