Electronic Supplementary Material (ESI) for Analyst

Annexin-V modified QCM sensor for label-free and sensitive detection of early stage apoptosis

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Materials and methods

1. Materials and instrumentation

O-phenylenediamine (*o*-PD) was purchased from J&K Scientific Ltd., China. Biotin-Annexin-V was purchased from Roche. Camptothecin (CAM) was purchased from Life Technology (USA). Other reagents were of analytical grade and used as received. Ultrapure water (18.2 MΩ·cm resistivity) produced from a Millipore Milli-Q system was used throughout. Phosphate-buffered saline (PBS) solution consisting of 136.7 mmol L^{-1} NaCl, 2.7 mmol L^{-1} KCl, 9.7 mmol L^{-1} Na_2HPO_4, 1.5 mmol L^{-1} KH_2PO_4 was used. The buffer used for the dilution of Annexin-V contains 10 mM HEPES/NaOH (pH 7.4), 140 mM NaCl and 5 mM $CaCl_2$.

AT-cut 9 MHz quartz crystals (12.5 mm diameter) with gold electrodes (6.0 mm diameter) formed uniformly on both sides were used. The resonant frequency of a quartz crystal resonator was measured with a Research Quartz Crystal Microbalance (RQCM) system (Maxtek INC., USA). Electrochemical experiments were performed on a CHI660A electrochemical workstation (Chenhua Instrument Company, Shanghai, China) with a conventional three-electrode system.

2. Cell culture

Jurkat cells (T-cell, human acute lymphoblastic leukemia) were purchased from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai,

China). The cells were cultured in 25 cm^2 cell culture flasks (Corning) at 37 °C with 5% CO_2 in air atmosphere. RPMI-1640 medium (Gibco) was supplemented with 10% fetal bovine serum (Gibco) and 100 U mL^{-1} penicillin-streptomycin. The cell density was determined with a hemacytometer. After the density of cells reached 5×10^6 cells mL^{-1}, they were collected by centrifugation at 1500 g for 5 min, washed with fresh medium, and resuspended in fresh medium for further use.

For fluorescence microscopy investigations, the cells captured on quartz crystal surface were treated with Ho33342 (10 μL, 0.1 mg mL^{-1})/PI (5 μL, 1 mg mL^{-1}) for 5 min. Then the cells were washed with PBS. The fluorescence microscopy images of cells were taken under UV and green light irradiation, respectively, with the Leica DMI4000B microscope.

3. P-oPD membrane synthesis and characterization

Recrystallization was applied before *o*-PD was used. Electroplate liquid containing *o*-PD 0.01 mol L^{-1}, NaCl 0.4 mol L^{-1}, HCl 0.1 mol L^{-1} was deoxidized by nitrogen (N_2) before use. P-*o*PD membrane was synthesized by electropolymerization of *o*-PD to one side gold electrode on the QCM with cyclic voltammetry (scan rate: 100 mV s^{-1}; potential range: –0.25 ~ 0.8 V; cycle number: 85). The surface morphology and the thickness of the P-*o*PD membrane were characterized with scanning electron microscopy (SEM).

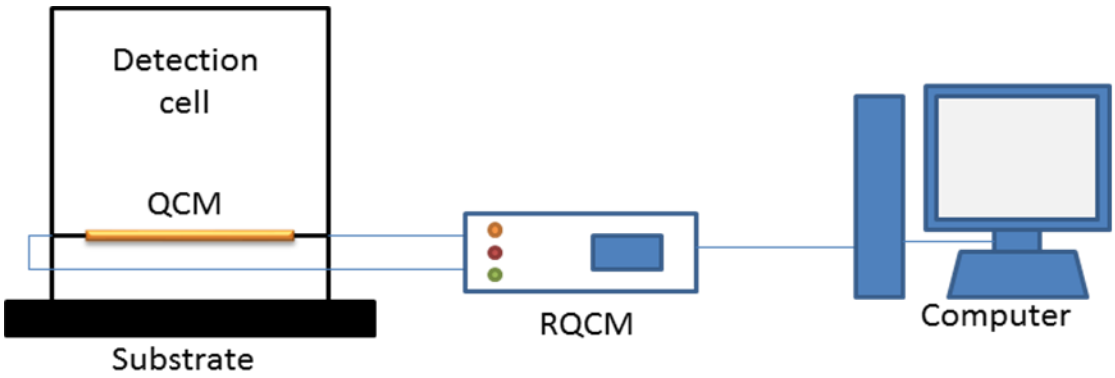

Fig. S1 Schematic diagram of the apparatus for QCM detection of apoptotic cells.

As shown in Fig. S1, after the P-*o*PD membrane was synthesized on the quartz crystal, the bare gold electrode side of the quartz crystal was sealed with a short plastic tube (2 mm length), and the other side was then combined with a long plastic tube (10 mm length; 12 mm inner diameter) which was used as the detection cell. The whole device was fixed on a substrate.

4. Combining Annexin-V to the P-oPD membrane

Streptavidin (SA, 300 μL, 0.1 mg mL^{-1}) was bound to the P-*o*PD membrane by glutaraldehyde (0.25%, *v*/*v*) crosslinking (1 h). The sensor was washed with PBS for 3 times, and 300 μL biotin-Annexin-V (20 μL in 1 mL incubation buffer) was then combined to SA. All the steps of modifications were recorded by QCM in real-time.

5. Apoptosis detection

One milliliter of the buffer used for the dilution of Annexin-V bulk solution was added into the QCM detection cell (shown in Fig. S1). When the resonant frequency became stable, apoptotic cells (10 μL in culture medium) that had been incubated with CAM (4 μg mL^{-1}) for 4 h were injected to the buffer. Once the resonant frequency came to stable again, the experiment was stopped and the data were recorded. Normal Jurkat cells were used as control.

After one test, the QCM sensor was treated with H_2SO_4/H_2O_2 (3:1) to get rid of the proteins (Annexin-V and streptavidin) and the P-*o*PD membrane bound on the surface, then the quartz crystal was washed with water, alcohol and water for 3 times, and dried under nitrogen. The frequency of the sensor was restored after these treatments and thus the quartz crystal resonator could be used repeatedly.

Results and discussion

1. Thickness of the P-oPD membrane

The thickness of the P-oPD membrane on the quartz crystal gold surface was characterized with SEM, it can be seen from Fig. S2 that the thickness of the membrane was about 5 μm.

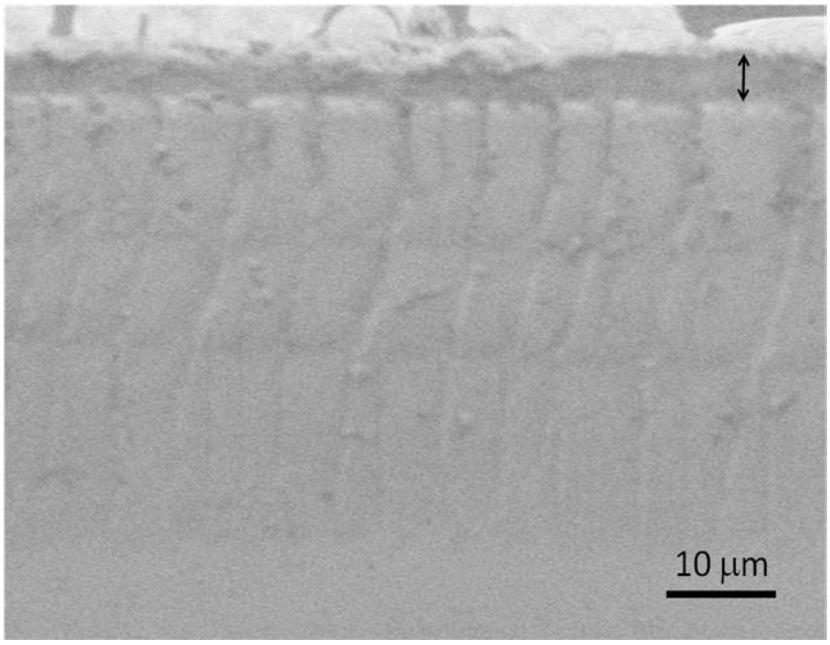

Fig. S2 SEM image of the side face of the P-*o*PD modified QCM. The double-sided arrow shows the thickness of the P-*o*PD membrane.

2. Flow cytometry analysis of apoptosis

Annexin-V-FITC/PI apoptosis analysis kit purchased from Roche Co. Ltd., Switzland was used according to the manufactory's instructions. For the Jurkat cells incubated with camptothecin for 4 h, the apoptosis/normal/death ratio was measured to be 40.0%/59.7%/0.3%.