Supplementary Material (ESI) for Chemical Communications

An Effective DNA-based Electrochemical Switch for Reagentless Detection of Living Cells

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Experimental Section

Chemicals and Materials

DNA oligonucleotides labeled with ferrocene were obtained from TaKaRa Biotechnology (Dalian) Co., Ltd. The sequences of employed oligonucleotides are as follows: probe, 5′-ferrocene-C6-AGACAAGGAAAATCCTTCAATGAAGTGGGT-C6-HS-3′.The probe DNA was modified at the 3′-terminus with a C_6-disulfide [HO-$(CH_2)_6$-S-S-$(CH_2)_6$-] linker and at the 5′-end with ferrocene. 6-Mercapto-1-hexanol (MCH), methyleneblue(MB) and tris(2-carboxyethyl) phosphine hydrochloride (TCEP) were from Sigma. Acridine orange (AO) and ethidium bromide (1% w/v, EB) were purchased from Amresco (USA). The phosphate buffered saline (PBS) (pH 7.4) contained NaCl (100 mM), Na_2HPO_4 •$12H_2O$ (10 mM), and NaH_2PO_4 (10 mM). All the other chemicals were of analytical grade and used as received. All aqueous solutions were prepared with ultrapure water.(Milli-Q, Millipore)

Cell Line and Culture.

The SMMC-7721 cell line was kindly provided by the Gulou Hospital, Nanjing, China. SMMC-7721 cells were cultured in RPMI 1640 medium (GIBCO) supplemented with fetal bovine serum (10%) (FBS, GIBCO), penicillin (60 μg mL^{-1}), and streptomycin (100 μg mL^{-1}) at 37℃ in a humidified atmosphere containing CO_2 (5%). After 48 h, the cells were collected and separated from the medium by centrifugation at 1500 rpm for 5 min and then washed twice with sterile PBS (pH 7.4). The sediment was resuspended in PBS to obtain a homogeneous cell suspension at a certain concentration. The cell number was determined using a Petroff Hausser cell counter (USA).

Preparation of the DNA-Modified Electrode.

The substrate gold electrodes (2 mm in diameter, CH Instruments Inc.) were first polished on microcloth (Buehler) with Gamma micropolish deagglomerated alumina suspension (0.05 μm) for 5 min. These electrodes were then thoroughly washed by

ultrasonicating in ethanol and Milli-Q water for 5 min, respectively. Finally, the electrodes were electrochemically cleaned in 0.5 M H_2SO_4 to remove any remaining impurities. After drying with purified nitrogen, the gold electrode was immersed in a solution of 1 μM probe DNA, 10 mM Tris HCl, 1 mM EDTA, 1.0 M NaCl, and 1 mM TCEP (pH 8.0), for 16 h, followed by a 1.5 h treatment with an aqueous solution of 1 mM spacer thiol molecules, MCH. Following this, the electrodes were rinsed with pure water and dried again with nitrogen for future use.

Cell Immobilization.

Transfection was based on a well-established procedure described by Sambrook et al[1] with a little modification. Briefly, the above-prepared DNA-modified gold electrode was immersed in the mixed solution which contains 12.5 μL of 2 × HBS (1.6 g of NaCl, 0.074 g of KCl, 40.2 mg of Na_2HPO_4 $7H_2O$, 0.2 g of glucose, 1.0 g of HEPES/100 mL, pH 7.05), 9.4 μL of H_2O, and 100 μL of SMMC-7721 cell suspension. Then, 3.1 μL of 1 M $CaCl_2$ was slowly added to the mixed solution with vigorous stirring. Finally, they were incubated at 37°C for 6 h. Since the DNA molecules, which had been immobilized on the surface of the gold electrode, were transfected into the SMMC-7721 cells, the cells were thus assembled onto the surface of the gold electrode. After carefully rinsing with 0.1 M PBS (pH7.4) containing 1 mM EDTA to remove the non-captured cells, the SMMC-7721 cell-assembled electrode was obtained and could be used for the following experiments.

Observation with an Inverse Microscope.

A DMIRE2 inverted fluorescence microscope (Leica, Germany) equipped with a DP71 CCD (Olympus, Japan) was used for fluorescence microimaging.

Electrochemical Measurements.

Electrochemical impedance spectroscopy (EIS) experiments were carried out on a PGSTAT30/FRA2 system (Autolab, Netherlands) in a $K_3Fe(CN)_6/K_4Fe(CN)_6$ (5 mM, 1:1) mixture with KCl (0.1M) as the supporting electrolyte, within the frequency range of 1×10^{-2}~10^6 Hz. Cyclic voltammetry (CV), and differential pulse voltammetry (DPV) were performed on a model 660C electrochemical workstation (CH Instruments), with 10 mM PBS (pH 7.4). A conventional three-electrode system consisting of the working electrode, saturated calomel reference electrode (SCE), and platinum counter electrode was used for all the electrochemical measurements.

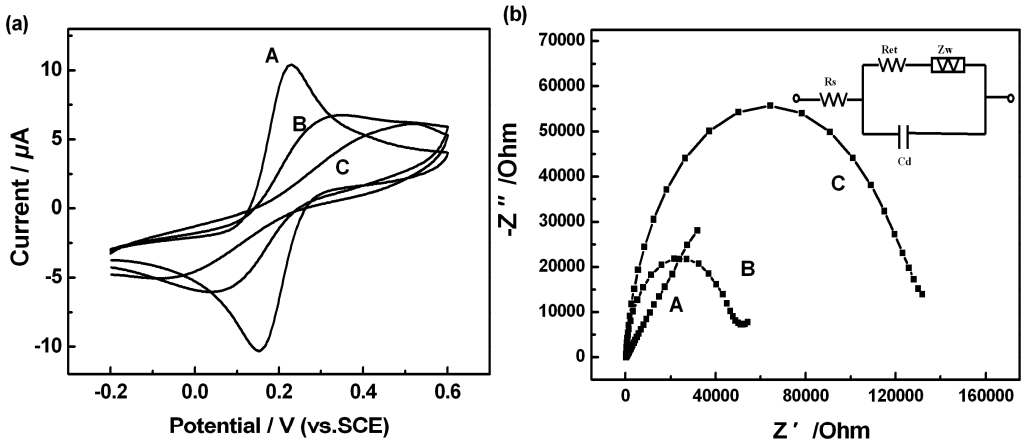

Figure S1. Cyclic voltammograms (a) and the Nyquist plots (b) obtained for bare (A), thiolated ssDNA and MCH immobilized (C), and incubation with SMMC-7721 modified (B) electrodes at 100 mVs^{-1} in 1 mM $[Fe(CN)_6^{3-/4-}]$ and 0.1M KCl solution. The concentration of SMMC-7721 was 10^5 cell mL^{-1} in PBS (0.1 M, pH 7.4).

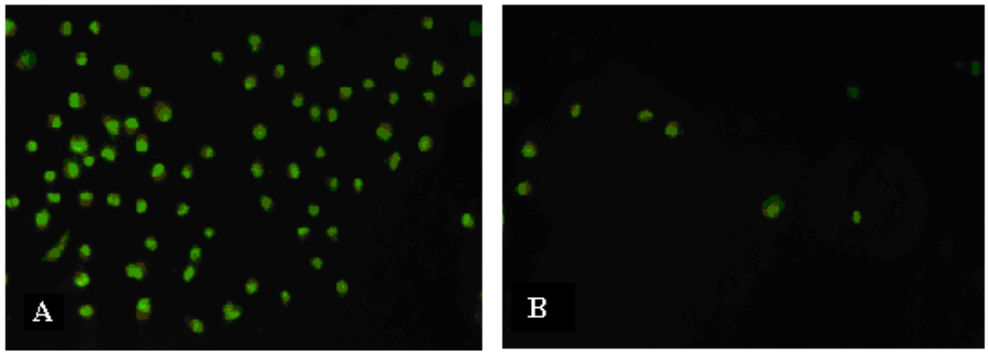

Figure S2. Photos of SMMC-7721 cells cultivated on A) Fc-DNA modified Au electrode B) bare Au electrode, observed with inverted fluorescence microscope.

REFERENCES

(1) J. Sambrook, D.W. Russell. Molecular Cloning: A Laboratory Mannual, 3rd ed.2001 by Cold Spring Harbor Laboratory Press.