Electronic Supplementary Materials for

Mesoporous silica film assisted amplified electrochemiluminescence for cancer cell detection

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EXPERIMENTAL SECTION

Materials. Bovine serum albumin (BSA), Tetraethyl orthosilicate (TEOS), (3-aminopropyl)triethoxysilane (APTES), luminol was purchased from Sigma-Aldrich (St. Louis, MO). Cetyltrimethylammonium bromide (CTAB) and other routine chemicals were purchased from Nanjing Chemical Co. Ltd.. $HAuCl_4$ was from Shanghai Chemical Reagent (Shanghai, China). ECL detection solution was 0.1 M phosphate buffered saline (PBS, pH 7.4) containing 3 mM luminol, 50 mM H_2O_2, and 0.1 M NaCl. All solutions were prepared using Millipore (model milli-Q) purified water and stored at 4 ºC in a refrigerator. All the other chemicals were of analytical grade. Indium tin oxide (ITO) -coated (thickness, ~100 nm; resistance, ~10 Ω/square) aluminosilicate glass slides were purchased from CSG (Shenzhen, China).

DNA oligonucleotides were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China) and their sequences are given below:

5'-amino-modified aptamer- DNA for HL-60 cells (aptamer): 5'-NH_2-TTT ATC CAG AGT GAC GCA GCA TGC CCT AGT TAC TAC TAC TCT TTT TAG CAA A CG CCC TCG CTT TGG ACA CGG TGG CTT AGT-3'

ssDNA: CCA CCG TGT CCA AAG CGA GGG CGT TTG CTA AAA.

Instruments. The electrochemical and ECL emission curves were obtained on a MPI-E multifunctional electrochemical and chemiluminescent analytical system (Xi'an Remax Electronic Science &Technology Co. Ltd., Xi'an, China) with a conventional three-electrode system at room temperature. It consists of ITO electrode as working electrode, a Pt wire counter electrode and a saturated calomel reference electrode (SCE). The spectral width of the photomultiplier tube (PMT) was 350–650 nm, and the voltage of the PMT was set at −500 V in the process of detection. Transmission electron microscopy (TEM) images were performed with a JEOL model 2000 instrument operating at 200 kV accelerating voltage to characterize the sensing surfaces. Scanning electron microscopy (SEM) images were obtained using an S-3000 N scanning electron microscope (Tokyo, Japan). The elemental compositions of deposited materials were determined using an SEM fitted with an energy dispersive X-ray analyzer (EDS). EIS measurements were performed on an Autolab PGSTAT12 (Ecochemie, BV, The Netherlands) controlled by GPES 4.9 and FRA 4.9 software.

Mesoporous silica thin film modified ITO electrode. Vertical silica array modified ITO electrode was prepared following a published protocol with minor modifications[S1]. Briefly, an ITO slice was cleaned by immersion in a boiling solution of 2 M KOH in 2-propanol for 20 min, followed by washing with milli-Q water. An adhesive tape was fixed on the ITO surface to restrict its working area (0.5 cm^2). Subsequently, silica solutions were prepared according to the following procedures. 13.6 mmol TEOS, 20 mL ethanol, 20 mL aqueous solution containing 0.1 M $NaNO_3$, 1.0 mM HCl and 4.35 mmol CTAB (98%, Fluka) were mixed and stirred for 2.5 h. After that, the ITO electrode was immersed in this solution and a voltage of -1.05 V was applied for a few seconds. Then the modified electrode was rinsed with water and dried in oven at 130 °C for 6 h. The mesoporous silica film modified electrode was immersed into 0.1 M HCl/ethanol solution for 5 min to remove the surfactant template.

Silica thin film modified ITO electrode was then immersed into a 10 % (v/v) 3-aminopropyltriethoxylane (APTES) solution in ethanol overnight in order to functionalize the surface with amino groups. The surface was then washed with ethanol and ultrapure water.

Luminol/Au /mesoporous silica film modified electrode. Electrodeposition of Au inside the

pores of silica thin film was performed in an aqueous solution of 0.5 % $HAuCl_4$ by applying a constant voltage of -0.5 V for 3 s. The electrode was then rinsed with ultrapure water. The as-prepared Au /silica/ITO electrode was then immersed into 3 mM luminol solution (pH was adjusted to 7.4 with 1 M HCl) for 45 min at 37 ◦C. After that, Luminol/Au / silica/ ITO electrode was washed with PBS to remove unbounded luminol molecules and stored at 4 ◦C before use.

Aptamer/Luminol/Au / silica/ ITO electrode. Conjugation of aptamer onto the electrode was achieved by the use of glutaraldehyde coupling reaction between amine groups on the silica film surface and the amine-functionalized aptamer. Luminol/Au / silica/ITO electrode was incubated with 2.5% glutaraldehyde solution at 37 ◦C for 2 h and then washed with PBS. Following this step, 50 μL of aptamer probes (1 μM) was dropped onto the electrode surface and incubated at 4 ° C for 12 h. After incubation, the electrode was incubated with ssDNA (50 μL, 1 μM) at 37 ° C for 1 h and then rinsed with PBS (pH 7.4, 0.1 M NaCl) carefully. Finally, the electrode was immersed in 2% BSA for 1 h at 37 ° C to block the nonspecific binding sites, and washed with PBS (pH 7.4, 0.1 M NaCl) thoroughly for subsequent cell capture.

Cell Culture, Capture and Detection. HL-60 cells, MCF-7 cells, and Hela cells were cultured in a flask in DMEM medium supplemented with 10% fetal calf serum, penicillin (100 μg/mL), and streptomycin (100μg/mL) in an incubator (5% CO_2, 37 ° C). Cells were collected by centrifugation at 1000 rpm for 5 min and then resuspended in the sterile PBS (10 mM, pH 7.4) containing 137 mM NaCl, 2.7 mM KCl, 87.2 mM Na_2HPO_4, and 14.1 mM KH_2PO_4) to obtain a homogeneous cell suspension. Cells number was measured using a hemacytometer.

50 μL of cell suspension at a certain concentration was dropped onto the dsDNA/luminol/Au / silica/ ITO electrode surface and incubated at 37 °C for 1 h. Then, the electrodes were rinsed with sterile PBS to remove the unbinding cells. The obtained cell-captured electrodes were used for subsequent ECL measurement.

The ECL responses of the electrode were recorded in 0.1M PBS (pH 7.4) containing 3 mM luminol, 50 mM H_2O_2, and 0.1 M NaCl before and after the capture of HL-60 cells. The voltage of the PMT was set at 500V.

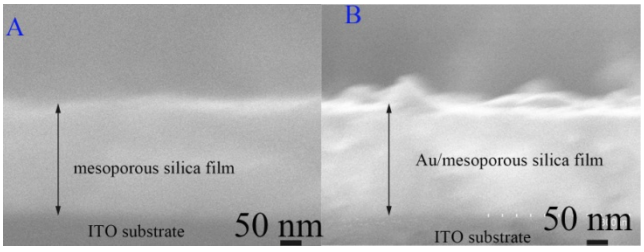

Fig. S1 SEM image of the cross section of silica film/ITO electrode (A) and Au/ silica film/ITO electrode (B).

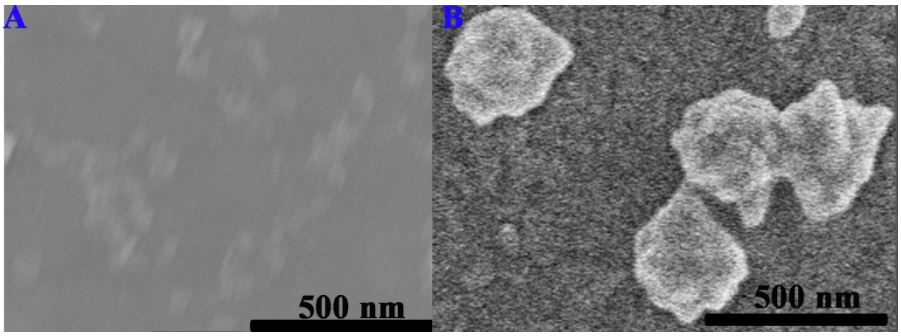

Fig. S2 SEM images of Au/silica film/ITO electrode (A) and Au /ITO electrode (B).

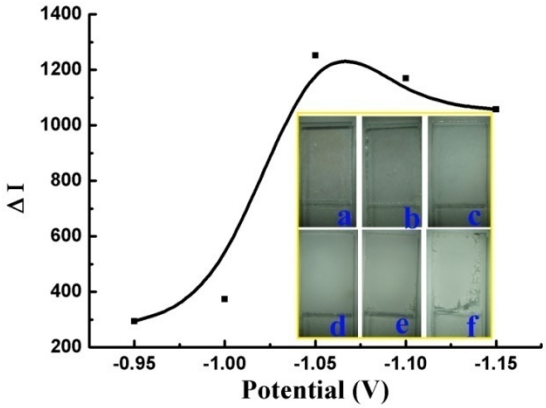

Fig. S3 Dependence of ΔI upon the deposition voltage of silica thin film. Inset was the photograph of silica film modified electrode under different deposition voltage (from a to f: -0.95, -1.00, -1.05, -1.10, -1.15, -1.20 V).

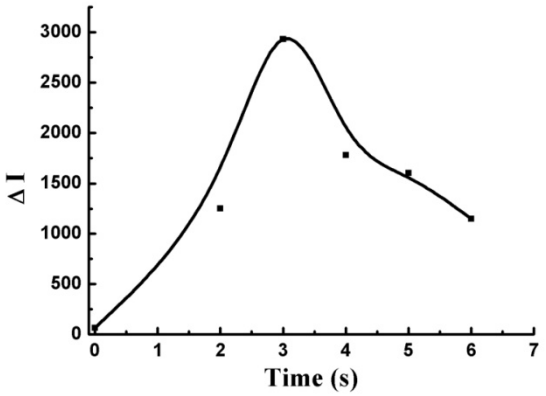

Fig. S4 the dependence of ΔI upon the deposition time of Au.

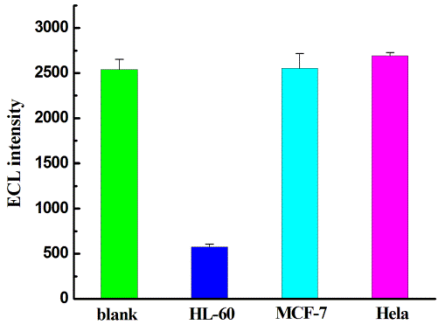

Fig. S5 ECL response of the biosensor in the absence (blank) and presence of HL-60 cells (target), MCF-7 cells (control), and Hela cells (control). Cells number was 1.0×10^6/mL.

Reference

S1 Walcarius, A., Sibottier, E., Etienne, M. & Ghanbaja, J. *Nature Materials* 2007, **6**, 602-608.