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Sensitive Electrochemiluminescence Biosensor Based on Au-ITO Hybrid Bipolar Electrode Amplification System for Cell Surface Protein Detection

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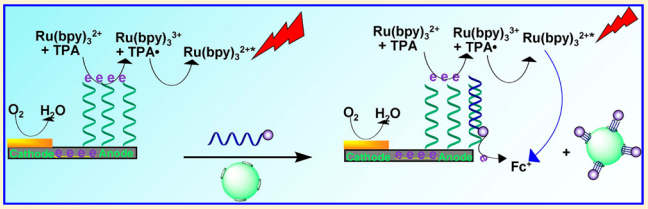
ABSTRACT: Here we developed a novel hybrid bipolar electrode (BPE)−electrochemiluminescence (ECL) biosensor based on hybrid bipolar electrode (BPE) for the measurement of cancer cell surface protein using ferrocence (Fc) labeled aptamer as signal recognition and amplification probe. According to the electric neutrality of BPE, the cathode of U-shaped ITO BPE was electrochemically deposited by Au nanoparticles (NPs) to enhance its conductivity and surface area, decrease the overpotential of O_2 reduction, which would correspondingly increase the oxidation current of $Ru(bpy)_3^{2+}$/tripropylamine (TPA) on the anode of BPE and resulting a ∼4-fold enhancement of ECL intensity. Then a signal amplification strategy was designed by introducing Fc modified aptamer on the anode surface of BPE through hybridization for detecting the amount of mucin-1 on MCF-7 cells. The presence of Fc could not only inhibit the oxidation of $Ru(bpy)_3^{2+}$ because of its lower oxidation potential, its oxidation product Fc^+ could also quench the ECL of $Ru(bpy)_3^{2+}$/TPA by efficient energy-transfer from the excited-state $Ru(bpy)_3^{2+*}$ to Fc^+, making the ECL intensity greatly quenched. On the basis of the cathodic Au NPs induced ECL enhancing coupled with anodic Fc induced signal quenching amplification, the approach allowed detection of mucin-1 aptamer at a concentration down to 0.5 fM and was capable of detecting a minimum of 20 MCF-7 cells. Besides, the amount of mucin-1 on MCF-7 cells was calculated to be 9041 ± 388 molecules/cell. This approach therefore shows great promise in bioanalysis.



Glycoproteins play essential roles in a wide variety of cell activities, including differentiation, proliferation, immune response, and cell−cell communication.[1] Alternations of glycoproteins on cell surface have been shown to be associated with many diseases. For example, mucin-1 is a glycoprotein often associated with colon, breast, ovarian, lung, and pancreatic cancers, whereas in normal cells, its expression is significantly low. Thus, accurate and sensitive determination of cell surface glycoprotein expression is critical to achieve early cancer detection and understanding the relevant biological processes of cancers. A variety of techniques have been developed for cell surface glycoproteins detection, including electrochemistry,[2] chemiluminescence,[3] and electrochemiluminescence (ECL).[4] Although some of the detection approaches are sensitive, it is still urgently to develop an efficient, rapid, low-cost, ease of operation and fabrication device to meet the increasing demand of glycoproteins detection.

Recently, ECL biosensor based on bipolar electrodes (BPEs), as a new class of molecular recognition platform, attracts great attention in bioanalysis.[5−9] The BPE-ECL system is usually composed of a microchannel with an electrode embedded in it. The miniaturized ECL platform on a microfluidic chip offers the advantages of low reagent consumption, portability, and disposability. Besides, there is no need for a direct external connection to the BPE, which simplifies the operational procedure. When a sufficiently high voltage is applied across the microchannel, oxidation and reduction reactions occur at the same rate on the BPE. Therefore, ECL reactions on the anode of BPE are greatly influenced by the cathodic reduction current. This type of electrochemistry makes it possible to further improve the sensitivity and selectivity, such as functionalize specific biological event on BPE surface,[5,6] change the configurations of microchannel and BPE,[9,10] and so on. The most recent efforts have focused on decreasing the driving voltage on both ends of BPEs.[7,8] For example, $Ru(bpy)_3^{2+}$ could be oxidized at a lower external voltage when the cathode was dipped in $Fe(CN)_6^{3-}$ than that in water.[7] The driving voltage could also be reduced by increasing the BPE length, because the driving voltage is proportional to the length of BPE. We have designed a target-guided deposition of Ag particles between two neighboring BPEs for sensitive and rapid visual bioanalysis. The gap between the two ITO bands could be regarded as an electrical switch. The electronic conductivity of the electrical switch could be tuned by prostate specific antigen (PSA) guided silver particles deposition via an immunosandwich assembly and a silver enhancement strategy.

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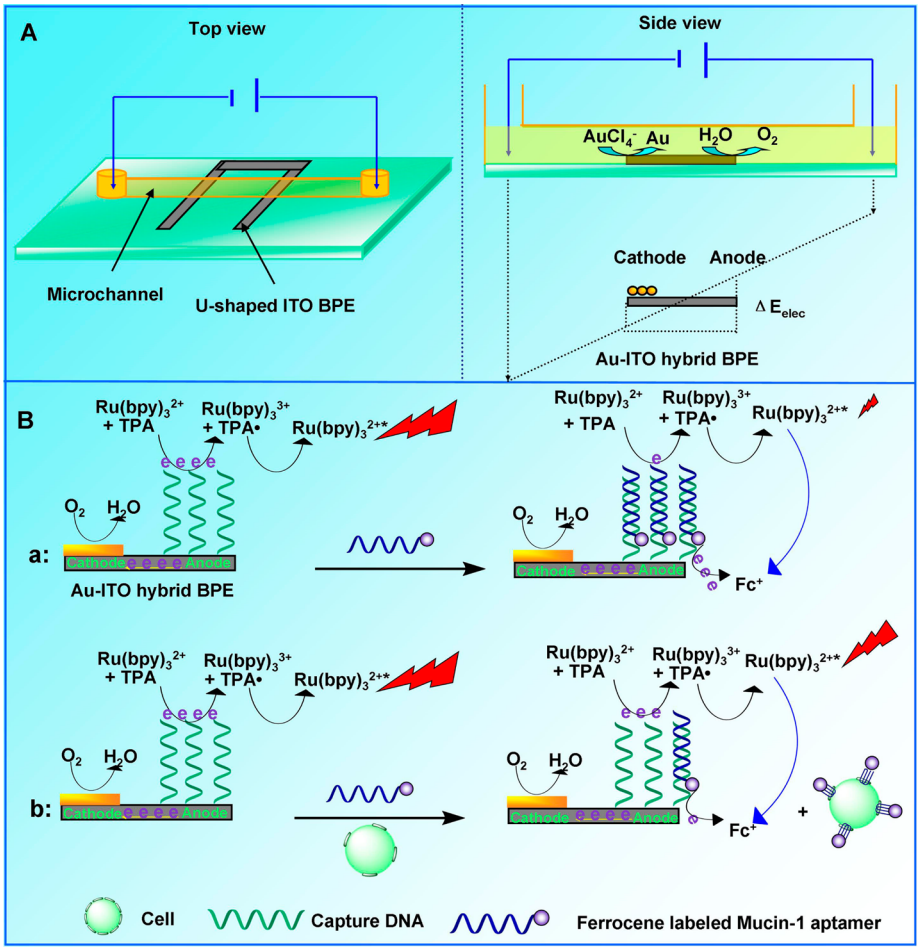
Scheme 1. Schematic Representation of the Prepared Hybrid BPE for Cancer Cells Detection



At the "on" state of the electrical switch, PSA induced deposition of silver particles forms an electronic circuit between the adjacent BPEs and makes them behave like a continuous H-shaped BPE, which results in a relative low external voltage for driving ECL.[8]

In this paper, a novel ultrasensitive hybrid BPE biosensor is proposed for the detection of cancer cell surface protein by using an Fc labeled aptamer as signal recognition and amplification probe. The hybrid BPE uses Au nanoparticles (NPs) as cathode which presents excellent conductivity, large surface area, as well as low overpotential for the reduction of oxygen,[11] and ITO glass as anode which is suitable for ECL determination due to its superior transparency. The enhanced cathodic current on Au NPs results in an increased ECL of $Ru(bpy)_3^{2+}$/TPA system on anode. Also, ECL signal is regulated by the electrochemical reactions of Fc on the anode. The oxidized species of Fc (Fc^+) showed efficient quench effect on the ECL intensity by energy transfer between $Ru(bpy)_3^{2+}$ and Fc^+. Besides, the oxidation of Fc at the anode could also significantly inhibit the oxidation of $Ru(bpy)_3^{2+}$ to maintain the electroneutrality of BPE. Then we employed mucin-1 (breast cancer biomarker), a kind of cell-surface associated glycoprotein, as a model analyte to demonstrate the utility of this approach in the determination of cancer cell surface proteins.[12] The anode is functionalized with capture DNA, which is complementary to Fc labeled mucin-1 aptamer (Fc-aptamer). In the presence of MCF-7 cells, Fc-aptamer could be combined with mucin-1 expressed on cell surface, resulting in a decreased number of Fc-aptamer hybridized with capture DNA at the anode.

EXPERIMENTAL SECTION

Materials. 3-Aminopropyl triethoxysilane (APTES), $Ru(bpy)_3^{2+}$, and tripropylamine (TPA) were obtained from Sigma-Aldrich (U.S.A.). MCF-7 cells (human breast cancer cell line) were purchased from KeyGEN Biotech (Nanjing, China). DMEM medium (Gibco Invitrogen Corp., U.S.A.), fetal bovine serum (FBS, Gibco Invitrogen Corp., U.S.A.), penicillin, and streptomycin were purchased from KeyGEN Biotech (Nanjing, China). Indium tin oxide (ITO)-coated (thickness, ~100 nm; resistance, ~10 Ω/square) aluminosilicate glass slides were purchased from CSG (Shenzhen, China). Sylgard 184 (including PDMS monomer and curing agent) was from Dow Corning (Midland, MI, USA). SG-2506 borosilicate glass (with 145 nm thick chrome film and 570 nm thick positive S-1805 type photoresist) was from Changsha Shaoguang Chrome Blank Co., Ltd. Ten millimolar sterile phosphate-buffered saline (PBS, pH 7.4) contained 137 mM NaCl, 2.7 mM KCl, 87.2 mM Na_2HPO_4, and 14.1 mM KH_2PO_4. ECL detection solution was 0.1 M PBS buffer solution (pH 7.4) containing 0.2 mM $Ru(bpy)_3^{2+}$, 0.1 M NaCl, and 10 mM TPA. All solutions were prepared using Millipore (model milli-Q) purified water and stored at 4 °C in a refrigerator. All the other chemicals were of analytical grade.

Mucin-1 aptamer probe was designed according to the literature in previous study.[13] Capture DNA probe (5′-NH_2-

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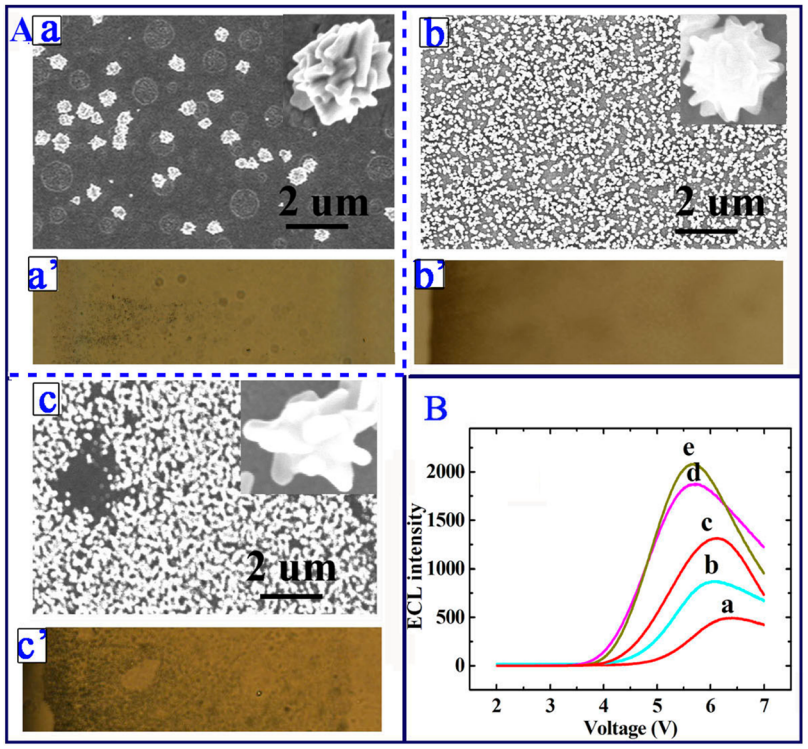

Figure 1. (A) SEM images (a, b, c) and the optical images (a′, b′, c′) of the Au NPs deposited ITO electrode obtained at 4.0 (a, a′), 5.0 (b, b′), and 6.0 V (c, c′). The inset images were the individual Au NP. (B) ECL performance of $Ru(bpy)_3^{2+}$ on ITO BPE (a) and Au-ITO hybrid BPE at a deposition voltage of 3.0 V (b), 4.0 V (c), 5.0 V (d), 6.0 V (e) in PBS (0.1 M, pH 7.4) that containing 0.2 mM $Ru(bpy)_3^{2+}$, 0.1 M NaCl, and 10 mM TPA. PMT was set at 600 V.

$(CH_2)_6$-TTT CCA GGG TAT CCA AAG) and Fc labeled mucin-1 aptamer probe (Fc-aptamer)(5′-GCA GTT GAT CCT TTG GAT ACC CTG G-$(CH_2)_6$-Fc-3′) were synthesized by Sangon Biotech Co. Ltd. (Shanghai, China).

Instruments. ECL signals were measured with MPI-E electrochemiluminescence analyzer (Xi'An Remax Electronic Science &Technology Co. Ltd., Xi'An, China, 350−650 nm). U-shaped ITO microelectrode embedded in microchannel was used for ECL detection.

Cell Culture. MCF-7 cells were cultured in DMEM medium with fetal bovine serum (10%), penicillin (1%) and streptomycin (1%) at 37 °C in 5% CO_2/95% air. The cells were collected by centrifugation at 1000 rpm for 5 min, followed by washing twice with a sterile PBS (10 mM, pH 7.4). The sediment was then resuspended in this solution.

Microfluidic Chip Design and Fabrication. Microfluidic channels and ITO BPE were fabricated by a previously reported procedure.[9] In brief, degassing PDMS was cast on the glass molds with a predesigned micropattern (straight line or U-shaped curve) for 30 min in 80 °C. After it was cooled, the PDMS layer was peeled off from the mold, and two holes (3.0 mm) were punched at both ends of the PDMS microchannel as reservoirs. The as-prepared U-shaped PDMS microchannel (arms 500 μm width, spacing 1.5 cm) was used to fabricate ITO BPE. ITO glass slide substrate (4 cm ×4 cm) 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 and dried at 80 °C. The ITO glass slide substrate was covered with U-shaped PDMS microchannel. After that, the channels were filled with carbon ink and allowed to dry in thermostat at 30 °C for 30 min. Then, the PDMS mold was peeled off and the exposed ITO surface was etched with an aqueous acid solution ($FeCl_3$/HNO_3/HCl = 0.5 M: 1 M: 1 M). The remaining ink was removed with ethanol.

Assembly of Capture DNA on ITO/Au Hybrid Electrode. U-shaped ITO electrode was immersed in an ethanol solution of APTES (5%, v/v) overnight to form reactive amine groups. The electrode was thoroughly rinsed with ethanol to remove loosely bound molecules and heated at 80 °C for 10 min. The resulting ITO electrode was irreversibly embedded into the as-prepared straight PDMS microchannel (500 μm wide, 3.5 cm long). Then, the cathodic pole of the U-shaped ITO BPE was deposited with Au NPs and the anodic pole of which was functionalized with capture DNA, as shown in Scheme 1. For electrodepositing Au NPs on the cathodic pole of BPE, the microchannel was filled with 1% $HAuCl_4$ and a voltage (3.0−6.0 V) was applied to platinum wires at the two reservoirs of microchannel for 60 s using CHI 660C electrochemical workstation. Then the color of the cathode changed from blue (ITO) to yellow (Au), indicating the formation of Au NPs. The as-prepared Au-ITO hybrid BPE was then washed with ultrapure water. After that, capture DNA was modified on the anode by the following procedure. Glutaraldehyde (GA) was pipetted into the microchannel from the right reservoir and incubated with the anode for 2 h at 37 °C. The microchannel was rinsed carefully with PBS thoroughly, after which amino-modified capture DNA (1.0×10^{-7} M) was added into the microchannel and incubated for 3 h at 37 °C. After incubation, the unoccupied sites of GA were blocked with 2% BSA for 30 min, then rinsed with ultrapure water and stored at 4 °C before use.

ECL Analysis of Mucin-1 Aptamer and MCF-7 Cell. Twenty microliters of sample solution containing Fc-modified mucin-1 aptamer and MCF-7 cell were introduced into the

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microchannel and incubated at 37 °C for 50 min. The microchannel was then washed twice with PBS. ECL experiments were performed using MPI-E electrochemical and electrochemiluminescence analyzer. The ECL-voltage curves were obtained by applying a linearly increasing voltage (from 2.0−7.0 V) on the two ends of microchannel with the scan rate of 0.1 V/s.

RESULTS AND DISCUSSION

Characterization of Au-ITO BPE. Recent studies have indicated that electrode materials play important roles in bipolar electrochemical and ECL processes.[11] Herein, we developed a novel Au-ITO hybrid BPE using bipolar electrodeposition approach for sensitive ECL assay, shown in Scheme 1A. For fabrication the Au-ITO hybrid BPE, the microchannel containing a U-shaped ITO electrode was filled with $HAuCl_4$ and an external voltage was applied at the two ends of the channel. When the voltage reached to a critical value, $HAuCl_4$ was reduced at the cathodic pole of the U-shaped ITO BPE to form Au NPs and H_2O was oxidized at the anode. The deposition of Au was illustrated as follows:

cathode reaction

$$AuCl_4^- + 3e \rightarrow Au + 4Cl^- \quad E^\circ = +1.002\ V \tag{1}$$

$$2H^+ + 2e \rightarrow H_2 \quad E^\circ = 0.00\ V \tag{2}$$

anode reaction

$$2H_2O \rightarrow O_2 + 4e + 4H^+ \quad E^\circ = +1.23\ V \tag{3}$$

Therefore, the potential difference between the anode and cathode should be higher than 0.23 V to drive the deposition of Au. The morphologies of the Au coated ITO obtained by varying the deposition voltage from 4.0 to 6.0 V were studied using scanning electron microscopy (SEM), shown in Figure 1A (a−c). Ideally, the potential difference on BPEs (ΔE_{elec}) was from 1.6 to 2.4 V which was sufficiently high to form Au NPs. The inset images in Figure 1A (a−c) illustrated that the individual Au NP was composed of highly aggregated Au particles. It can be seen that the nuclei of Au were the largest and scarce at 4.0 V (Figure 1A-a). As the deposition voltage increase (Figure 1A-b and 1A-c), large amounts of small-sized Au NPs formed, indicating that higher deposition voltage caused fast growth of nuclei on the cathode of BPE. Furthermore, if the external voltage reached 6.0 V (ΔE_{elec} =2.4 V) then the evolution of hydrogen would occur at cathode (eq 2 and 3), resulting in the formation of a nonuniform Au film (Figure 1A-c). Similar results obtained by CCD camera (Figure 1A-a′ to Figure 1A-c′) also confirmed that the porous structure of Au film correlated with the bipolar deposition voltage. Meanwhile, Au film displayed gradient distribution on ITO substrate due to the potential gradient along the BPE surface.

In this Au-ITO hybrid BPEs (Scheme 1B), ITO glass, which possesses superior optical transparent, serves as the anodic pole to facilitate the ECL signal collection by the detector, thereby effectively avoiding the loss of ECL signal. The pole covered with Au NPs, which presents a number of advantages over traditional BPE materials, including excellent electrical conductivity, low overpotential for oxygen reduction, and large surface area,[11] is employed as the cathodic pole to further improve the ECL response. To evaluate the electrochemical and ECL performances of the hybrid BPE, we performed ECL measurements on a bare ITO BPE and Au-ITO hybrid BPEs obtained under different deposition voltages. The reaction mechanism of $Ru(bpy)_3^{2+}$/TPA on BPE was shown in Scheme 1B. $Ru(bpy)_3^{2+}$ and TPA were oxidized at ITO anode (eq 5)

anode reaction

$$\begin{cases} \begin{cases} Ru(bpy)_3^{2+} - e \rightarrow Ru(bpy)_3^{3+} \\ TPA - e \rightarrow [TPA^\bullet]^+ \rightarrow TPA^\bullet + H^+ \\ Ru(bpy)_3^{3+} + TPA^\bullet \rightarrow Ru(bpy)_3^{2+*} \rightarrow h\nu \end{cases} & (5) \\ Fc - e \rightarrow Fc^+ & (6) \end{cases}$$

and oxygen was reduced at Au cathode (eq 4) at the same rate. Then the $TPA^\bullet$ radical species reduced $Ru(bpy)_3^{3+}$ to form $Ru(bpy)_3^{2+*}$.[14] As can be seen in Figure 1B, an obvious ECL peak can be observed on the ITO BPE and Au-ITO hybrid BPEs (curve a to e). The ECL intensity on Au-ITO hybrid BPEs (curve b to e) enhanced notably compared with that on ITO BPE (curve a). Meanwhile, the ECL peak voltage shifted toward negative gradually (curve b to e) with the increase of Au electrodeposition voltage, confirming that external voltage for driving the redox reactions on Au-ITO hybrid BPE was decreased. Although large deposition voltage could lead to the increased density of Au NPs, the rate of hydrogen evolution was also increased, which was responsible for the irregular pore structure inside Au film. Thus, a deposition voltage of 5.0 V was selected for all subsequent experiments. Compared with ITO BPE, this Au-ITO BPE provided a ∼4-fold enhancement of ECL intensity, showing a significant improvement of ECL sensitivity.

The anodic pole of the BPE was modified with capture DNA. Upon the hybridization with a complementary aptamer, Fc at the end of the aptamer strand was brought into sufficient proximity to the anode surface. The quenching effect of $Ru(bpy)_3^{2+}$/TPA ECL by Fc in three-electrode system has been well studied in previous reports,[15] however, has not yet been investigated on BPE. Here, we investigated the interaction between $Ru(bpy)_3^{2+}$ and Fc on BPE (Figure 2A) and compared

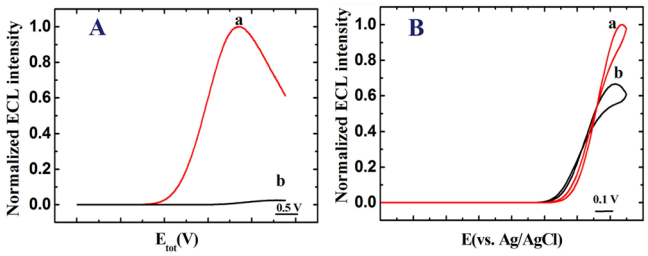

Figure 2. ECL curves of 0.2 mM $Ru(bpy)_3^{2+}$ in the absence (a) and presence (b) of 1.0×10^{-7} M Fc-mucin-1 aptamers/capture DNA/on BPE(A) and macroscopic ITO electrode with three-electrode cell (B). Electrode area = 0.25 cm^2.

it with that in three-electrode system using ITO glass as working electrode (Figure 2B). When Fc-labeled aptamer was captured on electrode surface, the ECL of $Ru(bpy)_3^{2+}$/TPA system was completely quenched on BPE. However, it decreased by 34% in the three-electrode system, suggesting that the ECL quenching mechanism on BPE was more complicated than that in three-electrode system.

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It is well-known that Fc has a lower (more negative) oxidation potential (about 0.4 V vs Ag/AgCl)[16] than $Ru(bpy)_3^{2+}$ (about 1.2 V vs Ag/AgCl). This clearly showed that Fc was easily oxidized to form Fc^+ prior to $Ru(bpy)_3^{2+}$. The corresponding faradaic reactions on BPE can be described as follows:

cathode reaction

$$O_2 + 4e + 4H^+ \rightarrow H_2O \quad (4)$$

As a result, the enhanced quenching effect of Fc on BPE can be explained by the following aspects. The potential difference between BPE and solution interface was controlled by the electric field in solution, electroneutrality should be maintained across the BPE; that was, the anodic and cathodic currents were equal. Therefore, the oxidation of Fc on anode would compete with that of $Ru(bpy)_3^{2+}$, which could decrease the ECL signal. The oxidation species of Fc (Fc^+) could further react with excited-state $Ru(bpy)_3^{2+*}$ to directly quench ECL light emission through an electron-transfer mechanism. Both of them results in the ECL signal inhibited greatly.

Analysis of Mucin-1 Expression on Cell Surface. As is well-known, an elevated level of mucin-1 plays crucial roles in poor prognosis, higher risk of recurrence and increased lymph node metastases in the breast cancer patients.[17] Thus, it is important to evaluate the concentration of mucin-1 for understanding the roles of mucin-1 peptides in cancer development. Scheme 1B showed the schematic representation of this novel ECL method based on Au-ITO hybrid BPE for the amplified sensing of cancer cells. As discussed above, the ECL quenching efficiency was dependent on the concentration of Fc-aptamer captured on anode. After the treatment of capture DNA/BPE with Fc-aptamer and MCF-7 cells, the solution-phase Fc-aptamer recognized mucin-1 expressed on MCF-7 cell surface with high affinity, leading to a decreased number of Fc-aptamer hybridized with capture DNA on anode (Scheme 1B-b). Consequently, the recovery of $Ru(bpy)_3^{2+}$ ECL peak intensity could reflect the amount of specific proteins on cell surface and the number of cancer cells.

Since many experimental parameters, such as the self-assembly time of capture DNA on BPE and hybridization time, can affect the detection sensitivity of this assay, we performed a series of experiments to optimize these parameters. Figure 3A displayed the influence of self-assembly time of capture DNA on the decrement of ECL peak intensity (ΔI, $\Delta I = I_0 - I$, where I_0 and I were the ECL peak intensities before and after hybridization, respectively). In this study, the anodic pole of Au-ITO hybrid BPE was incubated with capture DNA ranging from 0.5 to 3.5 h at 37 °C. It could be seen that ΔI increased with the increase of self- assembly time from 0.5 to 1.5 h and reached a maximum at 1.5 h. However, ΔI decreased with further increased self-assembly time, attributed to steric hindrance arising from the densely packed capture DNA probe on BPE which hindered the hybridization efficiency and decreased the electron transfer of $Ru(bpy)_3^{2+}$ on electrode surface. Therefore, the assembly time of 1.5 h was selected in the following experiments. The effect of the hybridization time was also investigated (Figure 3B). It showed that ΔI increased rapidly when the hybridization time increased between 10 and 40 min and then increased slightly from 40 to 50 min, indicating that the hybridization almost reached equilibrium. As a result, 50 min was employed for DNA hybridization.

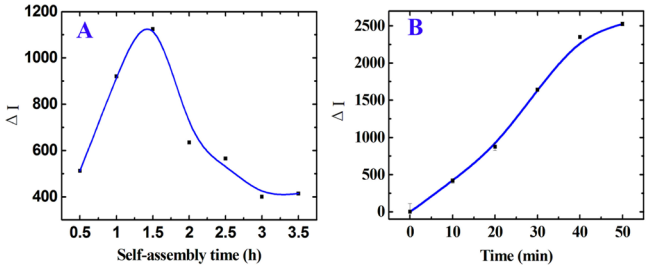

Figure 3. Effects of self-assembly time of capture DNA (A) and hybridization time (B) on ECL response of the sensor toward 1.0×10^{-15} M Fc-labeled mucin-1 aptamers. PMT was set at 700 V.

Since the decreased number of Fc-aptamer captured by BPE was dependent on the amount of mucin-1 expressed on cancer cells, a calibration curve for detection of mucin-1 aptamer was constructed. Figure 4A showed the ECL responses of the biosensor obtained upon different concentrations of mucin-1 aptamer. It could be seen that the ΔI decreased gradually with increasing concentrations of mucin-1 aptamer. As can be seen in the inset of Figure 4, it showed a linear relationship in the range of 0.5 fM to 1.0 pM with a correlation coefficient of 0.9964. The regression equation was lgI = −4.915 − 0.552 lgC_{apt}.

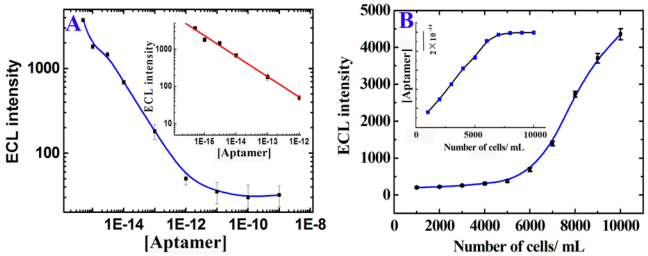

Figure 4. (A) Calibration curve of ECL peak intensity versus the concentration of mucin-1 aptamer from 0.5 fM to 1.0 nM. Inset is the amplification of the linear range from 0.5 fM to 1.0 pM for mucin-1 aptamer determination. (B) Calibration curves for ECL detection of cells. Concentrations of the cells: 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10 000 cells/mL. Sample volume: 20 μL. Inset: The mucin-1 aptamer concentration versus MCF-7 cells concentration. The error bars show the standard deviation of three replicate determinations.

The detection of mucin-1 on cell surface was carried out based on the competition between capture DNA and cancer cell for binding the aptamer because of the specific recognition between mucin-1 aptamer and mucin-1 on cell surface. First, amino-modified capture DNA (1.0×10^{-7} M) was added into the microchannel and modified at the anode of the Au-ITO hybrid BPE. Meanwhile, mucin-1 aptamer ($C_{apt,0} = 1.0 \times 10^{-13}$ M) and different amount of MCF-7 cell (C_{Cell}) were incubated with capture DNA modified electrode for 50 min, the results of which were shown in Figure 4B. It showed that the increased ECL peak intensity was related to the concentration of MCF-7 cell. The ECL peak intensity was converted into the concentration of mucin-1 aptamer hybridized with capture DNA on BPE surface (C_{apt}) according to the regression equation obtained in Figure 4A. The concentration of the aptamer binding on cell surface ($C_{apt,0}$ - C_{apt}) versus cell number (C_{Cell}) was shown in the inset of Figure 4B, which represented the expression level of mucin-1 on MCF-7 cells. It showed a linear relationship in the range of 1.0×10^3 to $7.0 \times$

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10^3 cells/mL (R^2 = 0.996) and reached a plateau corresponding to the excess of cells. The regression equation was $(C_{apt,0} - C_{apt}) = 1.413 \times 10^{-17}\ C_{Cell}$ (cells/mL) + 2.747×10^{-15}. The amount of mucin-1 on MCF-7 cell $((C_{apt,0} - C_{apt})/C_{Cell} \times N_A)$ was calculated to be 9041 ± 388 molecules/cell (N_A was Avogadro's number). The detection limit of MCF-7 cell was determined to be 20 cells (the volume of the added sample was 20 μL). The sensitivity of the device is comparable or more sensitive than most of the reported ECL methods.[18,19]

CONCLUSIONS

The present study demonstrates a novel microfluidic chip-ECL biosensor based on cathodic Au NPs induced signal enhancing amplification coupled with anodic Fc induced signal quenching amplification for evaluation of tumor cell surface proteins expression. Compared with other well-established ECL biosensor, this device offers ultrahigh sensitivity and broad linear range attributed to the strong enhancement from density Au NPs at cathode surface and the following amplified quenching effect via oxidation of Fc at anode. Furthermore, the detection of cell surface proteins is relatively simple without any pretreatment of cells. The high sensitivity of such an Fc-based ECL sensing protocol on Au-hybrid BPE could be expanded for detecting other biomarkers expression on cancer cells and thus provides a powerful tool for disease diagnostics and clinical analysis.

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Notes

The authors declare no competing financial interest.

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