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ARTICLE

Bipolar electrode-electrochemiluminescence (ECL) biosensor based on a hybridization chain reaction

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A novel electrochemiluminescence (ECL) closed bipolar electrode (BPE) chip was designed based on a hybridization chain reaction (HCR)-induced ECL amplification strategy for the detection of both DNA and H_2O_2. Without the utilization of a patterned ITO bipolar electrode (BPE), this chip platform consisted of an ITO glass coated with two layers of PDMS slices. The ITO cathode was modified with Au nanoparticles for further functionalization of biomolecules, which could also amplify the ECL signal at the anode of the BPE. Based on the specific hybridization and hybridization chain reaction (HCR), DNA sequences were greatly extended, leading to a significant increase in the resistance of the cathode. The reduction of H_2O_2 was inhibited on the cathode of BPE, resulting in a quenching effect on the ECL intensity on the anode of BPE. The designed biosensor displayed a satisfactory linear relationship for both the detection of DNA and H_2O_2. Therefore, the biosensor could not only be employed for DNA assays but also used in enzyme reactions based on the generation of H_2O_2.

Introduction

Bipolar electrochemistry[1, 2] is a powerful tool that has recently attracted a tremendous amount of interest due to its unique advantages. When the voltage applied to the two ends of the solution is sufficiently high, redox reactions can simultaneously occur at the two poles of the conductor. Usually, an electrochemiluminescence (ECL) reaction occurring at the anode of the BPE is used to monitor the reduction reaction occurring at the cathode.[3-13]The combination of a BPE and ECL techniques greatly broadens the application of ECL because the analytes do not need to participate in the ECL reactions. In addition, it enables modification of the catalyst at one pole of the BPE through bipolar deposition with a controllable area and density, resulting in the improvement of the electrochemical performance of the BPE.

Because no electric wire is connected to the BPE, versatile sensing devices have been developed based on the charge neutrality of BPEs[14-17]. Mehrgardi's group incorporated a 3D printed channel into a BPE for MCF-7 cell detection[13]. The cathode of the BPE was immersed in $Fe(CN)_6^{3-}$to improve the ECL signal at the anode of the BPE. Electroactive species can also be attached through specific recognition reactions to the terminal of biomolecules that are brought to the electrode surface to decrease the external voltage needed to drive the redox reactions[4], resulting in an improved ECL signal at the BPE. Moreover, the amplification effects of catalytic particles (Pt and Au) at the cathode of a BPE on the ECL intensity at the anode have been reported in previous studies[18, 19]. In our previous work, we deposited Au particles onto the cathode of a BPE in an open bipolar electrochemical chip and found that the Au-modified BPE could not only remarkably improve the ECL signal but also decrease the onset voltage for the ECL reaction in the BPE system[20]. To avoid chemical interference between the sensing solution and reporting solution, closed bipolar electrochemical platforms have commonly been used because the two ends of a BPE are immersed into two different solutions[21]. In addition, a BPE can be fabricated by wiring two electrodes together, such as glassy carbon electrodes. Studies have indicated that the interfacial difference between the electrode and solution is much higher than that in open BPE devices, leading to an improved electrochemical/ECL signal[22]. The cathode of a BPE can easily be modified with carbon materials (e.g., multi-walled carbon nanotubes) to facilitate electron transfer, fabricate various sensing interfaces and functionalize biomolecules[23]. Although all of these approaches are effective for achieving ECL assays, limited studies have been reported to integrate both electroactive species and DNA-assisted amplification strategies (e.g., hybridization chain reaction[24-27]) into BPE-ECL systems for dual-analyte detection.

In this work, we have proposed a novel sensitive ECL closed BPE chip based on an electroactive species and a hybridization chain reaction (HCR)-assisted amplification system. H_2O_2 is an excellent electrochemical indicator and has been employed to amplify ECL signals due to its higher reduction potential than that of dissolved oxygen, which can decrease the onset potential needed to drive the ECL reaction at the anode (Scheme 1). ITO slices were covered with two layers of PDMS slices, which eliminated the need for fabrication of a patterned ITO BPE. The ITO cathode was modified with Au particles

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through bipolar deposition for biomolecular modification and to catalyze the reduction reaction at the cathode of the BPE. The extension of a DNA sequence through the hybridization chain reaction (HCR) with two hairpin helper DNA probes (H1 and H2) remarkably increased the resistance of the cathode, leading to a quenched ECL signal at the anode.

Experimental section

2.1 Materials and reagents

$HAuCl_4$ was purchased from Sinopharm Chemical Reagent Company (Shanghai, China). $Ru(bpy)_3^{2+}$, tris(2-carboxyethyl) phosphine hydrochloride (TCEP), and 6-mercapto-1-hexanol (MCH) were obtained from Sigma-Aldrich. All DNA oligonucleotides were synthesized by Sangon Biotech Co. Ltd. (Shanghai, China), and the sequences are given below (cDNA was complementary to the HCR capture DNA. HCR ST was a single-base mismatched sequence to the HCR capture DNA).

Capture DNA: 5'-SH-$(CH_2)_6$-TAT TAA CTT TAC TCC- 3'

Target: 5'-*TCA GCG GGG AGG AAG*GGA GTA AAG TTA ATA- 3'

Hairpin helper DNA1 (H1): 5'-AAA AAA AAA *CTT CCT CCC CGC TGA* CAA AGT TCA GCG GGG- 3'

Hairpin helper DNA2 (H2): 5'-TCA GCG GGG AGG AAG *CCC CGC TGA ACT TTG* AAA AAA AAA- 3'

Complementary DNA (cDNA): 5'-GGA GTA AAG TTA ATA- 3'

Single base-mismatched DNA (ST): 5'-TCA GCG GGG AGG AAG GGA GT***T*** AAG TTA ATA- 3'

Indium tin oxide (ITO)-coated (thickness, ~100 nm; resistance, ~10 Ω/square) aluminosilicate glass slides were purchased from CSG (Shenzhen, China). Sylgard 184 (including a polydimethylsiloxane (PDMS) monomer and curing agent) was purchased from Dow Corning (Midland, MI, USA).

All solutions were prepared using ultrapure water and stored at 4 °C in a refrigerator. All other chemicals were of analytical grade.

2.2 Instruments

ECL signals were measured with an MPI-E multifunctional electrochemical and chemiluminescent analytical system (Xi'an Remax Electronic Science & Technology Co. Ltd., Xi'an, China). Pt wires were placed at the two reservoirs and connected to the above instrument to supply sufficient voltage for the electrochemical reactions occurring at the two poles of the BPE. The morphologies of the Au particles on the cathode of the ITO BPEs were characterized by scanning electron microscopy (S-3400N II). EIS measurements were performed on an Autolab PGSTAT12 (Ecochemie, BV, The Netherlands) controlled by GPES 4.9 and FRA 4.9 software. Electrochemical measurements were conducted on a CHI 660E potentiostat (CH Instruments, China) with a three-electrode system. An Au-modified ITO electrode was used as the working electrode, and Ag/AgCl and Pt wire served as the reference and counter electrodes, respectively.

2.3 Fabrication of the closed bipolar electrochemical cells

ITO glass (30 mm × 30 mm) was boiled in a mixture of 11.2 g KOH and 100 mL isopropyl alcohol for 20 min and dried at 60 °C. Two layers of PDMS slices were bonded to the ITO surface. Two holes were punched in each PDMS slice. The diameter of the holes in the bottom and top layer of the PDMS slices were 3 mm with a center to center distance of 5 mm and 7 mm with a center to center distance of 9 mm, respectively. Pt wires were placed in the two holes of the top PDMS slice and served as the driving electrodes. ITO electrodes in the two holes of the bottom PDMS slice acted as the cathodic and anodic poles of the BPE.

2.4 Preparation of the ECL biosensor

A total of 100 μL of a 10 mM $HAuCl_4$ aqueous solution was introduced into the two reservoirs, and two Pt electrodes were placed at the ends of the top two reservoirs. Pt electrodes were connected with CHI 660E potentiostat (CH Instruments, China) and used as driving electrodes for bipolar deposition. A constant voltage of 4.0 V was applied for 300 seconds for bipolar deposition of Au NPs onto the cathode of the BPE. The Au NP-modified ITO BPE was washed twice with water.

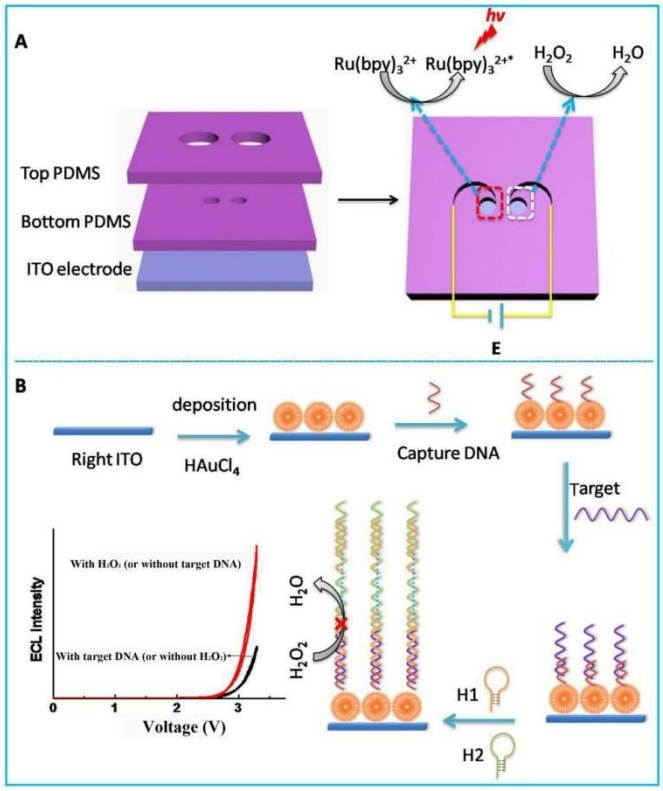
Capture DNA (30 μL of a 1.0 μM solution) was mixed with the same volume of a 10 mM tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) solution to cleave the disulfide bonds. Then, 30 μL of the mixed solution was added into the left reservoir and incubated at 37 °C for 2 h. After washing with 0.01 M PBS, the modified ITO electrode was blocked with 30 μL of MCH (1.0 μM) for 30 min. The electrode was washed thoroughly with 0.01 M PBS and soaked in 30 μL of the target solution at 37 °C for 1 h. Next, the electrode was washed with 0.01 M PBS and incubated with 30 μL of a mixture of H1 (0.5 μM) and H2 (0.5 μM) at 37 °C for 2 h. Before use, H1 and H2 were heated to 95 ºC for 2 min followed by cooling to room temperature. After rinsing with 0.01 M PBS, the electrode was rinsed with 0.01 M PBS and stored at 4 °C before use.

For the ECL assay, the reporting reservoir was filled with 100 μL of a 0.01 M PBS solution (pH 7.4) containing 1 mM $Ru(bpy)_3^{2+}$ and 10 mM DBAE, and the sensing reservoir was filled with 100 μL of a PBS solution (pH 7.4) containing 1 mM H_2O_2. A cyclic voltage from 0 to 3.3 V at a scan rate of 0.1 V/s was applied to the Pt electrodes to drive the redox reactions at the two regions of the ITO electrode in the sensing reservoir and reporting reservoir.

Results and discussion

3.1 Principle of the proposed BPE biosensor

Previous studies have indicated that the conductivity of a BPE and the addition of electrochemical species to the cathode of a BPE are two key roles in modulating the ECL signal of a BPE. In this device, dissolved oxygen was reduced at the cathode of the BPE, and $Ru(bpy)_3^{2+}$ was oxidized at the anode of the BPE to emit an ECL signal in the absence of H_2O_2. After the addition of H_2O_2tothe sensing reservoir, it was reduced at the cathode, leading to an improved ECL signal at the anode caused by the increased number of electrons flowing through the BPE (Scheme 1). To fabricate a sensitive biosensor, the resistance of the electrode was adjusted through the specific recognition of DNA on the cathode, and the length of DNA was extended



Scheme 1 (A) Configuration of the closed BPE-ECL device. (B) Electrochemical regulated electrochemiluminescence technology for DNA and H_2O_2detection.

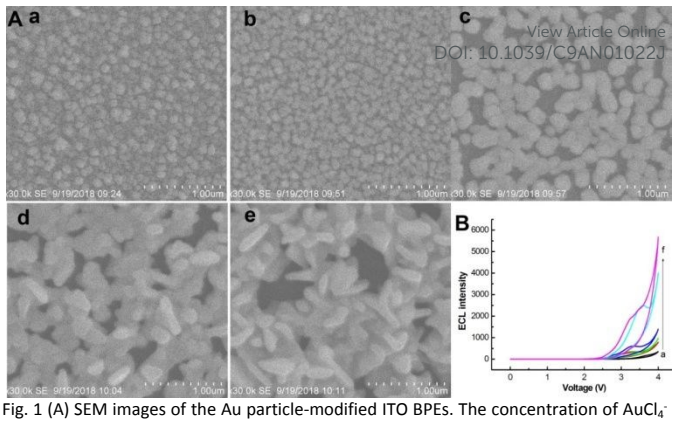


Fig. 1 (A) SEM images of the Au particle-modified ITO BPEs. The concentration of $AuCl_4^-$ in a-e was 1.0, 2.0, 5.0, 10.0, and 20 mM, respectively. Au particles was deposited under a constant voltage of 4 V for 300 s. (B) ECL-voltage curves of the ITO BPE (a) and the above Au particle-modified ITO BPEs (b to f). The reporting reservoir was filled with the ECL reagent, and the sensing reservoir was filled with 0.01 M PBS.

by a hybridization chain reaction (HCR). Scheme 1B illustrates the detailed mechanism of the proposed biosensor. The cathode of the ITO BPE was modified with Au particles through bipolar deposition to increase the cathode area, amplify the ECL signal, and afford anchor sites for the modification of the capture DNA. Then, target DNA, which was partially complementary to the capture DNA, was specifically recognized and functionalized on the capture DNA/Au/ITO electrode. In the presence of hairpin DNA (H1 and H2), the HCR process was initiated, leading to elongation of the DNA sequence. The increased interfacial resistance of the electrode decreased the reduction current of H_2O_2, resulting in a weak ECL signal at the anode. Therefore, the ECL signal was associated with the concentrations of both the target DNA and H_2O_2. That is, this biosensor could be employed to measure DNA and biomolecules that can generate H_2O_2 during chemical reactions.

3.2 Characterization of Au-ITO BPE

Fig.1A shows SEM images of Au particles modified on the cathode of the ITO BPE via bipolar electrodeposition under a voltage of 4.0 V. The concentration of HAuCl4 ranged from 1.0 to 20 mM (Fig. 1A, a-e). The Au particles on the ITO substrate exhibited a spherical morphology when the concentration of $AuCl_4^-$ was 1.0 (a) and 2.0 mM (b). As the $AuCl_4^-$ concentration increased, the Au particles displayed rod-like structures due to the aggregation of large number of Au particles , which was in accordance with the results of our previous work[20]; additionally, the size of the Au particles obviously increased.

Since the deposition of Au could improve the conductivity of the ITO electrode and amplify the ECL signal when serving as the cathode of the BPE, we investigated the ECL performance of the Au particle-modified ITO BPE. A cyclic voltage was applied to the two Pt wires. As shown in Fig. 1B, the ECL signals of the Au/ITO BPEs (from b to f) were higher than that of the bare ITO BPE (curve a). Additionally, the onset voltage needed to drive the ECL reactions decreased significantly. In addition, the ECL intensity remarkably increased with increasing deposition concentration of $AuCl_4^-$ due to the improved conductivity and enlarged surface area of the cathode.

3.3 Electrochemically modulated ECL performance

To confirm that the reduction of H_2O_2 could improve the number of electrons flowing through the BPE, we first investigated its electrochemical performance on an Au-modified ITO electrode, and the results are shown in Fig. 2A. The background current was very weak (curve a). After the addition of 5 mM H_2O_2 (curve b), a sharp increase in the redox current was observed, indicating that H_2O_2may improve the ECL signal when introduced into the cathode of the BPE. To verify this hypothesis, the corresponding ECL-potential curves of the Au particle-modified ITO BPE in the absence (curve a) and presence (curve b) of 1 mM H_2O_2 are displayed in Fig. 2B. As expected, not only was the ECL signal enhanced greatly after the immersion of the Au/ITO cathode into a H_2O_2 solution, but the onset voltage for driving the electrochemical reactions at the two poles of the BPE were shifted to a more negative value. These results confirmed that the reduction of H_2O_2 at the cathode of the BPE can be used to modulate the ECL signal in the BPE platform and improve the sensitivity of the device.

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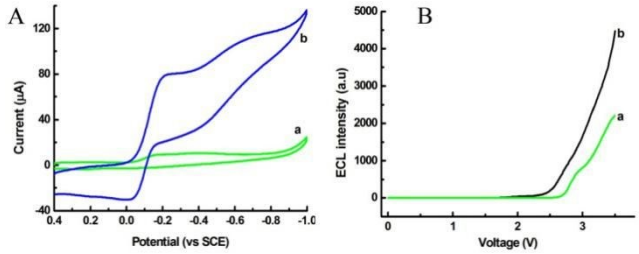

Fig. 2 (A) Cyclic voltammetry(CV) voltammograms of the Au particle-modified ITO electrodes in PBS (a) and a 5 mM H_2O_2 solution (b). (B) ECL-voltage curves of $Ru(bpy)_3^{2+}$/DBAE in a closed BPE device. The ECL reagent was in the left reservoir. The Au particle-modified ITO cathode was immersed in 0.01 M PBS (curve a) and 1 mM H_2O_2 (prepared in 0.01 M PBS, curve b). The concentration of $AuCl_4^-$ for the deposition of Au was 1 mM. The voltage of the PMT was set at 400 V.

3.4 Feasibility of the proposed biosensor for DNA detection

To validate the designed biosensor, we first evaluated the stepwise modification of the cathode by electrochemical impedance spectroscopy (EIS). Fig. 3A shows the EIS of different electrodes in the presence of a 5 mM $Fe(CN)_6^{3-/4-}$ solution containing 0.1 M KCl. The resistance obtained at the Au particle-modified electrode (curve b) had obviously decreased compared with that obtained at the bare ITO electrode (curve a) due to the excellent conductivity and large surface area of Au, which facilitated electron transfer. When capture DNA was modified at the Au surface through Au-S bonds, the semicircle in the EIS spectra was enhanced (curve c). After hybridization of the target DNA (curve d) and the further extension of the DNA sequence in the presence of hairpin DNA (H1 and H2, curve e), the resistance remarkably increased. All of these results indicated the successful modification of DNA and the extension of the DNA sequences during the HCR process.

Fig. 3B shows the ECL signal obtained for the designed BPE device after the step-by-step modification of the ITO cathode. The cathode was immersed in a H_2O_2 solution. The ECL signal obtained at the Au/ITO BPE (curve a) was significantly amplified compared to that obtained at the bare ITO BPE (inset in Fig. 3). After modification of the capture DNA and the blocking of MCH (curve b), the ECL intensity dropped to 6630, which was 77.7 % of that obtained at the Au/ITO BPE (curve a) due to the increased resistance of the electrode. The hybridization of the target DNA molecules with the capture DNA on the electrode surface led to a further decrease in the ECL intensity to 66.5 % of that obtained at the Au/ITO BPE. Subsequently, the ECL signal decreased again to 49.2 % after incubating with H1 and H2, confirming that the proposed platform can be used for DNA detection based on the reduction of H_2O_2 at the cathode of the BPE and the superiority of the HCR-assisted amplification strategy in the closed BPE platform.

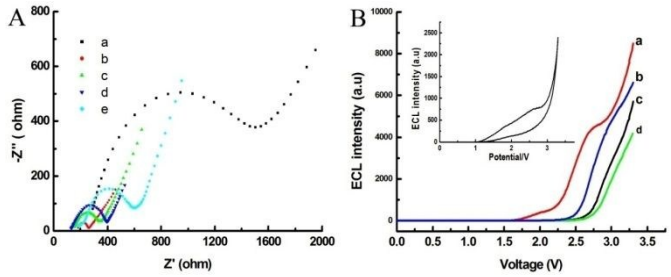

Fig. 3 (A) Nyquist plots of the cathodic ITO electrode in the presence of a 5.0 mM $Fe(CN)_6^{3-/4-}$ solution containing 0.1 M KCl in the frequency range of 0.1-10^5 Hz. (a) bare ITO, (b) Au/ITO, (c) MCH/capture DNA/Au/ITO, (d) target/MCH/capture DNA/Au/ITO and(e) H1/H2/target/MCH/capture DNA/Au/ITO electrode. (B) ECL-voltage curves obtained at the closed BPE platform when the cathode is bare ITO (inset, PMT was set at 800 V), Au/ITO (a), MCH/capture DNA/Au/ITO (b), target/MCH/capture DNA/Au/ITO (c) and H1/H2/target/MCH/capture DNA/Au/ITO (d). The PMT was set at 400 V. The concentration of H_2O_2 was 1 mM. The concentration of target DNA was 0.5 μM.

3.5 Optimizing the concentration of the ECL reagent and $AuCl_4^-$

To improve the sensitivity of the proposed biosensor, we first investigated the effect of the concentration of the ECL reagent on the ECL signal at the anode of the BPE. Fig. 4A shows the ECL signal recorded in the absence and presence of the target DNA. The anodic ECL intensity increased as the $Ru(bpy)_3^{2+}$concentration increased when the cathode of the BPE was incubated with the target solution as compared to no target solution. The highest ECL change could be obtained when the concentration of $Ru(bpy)_3^{2+}$ was 1.0 mM. Thus, this concentration was selected as the optimal concentration for the following experiments.

Fig. 4B shows the ECL signal obtained at the Au particle-modified ITO electrodes prepared under different concentrations of $AuCl_4^-$ in the absence and presence of the target. As expected, the ECL intensity at the Au/ITO electrode

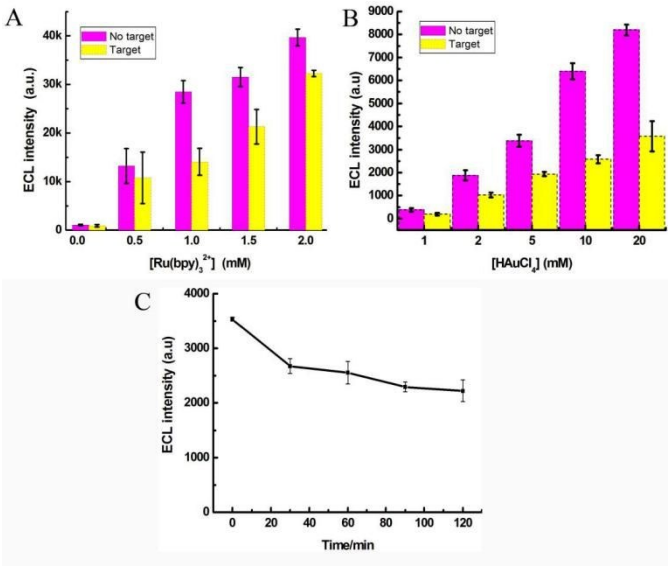

Fig. 4 (A) ECL intensity obtained at different concentrations of $Ru(bpy)_3^{2+}$ in the absence and presence of the target. The PMT was set at500 V. The concentration of $Ru(bpy)_3^{2+}$ was 0.1, 0.5, 1, 1.5, and 2.0 mM, respectively. The concentration of DBAE was 10-fold of $Ru(bpy)_3^{2+}$. (B) ECL intensity obtained at various concentrations of $AuCl_4^-$ deposited on the ITO electrode in the absence and presence of the target. 1 mM $Ru(bpy)_3^{2+}$ and 10 mM DBAE were added into the reporting reservoir. (C) Plot of ECL intensity as a function of the incubation time of H1 and H2. The concentration of target DNA was 0.5 μM. 1 mM H_2O_2 (0.01 M PBS) was added into the sensing reservoir.

was obviously enhanced when a high density of Au particles was present. The value of ΔI (ΔI=I_0-I, where I_0 and I represented the ECL intensity in the absence and presence of the target, respectively) increased as the concentration of $AuCl_4$ increased. However, the Au film was nonuniform, unstable and easily removed from the ITO electrode surface once the concentration of $AuCl_4^-$ reached 20 mM. Therefore, 10 mM $AuCl_4^-$ was selected for the following experiments.

The hybridization time of the HCR was also studied, and the results are displayed in Fig. 4C. As expected, the ECL intensity decreased as the HCR incubation time increased due to the increased resistance of the modified electrode and then leveled off when the incubation time reached 120 min. Thus, 120 min was selected as the optimal time for the following experiments. The ECL intensity could be reduced by 35.8 % after the HCR process.

3.6 Calibration curve

Under the optimized conditions, the ECL response toward the concentration of target DNA was measured, as shown in Fig. 5A. Increasing concentrations of target DNA led to a decrease in ECL intensity. A linear relationship between the ECL intensity and the logarithm of the target DNA concentration was found from 0.1 nM to 0.5 μM. The linear regression equation was I= 3888 - 394lg $C_{(pM)}$with a correlation coefficient of -0.9905. Compared with other HCR based amplified approaches, such as electrochemical assay[28], Surface-enhanced Raman spectroscopy[29], and fluorescent[30], the designed shows an acceptable linear range for the detection of DNA.

Then, quantitative analysis of the H_2O_2 concentration was carried out on the above BPE device. The cathode of BPE was H1/H2/target/MCH/capture DNA/Au/ITO electrode and the results are shown in Fig. 5B. The ECL signal increased as the concentration of H_2O_2 increased. A linear relationship between the ECL intensity and H_2O_2 concentration was found from 0 to 5 mM. The linear regression equation was I= 694 + 525C(H_2O_2) with a correlation coefficient of 0.9923. All these results indicated that the proposed biosensor could be used for both DNA and H_2O_2 detection.

The selectivity of the designed biosensor was evaluated by comparing the ECL signal in the absence of target DNA and in the presence of complementary DNA (cDNA), a mixed solution of helper DNA (H1) and single-base mismatched DNA (ST), and a mixed solution of different helper DNA probes (H1+H2). Fig.5C shows that the ECL intensity demonstrated an apparent decrease in the presence of helper DNA (H1+H2) compared to the ECL intensity in the presence of cDNA and the mixture of helper DNA (H1) and single-base mismatched DNA (ST), indicating a satisfactory selectivity and an improved sensitivity after the HCR process. Fig. 5D displays the excellent stability of the biosensor towards target detection.

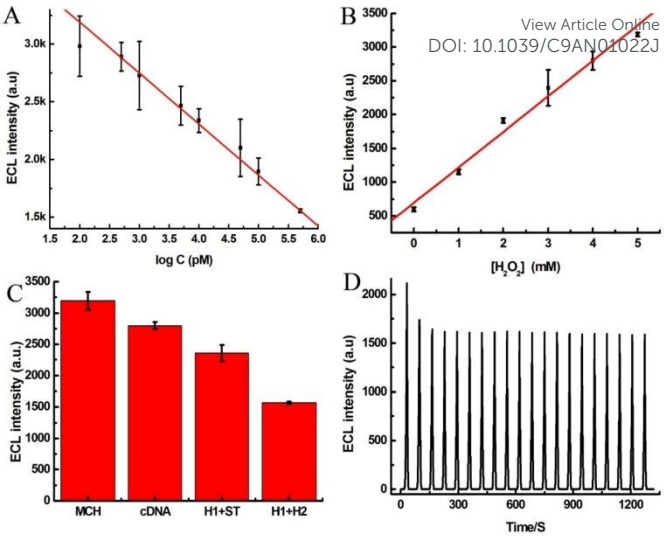

Fig. 5 Calibration curve corresponding to the ECL intensity as a function of the target DNA (A) and H_2O_2 (B) concentrations. 1 mM H_2O_2 (0.01 M PBS) was added into the sensing reservoir (A). H1/H2/target/MCH/capture DNA/Au/ITO electrode was used to obtain the calibration curve of H_2O_2 (B). (C) Selectivity of the ECL biosensor. ECL intensities were recorded at the MCH/capture DNA/Au/ITO, cDNA/MCH/capture DNA/Au/ITO, H1/ST/target/MCH/capture DNA/Au/ITO, and H1/H2/target/MCH/capture DNA/Au/ITO electrodes. (D) Stability of H1/H2/target/MCH/capture DNA/Au/ITO electrodes. The concentration of target DNA was 0.5 μM.

4. Conclusion

A convenient dual-analyte detection platform is constructed based on a closed-BPE chip. The ECL signal at the anode of the BPE is coupled with the electrochemical signal at the cathode of the BPE, which is triggered by target DNA and amplified by the extension of the DNA sequence during the HCR process. As a result, the designed biosensor can achieve sensitive detection of DNA and H_2O_2 with acceptable stability and reproducibility. Therefore, the proposed method displays great promise in biomolecular detection.

Conflicts of interest

There are no conflicts to declare.

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References

1 G. Loget, J. Roche and A. Kuhn, Adv. Mater. 2012, **24**, 5111-5116.
2 I. Malytska, C. Meziere, M. Kielar, L. Hirsch, G. Wantz, N. Avarvari, A. Kuhn and L. Bouffier, J. Phys. Chem. C, 2007, **121**, 12921-12927.
3 H.R. Zhang, Y.Z. Wang, W. Zhao, J.J. Xu and H.Y. Chen, Anal. Chem., 2016, **88**, 2884-2890.

View Article Online
DOI: 10.1039/C9AN01022J

4 M.S. Wu, Z. Liu, H.W. Shi, H.Y. Chen, J.J. Xu, Anal. Chem., 2015, **87**, 530-537.
5 Y.Z. Wang, W. Zhao, P.P. Dai, H.J. Lu, J.J. Xu, J. Pan and H.Y. Chen, Biosens. Bioelectron.,2016, **86**, 683-689.
6 Y.Z. Wang, C.H. Xu, W. Zhao, Q.Y. Guan and H.Y. Chen, J.J. Xu, Anal. Chem., 2017, **89**, 8050-8056.
7 H.W. Shi, W. Zhao, Z. Liu, X.C. Liu, J.J. Xu and H.Y. Chen, Anal. Chem., 2016, **88**, 8795-8801.
8 H.J. Lu, W. Zhao, J.J. Xu and H.Y. Chen, Biosens. Bioelectron., 2018, **102**, 624-630.
9 F. Yuan, L.M. Qi, T.H. Fereja, D.V. Snizhko, Z.Y. Liu, W. Zhang and G.B. Xu, Electrochi. Acta, 2018, **262**, 182-186.
10 C.L. Liu, D. Wang, C.S. Zhang, Sensor. Actuat. B-Chem., 2018, **270**, 341-352.
11 S.G. Ge, J.G. Zhao, S.P. Wang, F.F. Lan, M. Yan and J.H. Yu, Biosens. Bioelectron., 2018, **102**, 411-417.
12 S. Baek, S.R. Kwon, S.Y. Yeon, S.H. Yoon, C.M. Kang, S.H. Han and D. Lee, T.D. Chung, Anal.Chem., 2018, **90**, 4749-4755.
13 H. Motaghi, S. Ziyaee, M.A. Mehrgardi, A.A. Kajani and A.-K. Bordbar, Biosens. Bioelectron., 2018, **118**, 217-223.
14 X. Zhang, N. Bao, X. Luo and S.-N. Ding, Biosens. Bioelectron., 2018, **114**, 44-51.
15 J.-D. Zhang, L. Lu, X.-F. Zhu, L.-J. Zhang, S. Yun, C.-S. Duanmu and L. He, ACS Sensors, 2018, **3**, 2351-2358.
16 H. Motaghi, S. Ziyaee, M.A. Mehrgardi, A.A. Kajani and A.-K. Bordbar, Biosens. Bioelectron., 2018, **118**, 217-223.
17 C. Liu, D. Wang and C. Zhang, Sensor. Actuat. B-Chem., 2018, **270**, 341-352.
18 K.-F. Chow, F.o. Mavré and R.M. Crooks, J. Am. Chem. Soc., 2008, **130**, 7544-7545.
19 X.M. Lin, L.Y. Zheng, G.M. Gao, Y.W. Chi and G.N. Chen, Anal. Chem., 2012, **84**, 7700-7707.
20 M.S. Wu, D.J. Yuan, J.J. Xu and H.Y. Chen, Anal. Chem., 2013, **85**, 11960-11965.
21 Q. Zhai, X. Zhang, Y. Han, J. Zhai, J. Li and E. Wang, Anal. Chem., 2016, **88**, 945-951.
22 M.-S. Wu, Z. Liu, H.-W. Shi, H.-Y. Chen and J.-J. Xu, Anal. Chem., 2015, **87**, 530-537.
23 X. Zhang and S.-N. Ding, Biosens. Bioelectron., 2017, **94**, 47-55.
24 B. Zhang, B. Liu, D. Tang, R. Niessner, G. Chen and D. Knopp, Anal. Chem., 2012, **84**, 5392-5399.
25 H. Cheng, J. Liu, W. Ma, S. Duan, J. Huang, X. He and K. Wang, Anal. Chem., 2018, **90**, 12544-12552.
26 Y. Chen, J. Xu, J. Su, Y. Xiang, R. Yuan and Y. Chai, Anal. Chem., 2012, **84**, 7750-7755.
27 E.E. Augspurger, M. Rana and M.V. Yigit, ACS Sensors, 2018, **3**, 878-902.
28 T. Hou, W. Li, X. Liu, F. Li, Anal. Chem., 2015, **87**, 11368-11374.
29 X. Li, S. Ye, X. Luo, Chem. Commun. 2016, **52**, 10269-10272.
30 B. Wang, Y. Wu, Y. Chen, B. Weng, L. Xu, C. Li, Biosens. Bioelectron.2016, **81**, 125-130.

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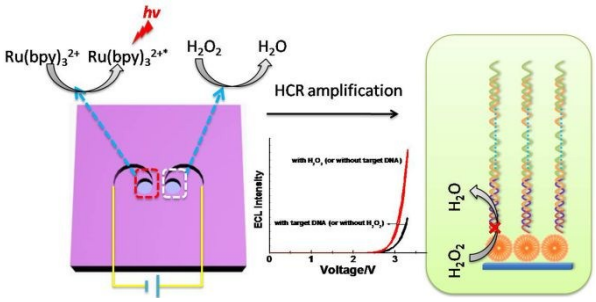
TOC



A electrochemiluminescence (ECL) closed bipolar electrode (BPE) chip was designed based on hybridization chain reaction (HCR)-induced amplification strategy