Article

An Ultrasensitive Electrochemiluminescence Biosensing Platform for Detection of Multiple Types of Biomarkers toward Identical Cancer on a Single Interface

Yamin Nie, Pu Zhang, Haijun Wang, Ying Zhuo, Ya-Qin Chai, and Ruo Yuan

Anal. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.analchem.7b03240 • Publication Date (Web): 03 Nov 2017

An Ultrasensitive Electrochemiluminescence Biosensing Platform for Detection of Multiple Types of Biomarkers toward Identical Cancer on a Single Interface

Yamin Nie, Pu Zhang, Haijun Wang, Ying Zhuo, Yaqin Chai,* Ruo Yuan*

Key Laboratory of Luminescent and Real-Time Analytical Chemistry (Southwest University), Ministry of Education, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China

* Corresponding author. Tel.: +86-23-68252277; Fax: +86-23-68253172.

E-mail address: yqchai@swu.edu.cn； yuanruo@swu.edu.cn

ABSTRACT

Electrochemiluminescence (ECL) with high sensitivity and excellent controllability provides a promising approach for ultrasensitive detection of multiple biomarkers. However, the detection for multiple types of biomarkers on a single interface remains considerable challenge owing to the functional differentiation of different types of biomarkers. Herein, we utilized "on-off-on" switching, target-induced cleavage of peptide, and TdT (terminal deoxynucleoside transferase)-mediated extension successfully constructing a novel ECL biosensor for the ultrasensitive detection of microRNA-141 (miRNA-141) and matrix metalloproteinase-2 (MMP-2). Importantly, the dual biomarkers are related with several identical cancers, which endow the biosensor with diagnostic accuracy and efficiency. In this protocol, target 1 (miRNA-141) firstly hybridized with probe DNA (pDNA) assembled on CdS QDs modified sensing surface. Afterwards, miRNA-141 captured trigger DNA (tDNA) to generate a long ssDNA nanotail *via* TdT-mediated DNA polymerization. Then the forming ssDNA could capture abundant Fc-peptide-ssDNA conjugates through the hybridization reaction, the ECL intensity quenched significantly due to the efficient quenching effect of Fc to CdS QDs, realizing the ultrasensitive detection of miRNA-141 with a detection limit of 33 aM (*S/N=3*). After incubated with target 2 (MMP-2) which specifically cleaved the Fc-peptide-ssDNA conjugates causing the releasing of Fc from the sensing surface, the ECL intensity had an obvious enhancement, achieving the ultrasensitive analysis of MMP-2 with a detection limit of 33 $fg \cdot mL^{-1}$ (*S/N=3*). More importantly, this

biosensor also realized the monitoring of biomarkers in different cancer cells and human serum, which indicated that the biosensing system could serve as applicable tools in clinical analysis.

INTRODUCTION

The detection for multiple biomarkers toward identical cancer could considerably improve diagnostic accuracy and efficiency, which holds critical promise in reducing the burden of cancer.[1] Up to now, numerous efforts have been devoted to constructing biosensors for the monitoring of multiple biomarkers in the field of fluorescence, surface-enhanced Raman spectroscopy, electrochemistry, electrochemiluminescence, and photoelectrochemistry, which usually depend on the functional differentiation of biomarkers, such as the specific recognition of antigen with antibody and the selective hybridization of DNA/microRNA(miRNA) with molecular beacons.[2-5] As a result, the existing literatures concerning the detection for multiple biomarkers usually get stuck in the analysis of the same type of biomarkers. However, there are many types of biomarkers, such as protein, nucleic acid, and the combination of different types of biomarkers could reflect more comprehensive information and play decisive role in clinical decision-making. Therefore, it is highly meaningful but full of challenge to develop a biosensor for the detection of multiple types of biomarkers.

Electrochemiluminescence (ECL), as a powerful analytical technique, has been received increasing concern owing to its merits of high sensitivity, low background,

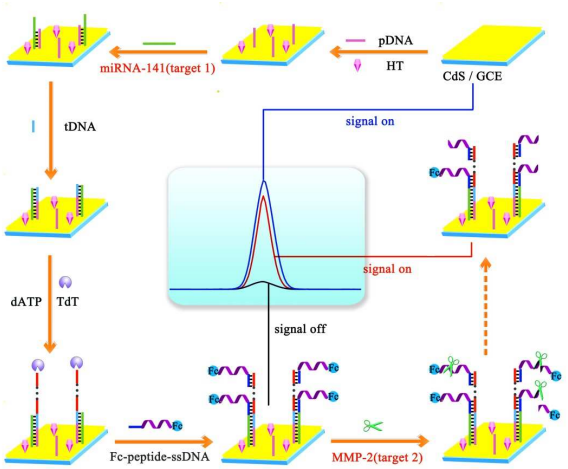
simple operation, and excellent controllability,[6,7,8] which enable prospective application in the detection of multiple biomarkers. Specifically, a potential-resolution ECL system labelling diverse luminophores with different ECL-potential responses, has been successfully applied to monitor multiple biomarkers. Jiang′s group has reported a potential-resolution ECL system achieving the determination of two antigens at the cell surface with $Ru(bpy)_3^{2+}$/luminol as ECL luminophores.[9] Unfortunately, the potential-resolution ECL system for multiple ECL detection has usually restricted with unavoidable cross reactions among different ECL luminophores. Recently, our group has devoted to circumvent this problem.[10-12] For example, the implementation of reasonable-regulated movement of DNA-based nanomachines achieves the multiple detecton of miRNAs,[11,12] and the application of multivariate linear algebraic equations realizes the multiple monitoring of proteins.[10] Although the above-mentioned assays have successfully overcome the inevitable cross reactions, these developed methods are limited to detect the same types of biomarkers. Therefore, it is extremely challenging to provide a feasible approach to analyzing multiple types of biomarkers on a single interface with the capacity to eliminate cross reactions.

MicroRNA-141 (miRNA-141), a small regulatory molecule with the function to modulate the activity of specific mRNA, plays an important role in cellular proliferation, differentiation and apoptosis.[13,14] Consequently, the dysregulated expression (downregulation or upregulation) of miRNA-141 usually correlated with various cancers, including colorectal cancer,[15] breast cancer,[16] pancreatic cancer,[17]

ovarian cancer,[18] prostate cancer,[19] and so on. In addition, matrix metalloproteinase-2 (MMP-2), a decisive member of the matrix metalloproteinases (MMPs), plays a vital part in the process of tumor invasion, angiogenesis and metastasis.[20] Moreover, MMP-2 upregulated in various malignant tumor, such as colorectal cancer,[21] breast cancer,[22] pancreatic cancer,[23] ovarian cancer,[24] prostate cancer,[25] cervical cancer[26]. Comparison with the corresponding cancers for miRNA-141 and MMP-2, we inferred that miRNA-141 and MMP-2 could serve as promising biomarkers for several identical cancers. Therefore, the construction of an ultrasensitive sensing platform which is aimed at detecting the dual types of biomarkers, is significantly meaningful for improving cancer diagnostic accuracy and efficiency.

Herein, we rationally developed a novel "on-off-on" ECL biosensor based on TdT-mediated extension and target-induced cleavage of peptide for the ultrasensitive detection of miRNA-141 and MMP-2 toward identical cancer on a single interface, improving the diagnostic efficiency and accuracy. As illustrated in Scheme 1, to begin with, target 1 (miRNA-141) hybridized with 5′-thiolated and 3′-phosphate group-blocked probe DNA (pDNA) modified on the CdS QDs surface which serve as the ECL emitters, and the pDNA could hybridize with trigger DNA (tDNA). With the help of TdT, a long ssDNA nanotail was generated at 3' OH terminal of tDNA, and then the forming nanotail could capture numerous Fc-peptide-ssDNA conjugates, leading to a signal switch "off" state owing to efficiently quenching effect of Fc to CdS QDs[27] and thus achieving the ultrasensitive detection of target 1 (miRNA-141). In the presence of target 2 (MMP-2), Fc-peptide-ssDNA conjugates

(HOOC-PLGVR-Fc) were specifically hydrolyzed at a particular site between G and V, leading Fc to release from the sensing surface and provoking a switch "on" state. Depending on the recovery of ECL intensity, the biosensor successfully realized the detection of target 2 (MMP-2). More importantly, as an example, this elaborated biosensor was successfully implemented for detect targets in spiked serum samples and cancer cells, indicating the platform holds considerable potential for the real application of cancer diagnosis.



Scheme 1. Fabrication of the ECL Biosensing Platform for the Detection of Multipe Types of Biomarkers on a Single Interface.

EXPERIMENTAL METHODS

The analytical apparatus, measurements, chemicals and materials, preparation and characterization of the ECL emitter, the synthetic process of Fc-peptide-ssDNA conjugates, cell culture and total RNA extraction， and polyacrylamide gel electrophoresis (PAGE) are displayed in the Supporting Information.

Fabrication of the Proposed Biosensor. The schematic manifestation

displaying the step fabrication of the prepared biosensor was shown in Scheme 1. To start with, the GCE was polished with alumina slurry, followed by rinsing with anhydrous ethanol and ultrapure water for 5 min, respectively. The well-polished GCE was first coated with 18 μL of CdS QDs with an air-drying treatment. 10 μL of 2.0 μM pDNA was incubated for 16 h on the CdS QDs surface at room temperature, followed by soaking in 1 mM HT for 40 min to block the nonspecific binding sites. Then 10 μL of target 1 (miRNA-141) was dropped onto the electrode for 2 h. After that, the above modified electrode was incubated with 10 μL of 2.0 μM tDNA for 2 h. Subsequently, the resultant electrode was immersed in the TdT extension solution (200 $U \cdot mL^{-1}$ TdT, 4 mM dATP, 50 mM KAc, 10 mM $Mg(Ac)_2$, 20 mM Tris−Ac, 0.25 mM $CoCl_2$, pH 7.9) and incubated at 37 °C for 60 min to form a long trigger nanotail. Following that, the Fc-peptide-ssDNA conjugates solution (The detailed synthetic process was showed in the Supporting Information.) was cast onto the modified electrode for 2 h to make the conjugates assemble on the sensing surface through the hybridization of ssDNA and trigger nanotail. Finally, the resultant modified electrode was incubated with 10 μL of MMP-2 for 60 min to specifically cleave the Fc-peptide-ssDNA conjugates and make quencher leave the biosensing surface. After each step, the modified electrode was thoroughly rinsed with PBS (pH 7.4) to get rid of physically absorbed species.

RESULTS AND DISCUSSION

Electrochemical Characterization of the Assembled Biosensing Platform. As an effective technique for interfacial investigation, electrochemical impedance

spectroscopy (EIS) was harnessed to characterize the fabrication of the biosensor. As illustrated in Figure 1A, a small semicircle was obtained from thoroughly cleaned bare GCE (curve a). When CdS QDs were dropped onto the electrode surface, a distinctly increased semicircle was acquired (curve b), resulting from the intrinsic semi-conductive property of CdS QDs. After the biosensing surface was incubated with negatively charged pDNA, the resistance augmented (curve c) owing to the electrostatic repulsion to negatively charged $[Fe(CN)_6]^{3-/4-}$ in test solution. Subsequently, blocking of nonspecific sites with HT generated a decreased resistance (curve d), because the modified HT compelled pDNA to undergo conformational changes whose strands stood up and oriented towards the solution.[28, 29] With the stepwise assembling of miRNA-141, tDNA and extension solution, the resistance sequentially increased (curve e, f, and g) due to the augment of interfacial negative charge causing an increased barrier for the $[Fe(CN)_6]^{3-/4-}$ to the biosensing interface. After the Fc-peptide-ssDNA conjugates were immobilized onto the electrode, the EIS signal significantly increased (curve h) since the charge transfer hindrance of peptide and ssDNA over the promotion of Fc. When the electrode was incubated with MMP-2, the resistance obviously reduced (curve i) because Fc-peptide-ssDNA conjugates (HOOC-PLGVR-Fc) were specifically cleaved at the recognition site (between G and V) by MMP-2 leading Fc release from the sensing surface. These above-mentioned results indicated that the designed biosensor was satisfactorily fabricated.

ECL Performance of the On-Off-On Switching Process. As shown in Figure 1B, there was a high ECL response when the electrode was coated with CdS QDs

(state on). However, after the fabrication of Fc-peptide-ssDNA conjugates, the ECL intensity significantly quenched due to the quenching effect of Fc to CdS QDs (state off). Surprisingly, the ECL intensity obviously augmented (state on) when target 2 (MMP-2) was incubated on the electrode, because MMP-2 specifically hydrolyzed the peptide of the Fc-peptide-ssDNA conjugates, leading to the release of the quencher from the sensing platform. The above results demonstrated the on-off-on switching system was successfully proposed.

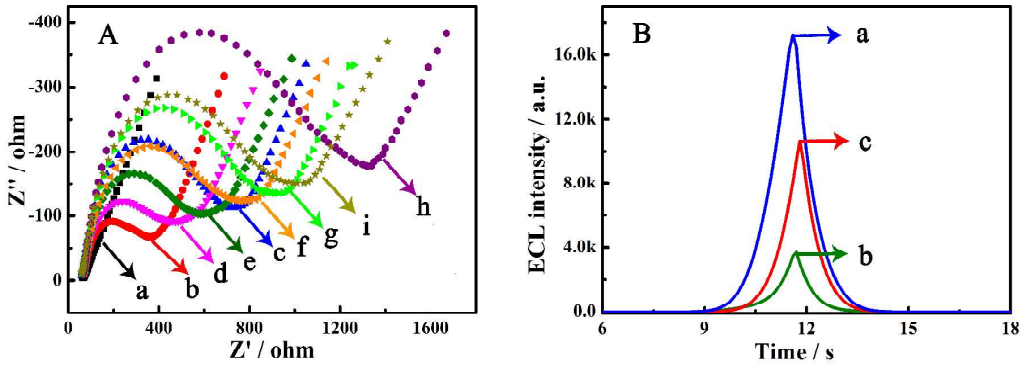


Figure 1. (A) The EIS characterization of the stepwise modified electrodes in 2 mL PBS (pH 7.4, 0.1 M) containing 0.1 M KCl and 5.0 mM $[Fe(CN)_6]^{3-/4-}$: (a) bare GCE; (b) CdS/GCE; (c) pDNA/CdS/GCE; (d) HT/pDNA/CdS/GCE; (e) miRNA-141/HT/pDNA/CdS/GCE; (f) tDNA/miRNA-141/HT/pDNA/CdS/GCE; (g) extension solution/tDNA/miRNA-141/HT/pDNA/CdS/GCE; (h) Fc-peptide-ssDNA conjugates/ extension solution/tDNA/miRNA-141/HT/pDNA/CdS/GCE; (i) MMP-2/Fc-peptide-ssDNA conjugates/extension solution/tDNA/miRNA-141/HT/pDNA/CdS/GCE. (B) ECL performance of the on-off-on switching process in PBS solution (pH 7.4) including 0.05 M of $K_2S_2O_8$:

coated with CdS QD (state on, a), incubated with Fc-peptide-ssDNA conjugates (state off, b), incubated with target 2 (MMP-2) (state on, c).

Optimized Conditions of the Proposed Biosensor. In order to improve the sensitivity and quenching efficiency of the constructed biosensor, the optimal concentration of the peptide during the process of the synthetic Fc-peptide-ssDNA conjugates was firstly investigated. Figure 2A showed that the ECL intensity decreased with an increasing concentration of peptide with 2 μM of ssDNA. Moreover, when the concentration of peptide was 100 μM, a minimum value emerged, thereby, 100 μM was selected as the optimizing concentration in the conjugates synthetic process of peptide.

Furthermore, the incubation time of MMP-2 also played a critical role in the performance of the developed biosensor. As illustrated in Figure 2B, the signal augmented with the passage of incubation time, and nearly became a constant value at 30 min. Therefore, the optimal incubation time for MMP-2 was chosen 30 min.

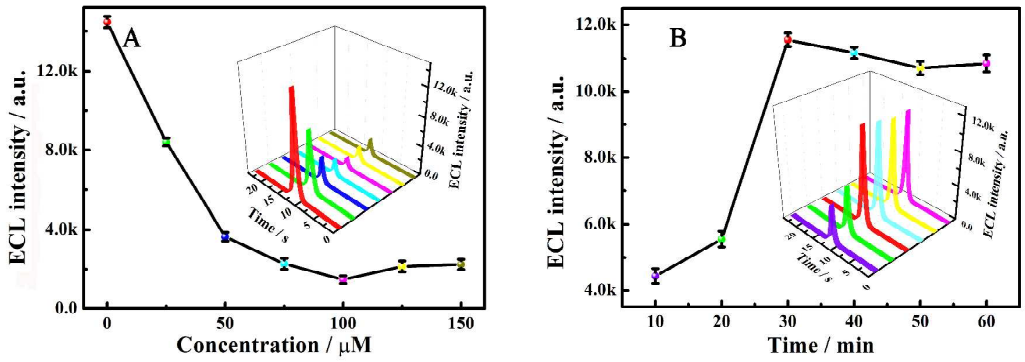


Figure 2. (A) Optimizing the concentration of the peptide in the Fc-peptide-ssDNA conjugates synthetic step. (B) Optimizing the incubation time of MMP-2. All ECL

intensity was measured in 0.05 M $K_2S_2O_8$ in 2 mL of PBS (0.1 M, pH 7.4) by scanning the potential from -1.2 to 0 V (vs Ag/AgCl) at a scanning rate of 0.1 $V \cdot s^{-1}$ with the voltage of 800 V.

Ultrasensitive Detection of Dual Types of Biomarkers with the Well-designed Biosensor. The proposed biosensor was exploited to detect the dual biomarkers with a series of concentration under the optimum conditions. As illustrated in Figure 3A, the ECL intensity gradually decreased with an incremental concentration of target 1 (miRNA-141) from 0.1 fM to 100 pM (curves a-g). Moreover, the quenching of ECL intensity linearly responds to the logarithmic value of miRNA-141 concentration with squared correlation coefficient of R^2 = 0.997. In addition, the equation was expressed as I = 3526.7 - 1206.9 lgc_1, where I was the ECL intensity and c was the concentration of target, and the estimated detection limit of target 1 was 33 aM.

Nevertheless, it was obviously seen from Figure 3B that the ECL intensity gradually increased with the variation of target 2 (MMP-2) concentration from 0.1 $pg \cdot mL^{-1}$ to 100 $ng \cdot mL^{-1}$ as the concentration of target 1 (miRNA-141) fixed with 100 pM. In addition, the linear equation was I = 8313.0 + 1168.2 lgc_2 with the squared correlation coefficient of R^2 = 0.998, and the detection limit was estimated 33 $fg \cdot mL^{-1}$. Impressively, compared with other reported literatures (Table 1 and Table 2), the elaborately designed biosensor not only realized multiple detection for dual cancer markers toward identical cancer, but also exhibited high sensitivity, paving an

promising way for clinical diagnosis.

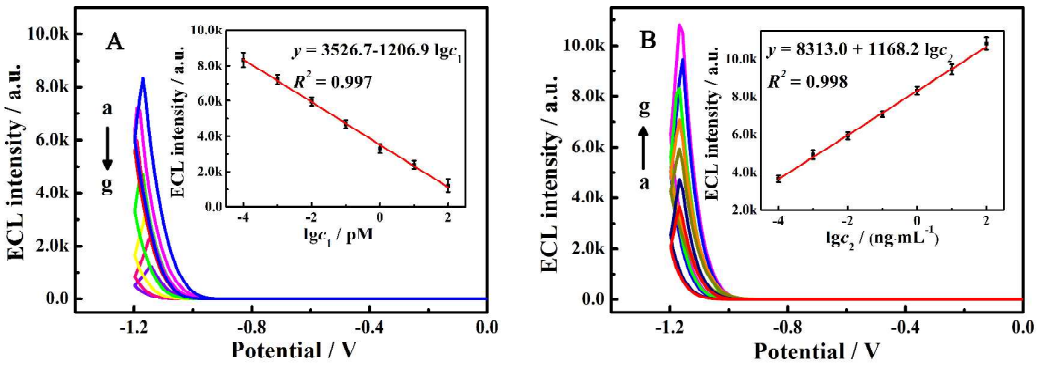


Figure 3. (A) ECL response of the developed biosensor with different concentrations of target 1 (miRNA-141): (a) 0.1 fM, (b) 1 fM, (c) 0.01 pM, (d) 0.1 pM, (e) 1 pM, (f) 10 pM, (g) 100 pM. (B) ECL intensity of the biosensor in various concentrations of target 2 (MMP-2): (a) 0.1 pg·mL^{-1}, (b) 1 pg·mL^{-1}, (c) 0.01 ng·mL^{-1}, (d) 0.1 ng·mL^{-1}, (e) 1 ng·mL^{-1}, (f) 10 ng·mL^{-1}, (g) 100 ng·mL^{-1}. The scanning potential ranged from -1.2 to 0 V, and the ECL signal was detected in PBS (pH 7.4) containing 0.05 M of $K_2S_2O_8$.

Table 1. Comparison of the Proposed Biosensor for Target 1 (miRNA-141) with Other Work

Method	Target	Linear range	Detection limit	References
photoelectrochemical	miRNA-141	0.1 fM-0.5 nM	27 aM	30
fluorescent	miRNA-21	10 fM-100 nM	10 fM	31
electrochemical	miRNA-122	0.1 fM-0.5 nM	0.1 fM	32
electrochemical	miRNA-122b	10 aM-1 pM	10 aM	33
ECL	miRNA-21	10 fM-10 pM	10 fM	34

ECL	miRNA-141	0.1 fM-100 pM	33 aM	This work

Table 2. Comparison of the Elaborated Biosensing Platform for Target 2 (MMP-2) with Other Work

Method	Target	Linear range	Detection limit	References
fluorescent	MMP-2	10 pg·mL^{-1}-500 pg·mL^{-1}	10 pg·mL^{-1}	35
fluorescent	MMP-2	1 pg·mL^{-1}-500 pg·mL^{-1}	1 pg·mL^{-1}	36
electrochemical	MMP-2	0.5 pg·mL^{-1}-50 ng·mL^{-1}	0.11 pg·mL^{-1}	37
electrochemical	MMP-2	0.5 pg·mL^{-1}-50 ng·mL^{-1}	0.15 pg·mL^{-1}	38
electrochemical	MMP-2	0.1pg·mL^{-1}-20 ng·mL^{-1}	0.03 pg·mL^{-1}	39
ECL	MMP-2	0.1 pg·mL^{-1}-100 ng·mL^{-1}	33 fg·mL^{-1}	This work

Performance of the Developed Biosensor. Stability is a vital factor to assess the performance of the developed biosensor. As illustrated in Figure 4A, the ECL response had scarcely any variation under continuous cyclic scans for 9 cycles with the relative standard deviation (RSD) of 1.37%, which indicated that the biosensor possessed the well-characterized of excellent stability.

To further estimate the reproducibility of the developed biosensor, the inter- and intra-assays were performed. As shown in Figure 4B, the intra-assay precision was explored by determining the ECL responses of four different sensing electrode made in the same batch, and the calculated relative standard deviation (RSD) was 3.42%. Moreover, the inter-assay precision was investigated by monitoring the ECL intensity of the same biosensing electrode under the different batches, and the RSD value was 3.31%. The RSD values of the intra- and inter-assays were all below 5%, indicating

the outstanding reproducibility of the ECL biosensor.

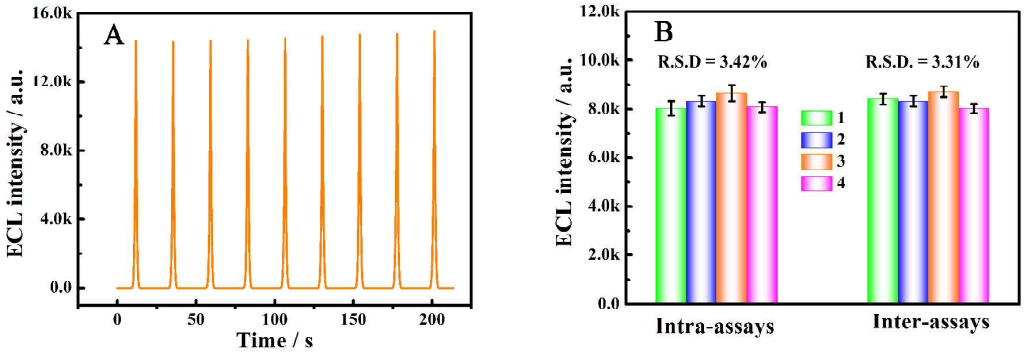


Figure 4. (A) ECL stability of the biosensor under continuous cyclic potential scans for 9 cycles under the biosensor incubated with TdT extension solution. (B) the reproducibility (intra-assays and inter-assays) of the proposed biosensor under the biosensor incubated with 1 ng·mL^{-1} MMP-2. All ECL intensity was measured in 0.05 M $K_2S_2O_8$ in 2 mL of PBS (0.1 M, pH 7.4) by scanning the potential from -1.2 to 0 V (vs Ag/AgCl) at a scanning rate of 0.1 V·s^{-1} with the voltage of 800 V.

To investigate the selectivity of the biosensor, multiple miRNAs and proteins were used as interfering substances. The comparative experiments for target 1 (miRNA-141) were conducted by using miRNA-21 (1 nM), miRNA-199a (1 nM), and miRNA-155 (1 nM) to replace miRNA-141(10 pM), respectively. As illustrated in Figure 6A, the interfering substances with little ECL response were detected, however, significant quenching was observed when detected the solution of miRNA-141 and the mixture containing 10 pM of miRNA-141. Therefore, the well-designed biosensor exhibited prominent specificity for target 1 (miRNA-141).

To investigate the selectivity for target 2 (MMP-2), interfering agents was used to substitute MMP-2 (10 ng·mL^{-1}), including MMP-7 (100 ng·mL^{-1}), lgG (100 ng·mL^{-1}), and CEA (100 ng·mL^{-1}), respectively. As described in Figure 6B, the ECL intensity of MMP-2 and the mixture containing MMP-2(10 ng·mL^{-1}) dramatically augmented, nevertheless, the signal of the interfering agents were no remarkable difference with the high concentration of 100 ng·mL^{-1}. According to the above results, we concluded that the biosensor displayed high specificity for target 2 (MMP-2). In summary, with desirable selectivity and stability, the biosensing platform showed potential applications in detecting different types of biomarkers toward identical cancer.

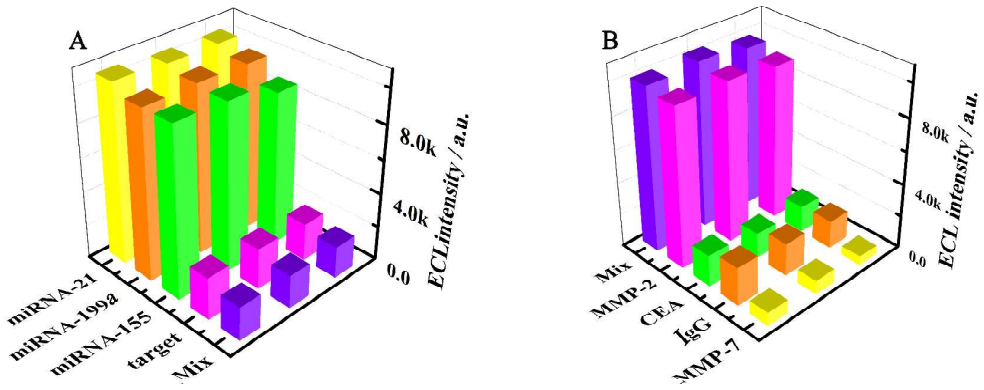


Figure 5. Investigated the selectivity of the designed biosensor with different substance: (A) miRNA-21 (1 nM), miRNA-199a (1 nM), miRNA-155 (1 nM), miRNA-141 (10 pM), and the mixture containing target 1 (10 pM); (B) MMP-7 (100 ng·mL^{-1}), lgG (100 ng·mL^{-1}), CEA (100 ng·mL^{-1}), MMP-2 (10 ng·mL^{-1}), and the mixture containing target 2 (10 ng·mL^{-1}).

Real Sample Analysis of the Proposed Biosensor. To investigate the

applicability of the biosensor in practical samples, the following experiments were conducted. To begin with, the sensor was applied to detect target 1 (miRNA-141) in the lysates from 22Rvl cancer cells and MCF-7 cancer cells. According to the experimental data in Figure 6, the ECL intensity decreased with the increasing of the cell numbers (b, c, d). The above-mentioned result showed that miRNA-141 was overexpressed in 22Rvl, which was compatible with the previous work.[19] However, ECL signal was slightly changed when incubating with the lysates from human breast (MCF-7) cancer cells (b, c, d), and the results were consistent with reported work.[16]

To further study the applicability of the biosensor for MMP-2 analysis, the biosensor was implemented to monitor the MMP-2 in the 20-fold diluted serum (serum from healthy human, serum from prostate cancer patient, and serum from breast cancer patient) and lysates from 10^5 22Rvl cancer cells and MCF-7 cancer cells. In order to ensure accuracy for the MMP-2 analysis, we introduced 100 pM of target 1 (miRNA-141) in advance. As showed in Figure 7, the ECL signal had a significant increase in all real samples (b, c, d, e, f) compared with the blank detection (a), and the ECL signal (c, d, e, f) was higher than serum from healthy human (b), which indicated that MMP-2 was overexpressed in cancer patients serum and lysates from cells, and the results were in agreement with the published reports.[22,25] Therefore, the biosensor holds the significant promise for ultrasensitive detection of MMP-2 in clinical diagnosis and prognostic evaluation. It can be concluded that the biosensor provided an efficient tool for monitoring different types of biomarkers toward identical cancer for improving cancer diagnostic efficiency and accuracy.

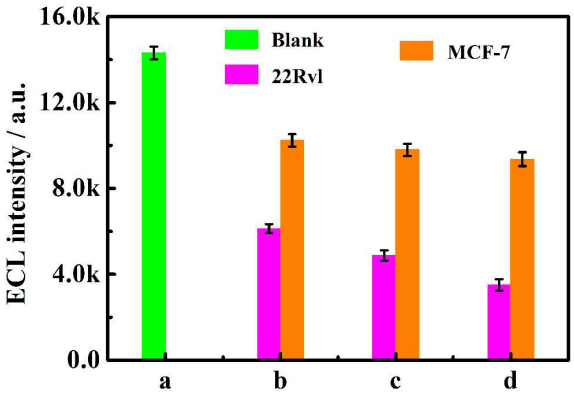


Figure 6. Analysis of miRNA-141 from the lysates from 22Rvl cancer cells and MCF-7 cancer cells: (a) blank detection without miRNA-141; (b) 10^3 cancer cells; (c) 10^4 cancer cells; (d) 10^5 cancer cells.

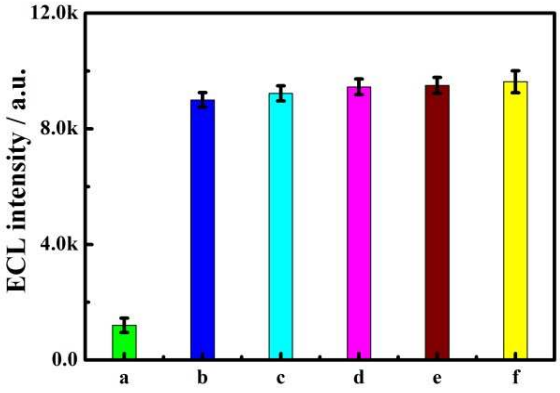


Figure 7. Analysis of MMP-2 in human serum and the lysates from cancer cells: (a) blank detection without MMP-2 (b) serum from healthy human; (c) serum from prostate cancer patients; (d) lysates from 10^5 22Rvl cancer cells; (e) serum from breast cancer patients; (f) lysates from 10^5 MCF-7 cancer cells.

CONCLUSIONS

In conclusion, we developed a novel electrochemiluminescence (ECL) biosensor

based on “on-off-on” switching, TdT-mediated extension and target-induced cleavage of peptide for the ultrasensitive detection of multiple types of biomarkers toward identical cancer on a single interface with diagnostic efficiency and accuracy. Furthermore, this elaborated biosensor was successfully used for monitoring multiple biomarkers on a single interface with one luminophore, which efficiently avoided the potential cross-reaction and operated simply. More importantly, the biosensor was implemented in real sample with sensitivity and could be applied as an ideal sensing platform for the detection of other different types of cancer biomarkers by changing the corresponding sequences of the pDNA, tDNA and peptide, paying a new avenue for cancer diagnosis and prognostic evaluation.

ASSOCIATED CONTENT

Supporting Information

Additional electronic information as pointed in the essay. This information is available free of charge via the Internet at http://pubs.acs.org

Chemicals and materials, apparatus and measurements, preparation and characterization of the ECL emitter, synthesis of Fc-peptide-ssDNA conjugates, cell culture and total RNA extraction, polyacrylamide gel electrophoresis (PAGE).

ACKNOWLEDGMENT

This work was financially supported by the National Natural Science Foundation (NNSF) of China (Grants 21775124, 21575116, 21675129 and 51473136), the Fundamental Research Funds for the Central Universities of China (Grant

XDJK2016E055), and Chongqing Postdoctoral Science Foundation (Xm2017016).

REFERENCES

(1) Liu, C. L.; Liu, A.Y.; Halabi, S. *Stat. Med.* **2011**, *30*, 2005-2014.

(2) Song, C. Y.; Yang ,Y. J.; Yang, B. Y.; Sun,Y. Z.; Zhao Y. P.; Wang H. *Nanoscale* **2016**, *8*, 17365-17373.

(3) Wang, C. G.; Zhang, H.; Zeng, D. D.; Sun, W. L.; Zhang, H. L.; Aldalbahi, A.; Wang, Y. S.; San, L. L.; Fan, C. H.; Zuo, X. L. *Nanoscale* **2015**, *7*, 15822-15829.

(4) Zheng, Y. N.; Liang, W. B.; Yuan, Y. L.; Xiong, C. Y.; Xie, S. B.; Wang, H. J.; Chai, Y. Q.; Yuan. R. *Biosens. Bioelectron.* **2016**, *81*, 423-430.

(5) Liu, X.; Jiang, H.; Fang, Y.; Zhao, W.; Wang, N. Y.; Zang, G. Z. *Anal. Chem.* **2015**, *87*, 9163-9169.

(6) Ding, C. F.; Zhang, W.; Wang, W; Chen, Y. Y.; Li, X. Q. Trends Anal. Chem. **2015**, *65*, 137-150.

(7) Xu, J. J.; Huang P. Y.; Qin, Y.; Jiang, D. C.; Chen H. Y. *Anal. Chem.* **2016**, *88*, 4609-4612.

(8) Zhang, Y.; Li, L.; Zhang, L. N.; Ge, S. G.; Yan, M.; Yu, J. H. *Nano Energy* **2017**, *31*, 174-182.

(9) Han, F. F.; Jiang, H.; Fang, D. J.; Jiang, D. C. *Anal. Chem.* **2014**, *86*, 6896-6902.

(10) Liang, W. B.; Fan, C.; Zhuo, Y.; Zheng, Y. N.; Xiong, C. Y.; Chai, Y. Q.; Yuan, R. *Anal. Chem.* **2016**, *88*, 4940-4948.

(11) Peng, L. C.; Zhang, P.; Chai, Y. Q.; Yuan, R. *Anal. Chem*. **2017**, *89*, 5036-5042.

(12) Zhang, P., Lin Z. F.; Zhuo, Y.; Yuan, R.; Chai, Y. Q. *Anal. Chem.* **2017**, *89*,

1338-1345.

(13) Shukla, H. D.; Mahmood, J.; Vujaskovic, Z. *Cancer Lett.* **2015**, 369, 28-36.

(14) Croce, C. M.; Calin, G. A. *Cell* **2005**, *122*, 6-7.

(15) Feng, L.; Ma, H. Q.; Chang, L.; Zhou, X. L.; Wang, N.; Zhao, L. M.; Zuo, J.; Wang, Y. D.; Han, J.; Wang, G. Y. *Exp. Ther. Med.* **2016**, *12*, 3405-3410.

(16) Li, P.; Xu, T.; Zhou, X.; Liao, L. Y.; Pang, G. L.; Luo, W.; Han, L.; Zhang, J. K.; Luo, X. Y.; Xie, X. B.; Zhu, K. C. *Cancer Med.* **2017**, *6*, 662-672.

(17) Zhao, G.; Wang, B.; Liu, Y.; Zhang, J. G.; Deng, S. C.; Qin, Q.; Tian, K.; Li, X.; Zhu, S.; Niu, Y.; Gong, Q.; Wang, C. Y. *Mol. Cancer Ther.* **2013**, *12*, 2569-2580.

(18) Gao, Y. C.; Wu, J. *Tumor Biol.* **2015**, *36*, 4843-4850.

(19) Brase, J. C.; Johannes, M.; Schlomm, T.; Falth, M.; Haese, A.; Steuber, T.; Beissbarth, T.; Kuner, R.; Sultmann, H. *Int. J. Cancer* **2011**, *128*, 608-616.

(20) Roy, R.; Yang, J.; Moses, M. A. *J. Clin. Oncol.* **2009**, *27*, 5287-5297.

(21) Deng, J. L.; Chen, W. J.; Du, Y.; Wang, W. M.; Zhang, G. Q.; Tang, Y. H.; Qian, Z. J.; Xu, P.; Cao, Z. H.; Zhou, Y. *Cancer Biomark.* **2017**, *19*, 57-64.

(22) Radenkovic, S.; Konjevic, G.; Jurisic, V.; Karadzic, K.; Nikitovic, M.; Gopcevic, K. *Cell Biochem. Biophys.* **2014**, *68*, 143-152.

(23) Xu, L. H.; Ding, X. M.; Tan, H.; Qian, J. J. *Cancer Cell Int.* **2013**, *13*, 1-7.

(24) Kenny, H. A.; Lengyel, E. *Cell Cycle* **2009**, *8*, 683-688.

(25) Reis, S. T.; Antunes, A. A.; Pontes, J.; de Sousa-Canavez, J. M.; Dall'Oglio, M. F.; Piantino, C. B.; da Cruz, J. A. S.; Morais, D. R.; Srougi, M.; Leite, K. R. M. *Int. Braz. J. Urol.* **2012**, *38*, 167-174.

(26) Rauvala, M.; Aglund, K.; Puistola, U.; Turpeenniemi-Hujanen, T.; Horvath, G.; Willén, R.; Stendahl, R. *Int. J. Gynecol. Cancer* **2006**, *16*, 1297-1302.

(27) Deng, S. Y.; Lei, J. P.; Liu, Y.; Huang, Y.; Ju, H. X. *Chem. Commun.* **2013**, *49*, 2106-2108.

(28) Gebala, M.; Stoica, L.; Neugebauer, S.; Schuhmann, W. *Electroanal.* **2009**, *21*, 325-331.

(29) Shi, K.; Dou, B. T.; Yang, C. Y.; Chai, Y. Q.; Yuan, R.; Xiang, Y. *Anal. Chem.* **2015**, *87*, 8578-8583.

(30) Ye, C.; Wang, M. Q.; Gao, Z. F.; Zhang, Y.; Lei, J. L.; Luo, H. Q.; Li, N. B. *Anal. Chem.* **2016**, *88*, 11444-11449.

(31) Duan, R. X.; Zuo, X. L.; Wang, S. T.; Quan X. Y.; Chen, D. L.; Chen, Z. F.; Jiang, L.; Fan, C. H.; Xia, F. *J. Am. Chem. Soc.* **2013**, *135*, 4604-4607.

(32) Wang, T. Y.; Viennois, E.; Merlin, D.; Wang, G. L. *Anal. Chem.* **2015**, *87*, 8173-8180.

(33) Ge, Z. L.; Lin, M. H.; Wang, P.; Pei, H.; Yan, J.; Shi, J. Y.; Huang, Q.; He, D. N.; Fan, C. H.; Zuo, X. L. *Anal. Chem.* **2014,** 86, 2124-2130.

(34) Liao, Y. H.; Huang, R.; Ma, Z. K.; Wu, Y. X.; Zhou, X. M.; Xing, D. *Anal. Chem.* **2014**, *86*, 4596-4604.

(35) Wang, Y. H.; Shen, P.; Li, C. Y.; Liu, Z. H. *Anal. Chem.* **2012**, *84*, 1466-1473.

(36) Zheng, T.; Zhang, R.; Zhang, Q.; Tan, T.; Zhang, K.; Zhu, J. J.; Wang, H. *Chem. Commun.* **2013**, *49*, 7881-7883.

(37) Yang, G.; Li, L.; Rana, R. K.; Zhu, J. J. *Carbon* **2013**, *61*, 357-366.

(38) Wang, D.; Yuan, Y. L.; Zheng, Y. N.; Chai, Y. Q.; Yuan, R. *Chem. Commun.* **2016**, *52*, 5943-5945.

(39) Kou, B. B.; Chai, Y. Q.; Yuan, Y. L.; Yuan, R. *Anal. Chem.* **2017,** *89,* 9383-9387.

for TOC only