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ORIGINAL PAPER

Electrochemical determination of the activity and inhibition of telomerase based on the interaction of DNA with molybdate

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Abstract

An ultrasensitive electrochemical sensor is described for the determination of the activity of telomerase. It is based on a DNA-generated current that is due to the reaction of the phosphate groups on DNA with molybdate to form a redox-active molybdophosphate. A telomerase substrate primer was first immobilized on a gold electrode. In the presence of telomerase and deoxyribonucleoside triphosphates (dNTPs), the primer can be extended with repetitive nucleotide sequences (TTAGGG). The subsequent reaction of the sensor with molybdate results in the enhancement of electrochemical current intensity due to an increased amount of nucleotides on the electrode. Sensitivity can be further improved by introducing a hairpin probe that partially hybridizes with the repetitive TTAGGG sequence and further enhances the amount of DNA on the electrode. The biosensor, best operated at 0.2 V (vs. Ag/AgCl) shows a linear response to telomerase activity from 1×10^2 to 10^7 Hela cells mL^{-1}. The assay was applied to the detection of telomerase activity in HeLa cancer cells treated with the anticancer drug epigallocatechin gallate, and the results indicate that it holds great potential in anticancer drug screening.

Keywords Electrochemistry · Telomerase · Anticancer drug · Inhibition · DNA amplification

Introduction

Telomerase is a ribonucleoprotein that responsible for maintaining the telomere length in cells through adding telomere repeat sequence $(TTAGGG)_n$ to the end of the human chromosomes [1–3]. Telomerase is a good biomarker for cancer detection as research has discovered telomerase is overexpressed in over 85% of all known cancer cells [4, 5]. In addition, telomerase activity is necessary to preserve many cancer types. Therefore, drugs that inhibit telomerase activity can repress the growth of cancer cells with minimal side effects. There for, it is of great importance to develop sensitive and selective methods for early cancer diagnosis, telomerase related anticancer drug screening and therapeutic research [6–8].

Polymerase chain reaction (PCR)-based telomeric repeat amplification protocol (TRAP) was developed as the most widely used method to measure telomerase activity [9]. What more, modified TRAP techniques have also been reported by combing TRAP with other detection strategies, such as TRAP-enzyme-linked immunosorbent assay and TRAP-polyacrylamide gel electrophoresis with the silver staining assay [10, 11]. However, compared to these methods, electrochemical methods have the advantages of simple instrumentation, low cost and high sensitivity [12, 13].

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Previously, our group reported electrochemical sensors for detection of different protein biomarkers based on the reaction of DNA molecules with molybdate to form redox molybdophosphate and generate electrochemical current [14, 15]. The formation of redox molybdophosphate is actually due to the reaction of phosphate groups on DNA with molybdate [16, 17]. Through various binding events, for example, the binding between protein biomarkers with aptamer and the binding between different DNA strands, DNA molecules will be captured onto electrode surface [18, 19]. The generated electrochemical current intensity is proportional to the amount of DNA molecules on electrode surface.

In this paper, we reported electrochemical sensor for measuring the activity and inhibition of telomerase based on the above mentioned DNA generated electrochemical current. In the presence of telomerase and deoxyribonucleoside triphosphates (dNTPs), the telomerase primer that immobilized on the gold electrode surface was elongated with repetitive nucleotide sequences (TTAGGG). The repetitive nucleotide sequences will then open the structure of a hairpin probe (HP) in solution and partially hybridize with the HP. This will significantly increase the loading of DNA molecules onto electrode and increase the electrochemical current intensity of the electrode after reacting of the electrode with molybdate, improving the sensitivity of the sensor for telomerase detection. The analytical performance of the sensor was studied in detail. The sensor was also applied for testing the inhibition effect of drugs on the activity of telomerase.

Experimental section

Materials and apparatus

Telomerase substrate primer (5'-SH-(CH2)6-TTT TTT AAT CCG TCG AGC AGA GTT-3′) and hairpin probe (5′-GGT TAG GGT TAC CTC AGC TTT CCT TTA ACC CTA ACC CTA A-3′) was synthesized by Sangon Biotech Co., Ltd. (Shanghai, China, http://www.sangon.com/). Dulbecco's modified eagle's medium (DMEM), fetal bovine serum, penicillin, and streptomycin were obtained from Gibco Laboratories Life Technologies Inc. (Grand Island, NY, https://www.thermofisher.com/cn/zh/home/brands/gibco.html). Sodium molybdate dehydrate ($Na_2MoO_4 \cdot 2H_2O$), ethylene glycol-bis(β-aminoethyl ether)-N, N, N′, N′-tetraacetic acid (EGTA), 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonic acid (CHAPS), deoxyribonucleoside triphosphates (dNTPs, 10 mM), phenylmethylsulfonyl fluoride (PMSF) and epigallocatechin gallate (EGCG) were obtained from Sigma-Aldrich (https://www.sigmaaldrich.com. The buffer employed in this study was Tris buffer (10 mM Tris-HCl, pH 7.4). All other reagents were of analytical grade and used without further purification and all aqueous stock solutions were prepared with ultrapure water.

Electrochemical measurements were carried out by a CHI-650D electrochemical workstation (Shanghai CH Instruments Co., China) equipped with a conventional threeelectrode configuration utilizing a working electrode (gold electrode, 2 mm in diameter), a saturated Ag/AgCl reference electrode, and a platinum auxiliary electrode.

Cell culture and telomerase extraction

Hela cells were cultured in DMEM medium supplemented with 10% fetal bovine serum and maintained at 37 °C in a humidified atmosphere (95% air and 5% CO_2). Hela cells were collected in the exponential phase and washed twice with ice-cold sterile Tris buffer. After that, 10^6 cells were re-suspended in 1 mL of ice-cold CHAPS lysis buffer (10 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, 1 mM EGTA, 0.5% CHAPS, 10% glycerol, 0.1 mM PMSF) and stored in a 4 mL eppendorf tube. Then, the cell lysis was frozen at −80 °C for 30 min. Subsequently, the cell lysis was centrifuged for 20 min at 4 °C (12 000 rpm). The finally clean lysis was stored at −80 °C for further use.

Preparation of the electrochemical sensor

Initially, to link the telomerase substrate primer onto gold electrode, the cleaned gold electrode was immersed into 100 μM of primer solution overnight at 4 °C. After extensive washing, the electrode was then immersed into 100 μL of telomerase reaction solution which contained 5 μL of telomerase extracts and 95 μL of telomerase reaction buffer (20 mM Tris−HCl buffer, pH 7.4, 1.5 mM $MgCl_2$, 63 mM KCl, 0.05% Tween 20, 1 mM EGTA, 1.0 mM dNTPs) at 37 °C for 1 h. Sequentially, the electrode was incubated with hairpin probe (2.5 μM, 20 μL) at 37 °C for 2 h. After washing again, the electrode was ready for measurement.

Electrochemical detection

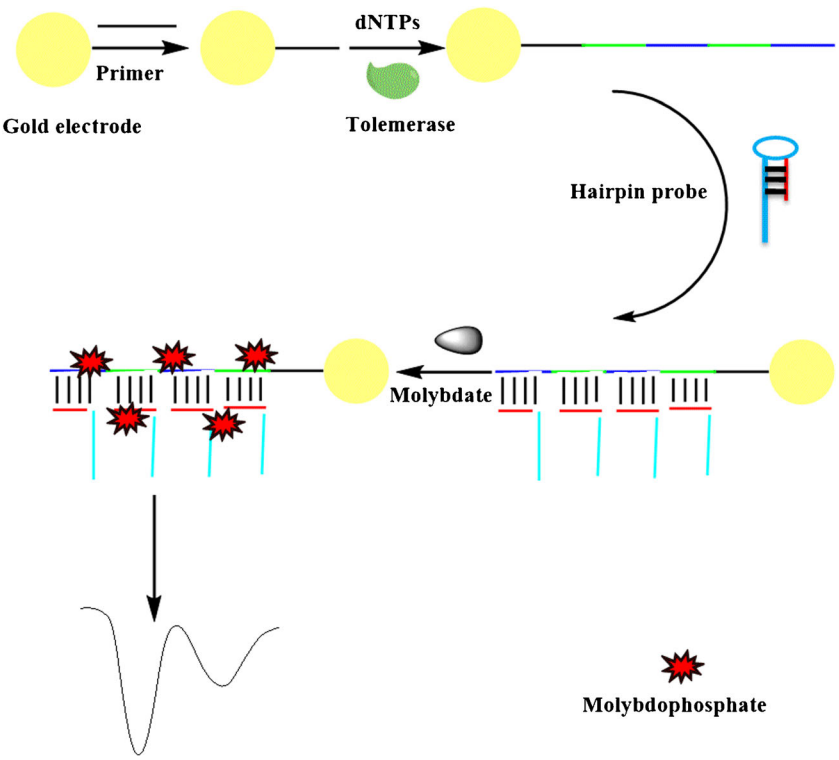
For electrochemical measurement, 5 μL of 5 mM Na_2MoO_4 solution was added onto the sensor surface and incubated for 20 min before electrochemical analyzed in 0.5 M H_2SO_4.

Results and discussion

Scheme 1 shows the schematic representation of the sensor preparation and testing process. The idea of the paper is utilizing the reaction between DNA molecules with molybdate to form redox-active molybdophosphate and generate electrochemical current. Telomerase can extend the length of telomerase substrate primer on gold electrode by adding repetitive nucleotide sequences (TTAGGG) to the primer, leading to increased loading of DNA on electrode. The increased loading of DNA on electrode will then enhance the electrochemical current intensity of the electrode after reaction with molybdate. In addition, the produced repetitive TTAGGG sequence will open the hairpin probe in solution and partially hybridize with the probe, further increasing the amount of DNA on electrode surface, enhancing the sensitivity of the sensor. The electrochemical current intensity of the sensor is proportional to the activity of telomerase.

Initially, the feasibility of the sensor for measuring the telomerase activity was studied. After the linking of the telomerase substrate primer onto gold electrode via the thiol group at the end of the primer, the primer was treated with telomerase extract from Hela cells and dNTPs. Finally, the electrode was incubated with hairpin probe solution to hybridize the probe onto electrode. After each modification step, the electrode was characterized in 0.5 H_2SO_4 solution by square wave voltammetry (SWV). As shown in Fig. 1, for the bare gold electrode, after the reaction of the electrode with molybdate, the electrode displays a small pair of current peaks in the H_2SO_4 solution at around 0.20 and 0.32 V (curve a). The generated small current is ascribed to the reaction of molybdate itself on the electrode. With the linking of the primer onto gold electrode, the current intensity is increased (curve b). The current intensity increased is due to the reaction of the substrate primer with molybdate that formed molybdophosphate, which indicated the successful linking of the primer onto electrode. Then, after the reaction of the electrode with telomerase extract from 10^7 Hela cells mL^{-1} in the presence of dTNPs, the electrochemical current of the electrode is increased again (curve c). The increase of electrochemical current proved the telomerase added repetitive TTAGGG sequence to the primer. Finally, when the electrode is incubated with hairpin probe solution, there is another significant increase of the electrochemical current (curve d). However, when the telomerase is absent, there is no obvious enhancement of the electrochemical current intensity. The above data demonstrated the feasibility of the sensor for detecting the activity of telomerase activity and that the introduction of the hairpin probe can significantly enhance the sensitivity of the assay.

After proved the feasibility of the sensor, parameters that affect the sensitivity of the sensor, such as the reaction time of the substrate primer with



Scheme 1 Schematic representation of the telomerase activity detection process

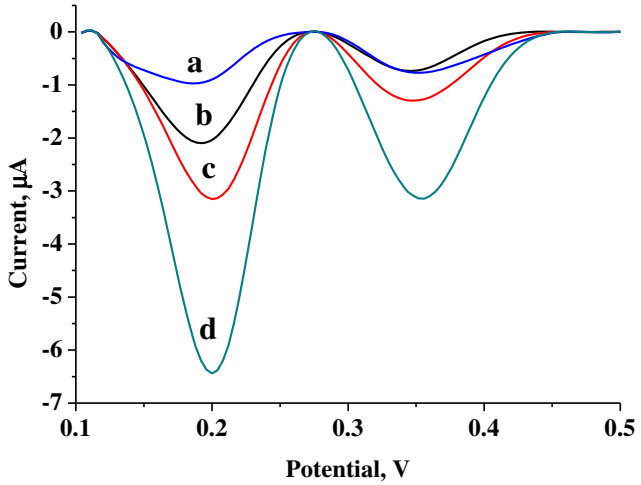


Fig. 1 SWV responses of different electrodes after reaction with molybdate. (**a**) Bare electrode; (**b**) primer modified electrode; (**c**) primer modified electrode after reaction with telomerase; (**d**) electrode c after incubation with the hairpin probe

telomerase and the reaction time of the sensor with hairpin probe were studied. For detection of telomerase extract from 10^7 Hela cells mL^{-1}, the following parameters were found to result in the optimized performance, the reaction of the substrate primer with telomerase for 1 h and the reaction of the sensor with hairpin probe for 2 h (Electronic Supporting Material, Fig. S1 a and b).

Under optimized experimental conditions, the telomerase extracted from different concentrations of Hela cells were analyzed by the sensor. From Fig. 2, it can be seen electrochemical current intensity of the sensor is increased with the increase of cell numbers. A linear relationship between the current intensity and the logarithm of cell number in the range from 10^2 to 10^7 cells mL^{-1} is obtained. The limit of detection, calculated based on signal-to-noise ratio of 3 is 40 cells mL^{-1}.

The performances of the sensor are compared to previous reported methods for telomerase detection. From Table 1, it can be seen the overall performance of the sensor are better than previous reports.

To study the selectivity of the assay, the responses of the sensor to various other enzymes were tested. Glucose oxidase (GOx), horseradish peroxidase (HRP), β-site amyloid precursor protein cleaving enzyme 1 (BACE1) and protein kinase A (PKA) were selected as potential interfering enzymes. For 1 $U.mL^{-1}$ of the above enzymes, from Fig. 3, it can be seen, the responses of the sensor to these enzymes are close the blank sample. In addition, the reproducibility of the sensor was studied by analyzing telomerase extract from 10^7 cells mL^{-1} independently 5 times. The relative standards deviation (RSD) of the analyzing results is 4.7%. These data indicate the sensor display good performance towards detection for telomerase activity.

Performance of the sensor in different cell lines

The performance of the sensor was also tested by using three kinds of different cell lines, such as Hela, MCF-7 and leukemia cells (K562) to evaluate the universality of the sensor. The telomerase were extracted from the three cell lines by exactly the same procedure. As shown in Fig. 4, it can be seen that the sensor are effective for detection of telomerase in these three cancer cell types. However, when the cancer cells

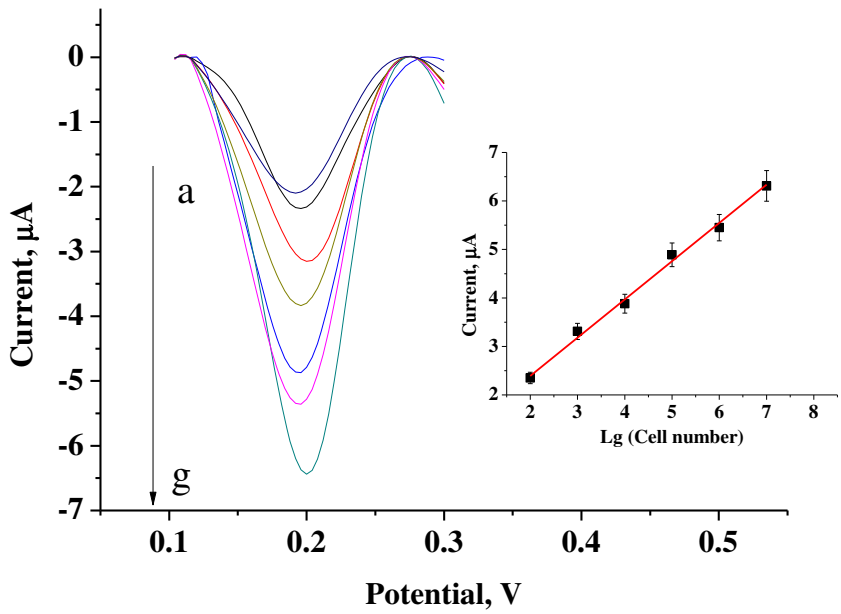


Fig. 2 Responses of the sensor to telomerase that extracted from different Hela cells, from a to g, 0, 10^2,10^3,10^4,10^5,10^6, 10^7 cells mL^{-1}. The inset is the calibration plot of the sensor to telomerase activity. Current intensity at 0.2 V was acquired to draw the calibration plot

Table 1 Compare the performance of the sensor towards telomerase activity detection with literature reports

Detection method	Materials used	Linear range (cells mL^{-1})	Limit of detection (cells mL^{-1})	Reference
Electrochemistry	Platinum nanoparticles	5×10^2 to 10^7	100	[20]
Electrochemistry	Magnetic mesoporous silica nanocontainer	50–5000	12	[21]
Electrochemiluminescence	Luminol	100–9000	62	[1]
Electrochemiluminescence	Ruthenium Complex	50 to 10^6	11	[22]
Colorimetric	Gold nanorod	200 to 1.5×10^4	90	[2]
Electrochemistry	Hairpin probe	10^2 to 10^7	40	This work

were heated at 95 °C, the telomerase in cancer cells was denatured as the telomerase activity is sensitive to heat, which can destroy the essential RNA template and the reverse transcriptase protein of telomerase [23]. As a result, the response of the sensor is close to background current. These data indicated the reliability of the sensor.

Testing the inhibition of telomerase activity by drugs

The sensor was then applied for dynamic monitoring of the variation of telomerase activity after treating with a typical telomerase-inhibiting drug, epigallocatechin gallate (EGCG) [24]. The telomerase that extracted from 10^7 Hela cells mL^{-1} was treated with different concentration of EGCG (0, 50, 100, 150, 200 $\mu g.mL^{-1}$) for 12 h and then tested by the sensor. As the concentration of EGCG increased, the current response of the sensor was decreased, indicating the activity of telomerase was inhibited that prevented the adding of TTAGGG sequence to the primer. As can be seen from Fig. 5, the inhibition of EGCG toward telomerase activity also shows dose-dependent behavior. These results demonstrated the sensor has potential to be applied for screening of telomerase-related drugs.

Conclusion

In summary, we reported electrochemical sensor for measuring the activity and inhibition of telomerase based on DNA generated electrochemical current. The introduction of the hairpin probe that can partially hybridize with the repetitive TTAGGG sequence significantly enhanced the sensitivity of the sensor. This sensor is sensitive with low cost. The successful application of the sensor for measuring the telomerase activity in different cell lines and the inhibition effect of EGCG on the telomerase activity demonstrated wide potential application of the sensor in clinical diagnosis and drug screening. However, the current method, including most recent reported works for telomerase activity detecting, did not measure the exact telomerase activity quantitively, due to the lack of commercial standard telomerase sample.

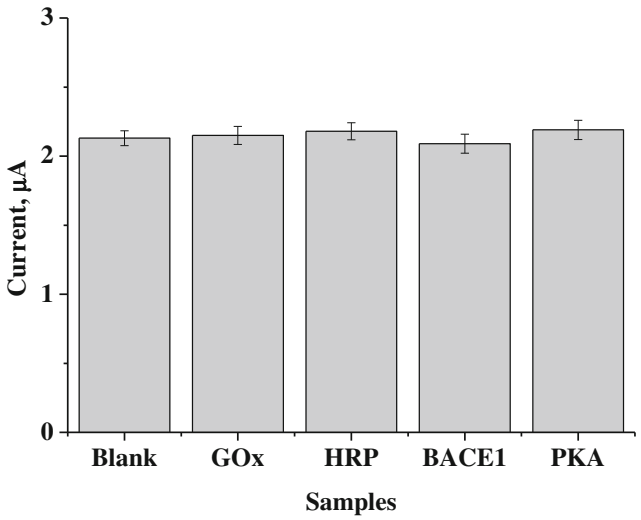


Fig. 3 Responses of the sensor to blank sample and to 1 $U.mL^{-1}$ of different proteins

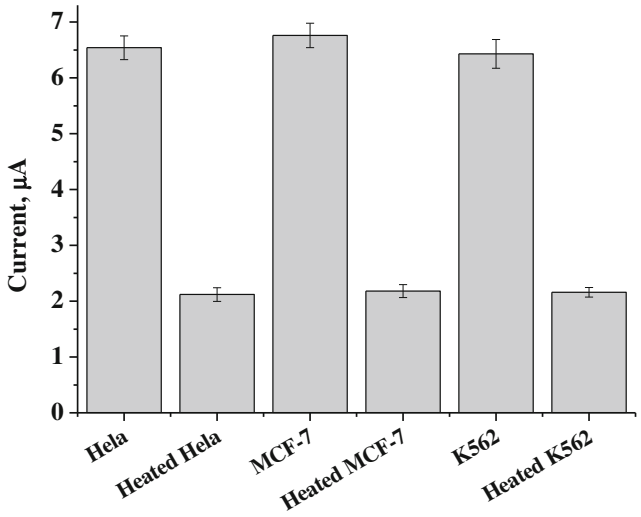


Fig. 4 Responses of the sensor to telomerase that extracted from different cells. Cell concentrations are 10^7 mL^{-1}

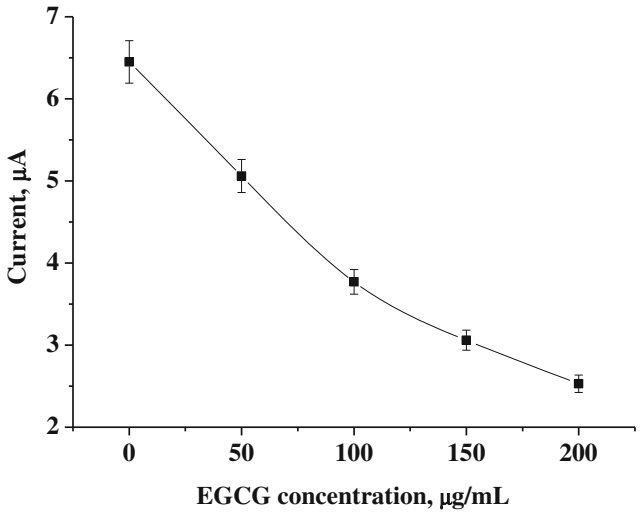


Fig. 5 Responses of the sensor to telomerase that treated with different concentration of EGCG

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Compliance with ethical standards

The author(s) declare that they have no competing interests.

References

1. Zhang H-R, Wu M-S, Xu J-J, Chen H-Y (2014) Signal-on dual-potential Electrochemiluminescence based on Luminol-gold bifunctional nanoparticles for telomerase detection. Anal Chem 86(8):3834–3840
2. Yang H, Liu A, Wei M, Liu Y, Lv B, Wei W, Zhang Y, Liu S (2017) Visual, label-free telomerase activity monitor via enzymatic etching of gold Nanorods. Anal Chem 89(22):12094–12100
3. Su D, Huang X, Dong C, Ren J (2018) Quantitative determination of telomerase activity by combining fluorescence correlation spectroscopy with telomerase repeat amplification protocol. Anal Chem 90(1):1006–1013
4. Liu X, Wei M, Liu Y, Lv B, Wei W, Zhang Y, Liu S (2016) Label-free detection of telomerase activity in urine using telomerase-responsive porous anodic alumina Nanochannels. Anal Chem 88(16):8107–8114
5. Graham MK, Meeker A (2017) Telomeres and telomerase in prostate cancer development and therapy. Nat Rev Urol 14(10):607–619
6. Chen C, Wei M, Liu Y, Xu E, Wei W, Zhang Y, Liu S (2017) Visual and fluorometric determination of telomerase activity by using a cationic conjugated polymer and fluorescence resonance energy transfer. Microchim Acta 184(9):3453–3460
7. Xu Y, Zhang P, Wang Z, Lv S, Ding C (2018) Determination of the activity of telomerase in cancer cells by using BSA-protected gold nanoclusters as a fluorescent probe. Microchim Acta 185(3) Unsp 198
8. Zhang L, Peng J, Hong M-F, Chen J-Q, Liang R-P, Qiu J-D (2018) A facile graphene oxide-based fluorescent nanosensor for the in situ "turn-on" detection of telomerase activity. Analyst 143(10):2334–2341
9. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL, Shay JW (1994) Specific association of human telomerase activity with immortal cells and cancer. Science 266(5193):2011–2015
10. Cheung DH-C, Ho S-T, Lau K-F, Jin R, Wang Y-N, Kung H-F, Huang J-J, Shaw P-C (2017) Nucleophosmin interacts with PIN2/TERF1-interacting telomerase inhibitor 1 (PinX1) and attenuates the PinX1 inhibition on telomerase activity. Sci Rep 7(43650)
11. Zhang L, Zhang S, Pan W, Liang Q, Song X (2016) Exonuclease I manipulating primer-modified gold nanoparticles for colorimetric telomerase activity assay. Biosens Bioelectron 77:144–148
12. Luo J, Zhao D, Yang M, Qu F (2018) Porous Ni3N nanosheet array as a catalyst for nonenzymatic amperometric determination of glucose. Microchim Acta 185(4) Unsp 229
13. Deng C, Zhang M, Liu C, Deng H, Huang Y, Yang M, Xiang J, Ren B (2018) Electrostatic force triggering elastic condensation of double-stranded DNA for high-performance one-step immunoassay. Anal Chem 90(19):11446–11452
14. Li X, Shen C, Yang M, Rasooly A (2018) Polycytosine DNA electric-current-generated Immunosensor for electrochemical detection of human epidermal growth factor Receptor2 (HER2). Anal Chem 90(7):4764–4769
15. Shen C, Liu S, Li X, Zhao D, Yang M (2018) Immunoelectrochemical detection of thehuman epidermal growth factor receptor 2 (HER2) via gold nanoparticle-based rolling circle amplification. Microchim Acta 185(12):547
16. Shen C, Zeng K, Luo J, Li X, Yang M, Rasooly A (2017) Self-assembled DNA generated electric current biosensor for HER2 analysis. Anal Chem 89(19):10264–10269
17. Xiang W, Wang G, Cao S, Wang Q, Xiao X, Li T, Yang M (2018) Coupling antibody based recognition with DNA based signal amplification using an electrochemical probe modified with MnO2 nanosheets and gold nanoclusters: application to the sensitive voltammetric determination of the cancer biomarker alpha fetoprotein. Microchim Acta 185(7):335
18. Hu L, Hu S, Guo L, Shen C, Yang M, Rasooly A (2017) DNA generated electric current biosensor. Anal Chem 89(4):2547–2552
19. Feng K, Liu J, Deng L, Yu H, Yang M (2018) Amperometric detection of microRNA based on DNA-controlled current of a molybdophosphate redox probe and amplification via hybridization chain reaction. Microchim Acta 185(1) Unsp 28
20. Ling P, Lei J, Jia L, Ju H (2016) Platinum nanoparticles encapsulated metal-organic frameworks for the electrochemical detection of telomerase activity. Chem Commun 52(6):1226–1229
21. Lu M (2017) Bioresponsive-controlled release of methylene blue from magnetic mesoporous silica from the electrochemical detection of telomerase activity. Analyst 142(18):3477–3483
22. Xiong C, Liang W, Zheng Y, Zhuo Y, Chai Y, Yuan R (2017) Ultrasensitive assay for telomerase activity via self-enhanced Electrochemiluminescent ruthenium complex doped metal-organic frameworks with high emission efficiency. Anal Chem 89(5):3222–3227
23. Liu X, Li W, Hou T, Dong S, Yu G, Li F (2015) Homogeneous electrochemical strategy for human telomerase activity assay at single-cell level based on T7 exonuclease-aided target recycling amplification. Anal Chem 87(7):4030–4036
24. Yang X-J, Zhang K, Zhang T-T, Xu J-J, Chen H-Y (2017) Reliable Forster resonance energy transfer probe based on structure-switching DNA for Ratiometric sensing of telomerase in living cells. Anal Chem 89(7):4216–4222