Int. J. Electrochem. Sci., 15 **(2020)** 11371 – 11386, doi: 10.20964/2020.11.28

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Mini review

Electrochemical, Electrochemiluminescent and Photoelectrochemical Methods for Detection of Telomerase Activity: A Review

Dandan Liu and Ming La[*]

School of Chemical and Environmental Engineering, Pingdingshan University, Pingdingshan, Henan 467000, People's Republic of China
[*]E-mail: mingla2011@163.com

Received: 4 July 2020 / *Accepted:* 25 August 2020 / *Published:* 30 September 2020

Telomerase can be activated in 85% - 90% of cancer cells. It has attracted extensive attention and become an important breakthrough in tumor diagnosis and clinical treatment. Therefore, it is of importance to determine telomerase activity. In recent years, many sensitive and accurate detection techniques in vitro have been developed for the detection of telomerase activity. This paper reviews the progress of electrochemistry-based methods for telomerase detection.

Keywords: Telomerase; electrochemistry; electrochemiluminescence; photoelectrochemistry

1. INTRODUCTION

Telomere and telomerase play a key role in ensuring the complete replication of chromosomes during cell division. Telomerase is the natural exfoliation of chromosomes, which can cause aging and cancer. In new cells, telomeres are shortened every time a cell divides. When the telomere cannot be shortened, the cell cannot continue to divide and die. About 90% of cancer cells have a growing number of telomeres and a relatively large number of telomerase. A large number of investigations have proved that the activity of telomerase is inhibited in normal somatic cells, and almost no active telomerase can be detected in normal tissue cells [1,2]. However, telomerase is activated and highly expressed in tumor cells. About 90% of cancer cells such as bladder cancer, breast cancer and gastric cancer can be detected with highly active telomerase [1]. Therefore, the detection of telomerase is of great significance for the prevention, early detection and prognosis of cancer. The conventional methods for the detection of telomerase activity mainly include telomere repeat amplification (TRAP), telomere repeat extension, chemiluminescence, and real-time quantitative fluorescent polymerase chain reaction (PCR) [3,4]. In recent years, many methods based on various nanomaterials and new

technologies have been developed to detect telomerase activity with high sensitivity, such as electrochemistry, colorimetry, fluorescence, surface enhanced Raman spectroscopy and so on. This paper mainly reviews the recent progress in electrochemistry-based techniques for telomerase detection, including electrochemistry, electrochemiluminescence and photoelectrochemistry.

2. ELECTROCHEMICAL DETECTION

2.1 Direct detection

Since it was found that nucleic acids show well-defined electrochemcial signal, many label-free electrochemical methods for DNA detection have been reported [5,6]. As the most easily oxidized base in DNA, guanine produces an irreversible oxidation peak at 1.00 V (vs. Ag/AgCl), and the peak intensity is related to the nucleic acid concentration and guanine content. Based on this principle, Eskiocak et al. reported an electrochemical method for the detection of telomerase activity by differential pulse voltammetry (DPV) [7]. Telomerase can extend a large number of $(TTAGGG)_n$ repeats at the end of its primers, and 50% of the bases are guanine. Therefore, the electrochemical oxidation signal of guanine can be used as an indicator for the detection of telomerase activity. As shown in Fig. 1, in contrast to the primer dimer with low guanine content, the samples amplified by PCR with medium and active telomerase produced stronger electrochemical oxidation current. In addition to the advantages of time-saving and low cost, the method can detect telomerase as low as 100 ng/μL in the cell extract. Subsequently, Shao et al. reported an electrochemical method for telomerase detection by lengthening the DNA strand on electrode surface without PCR amplification [8], which could detect active telomerase in 3000 HeLa cells.

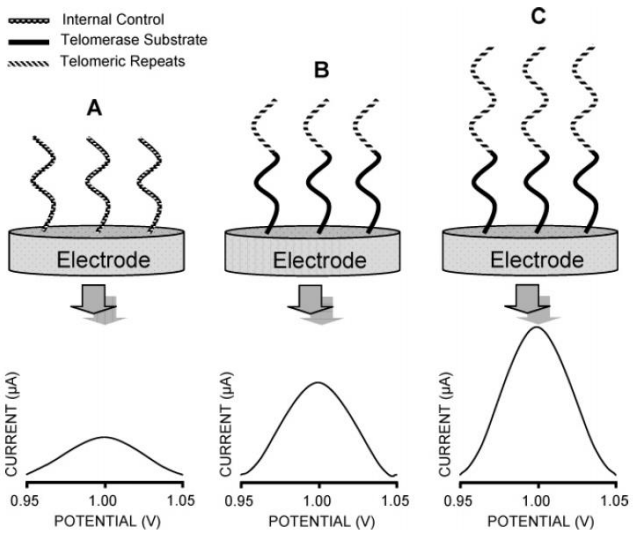

Figure 1. Schematic illustration of guanine oxidation signals of PCR products from (A) primer-dimer and heat inactivated negative controls, telomerase positive cell extracts with (B) moderate, and (C) strong telomerase activity. Reprinted with permission from reference [7]. Copyright 2008 American Chemical Society.

Electrochemical impedance spectroscopy (EIS) is a simple, convenient and sensitive electrochemical technique, which is widely used in DNA analysis [9,10]. In 2011, Yang et al. first reported the detection of telomerase activity in HeLa cells using electrochemical impedance technology [11]. In this work, the extended DNA precursor can prevent the electron transfer of $[Fe(CN)_6]^{3-/4-}$ on electrode surface, which led to the gradual increase in impedance. Cunci et al. designed an electrochemical impedance microchip, which did not need the additional redox molecules but directly utilized a large number of other molecules in Jurkat T cell extract [12]. This method can determine telomerase activity within 20 min. Liu et al. reported a rapid, label-free assay for telomerase activity based on a porous anodic alumina nanochannel (Fig. 2) [13]. Telomerase substrate was immobilized on the inner wall of the nanochannel via a Schiff base reaction. Under the catalysis of telomerase, the repeated G-rich sequence was extended at the end of the substrate, and a polyploid G-tetrahedral fold structure was formed under the action of K^+ ions. The change of DNA configuration lead to a sharp increase in the pore resistance and a significant decrease in the anodic current produced by $[Fe(CN)_6]^{3-/4-}$. There was a linear relationship between the ratio of the current and the logarithm of the number of HeLa cells. The detection limit of this method is 7 HeLa cells. Moreover, Díaz-Cartagena developed a label-free electrochemical impedance method for the detection of telomerase activity and its inhibitors using a gold cross finger electrode [14].

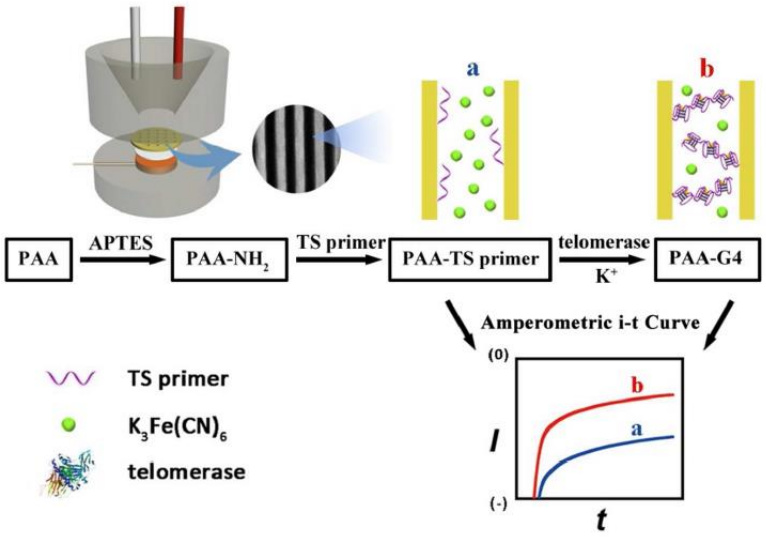

Figure 2. Schematic illustration of the telomerase activity detection strategy via nanochannel-based electrochemical platform. Reprinted with permission from reference [13]. Copyright 2016 American Chemical Society.

As a common indicator of electrochemistry, positively charged $[Ru(NH_3)_6]^{3+}$ can interact with negatively charged phosphate in DNA chain. Sato et al. reported an electrochemical method for the detection of telomerase activity on solid phase surface [15]. As shown in Fig. 3, the amount of $[Ru(NH_3)_6]^{3+}$ adsorbed on the DNA chain before and after telomerase treatment was significantly different, which resulted in a dintingushed current. With this method, the team investigated the possible mechanism of different drugs to inhibit telomerase activity.

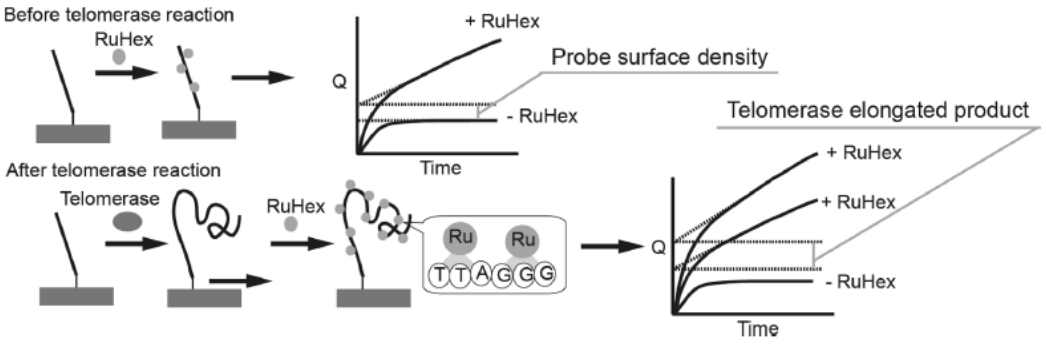

Figure 3. Electrochemical telomerase assay based on chronocoulometry with RuHex. This system enables one to estimate telomerase activity from the DNA length elongated from the TS-primer. Reprinted with permission from reference [15]. Copyright 2012 American Chemical Society.

Moreover, Li et al. detected human telomerase activity by using nanoparticle-based bio-coding technology. The gold nanoparticles (AuNPs) were immobilized on the surface of electrode by hybridization with the captured DNA and signal DNA. Other DNA molecules on the surface of AuNPs can adsorb a large amount of $[Ru(NH_3)_6]^{3+}$ and produce strong chronoamperometric signals [16]. Wang et al. established an enzyme-free, double signal amplification method for electrochemical detection of telomerase activity by combining this method with DNA hybridization chain reaction technology [17]. As shown in Fig. 4, when DNA-AuNPs are captured on the electrode surface, the DNA on the nanoparticles surface can induce the hybridization chain reaction, thus generating a large number of long double stranded DNA for adsorbing a large amount of $[Ru(NH_3)_6]^{3+}$. In addition, because of their good conductivity, noble metal nanoparticles can also reduce the resistance of charge transfer on electrode surface. For example, Meng et al. constructed a voltammetric method for detection of telomerase activity without PCR amplification by employing telomerase extension products to capture AuNPs [18].

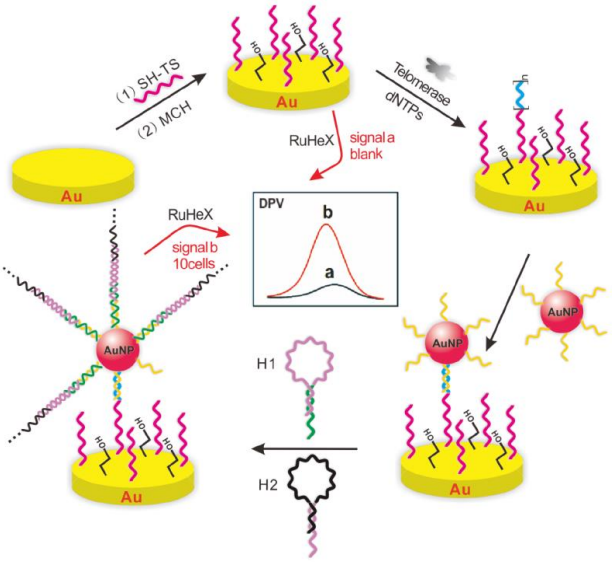

Figure 4. Schematic illustration of SNAs AuNPs triggered mimic-HCR dual signal amplification electrochemical assay for telomerase activity detection. Reprinted with permission from reference [17]. Copyright 2015 American Chemical Society.

G-rich substrate chains produced by telomerase-catalyzed extending can form G-tetrahedral folded structures under the action of K^+ ions. Up to now, many molecules have been confirmed to have specific effects on G-tetrahedral folding structure. Typically, Takenaka's group suggested that ferrocenyl naphthalimide can be threaded into the double stranded DNA. Based on this fact, some electrochemical methods for detecting DNA have been reported [19-21]. Takenaka's group also reported that ferrocenyl naphthalimide could be embedded into G-tetrahedral fold structure at a stoichiometric ratio of 3:1 at high K^+ concentration [22]. Then, an electrochemical method was developed to detect telomerase activity without PCR amplification (Fig. 5). Lately, the group synthesized a series of similar compounds and used them to detect telomerase activity in cancer cells [23-26].

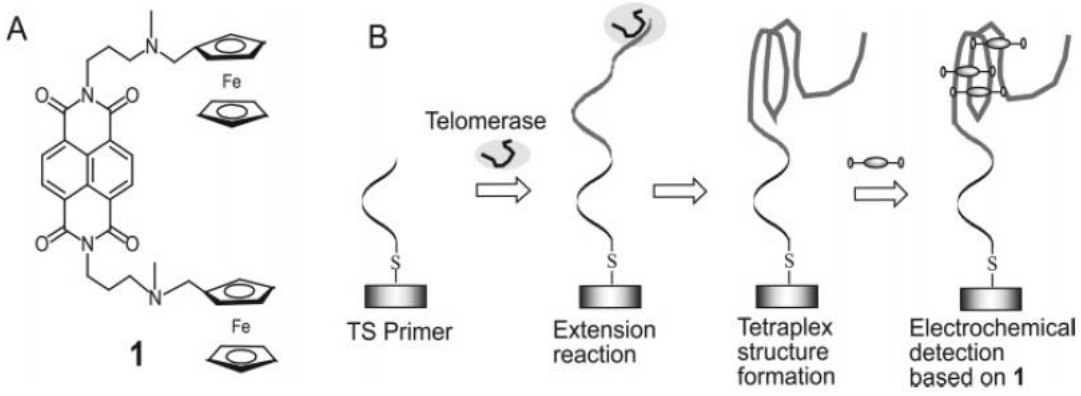

Figure 5. (A) Structure of ferrocenylnaphthalene diimide (**1**). (B) Principle of the electrochemical assay of telomerase activity. Reprinted with permission from reference [22]. Copyright 2005 American Chemical Society.

Molybdate can react with phosphate in DNA molecule to produce phosphomolybdate with redox activity on electrode surface. Based on this principle, Wang et al. constructed an electrochemical sensor for detecting telomerase activity [27]. In this work, the current intensity generated by phosphomolybdate was proportional to the base number of DNA assembled on the electrode surface by telomerase-catalytic reaction. In addition, Wu et al. established an electrochemical method for the detection of telomerase activity in circulating tumor cells by DNA metallization and the unique solid-state electrochemical characteristics of silver nanoparticles [28].

2. 2 Signal amplification

In 2004, Pavlov et al. developed an electrochemical and quartz microcrystalline balance method for the detection of telomerase by employing alkaline phosphatase (ALP) to catalyze the hydrolysis of 5-bromo-4-chloro-3-indolylphosphate to generate precipitate products [29]. In electrochemical biochemical analysis, the electrode surface is usually modified with mercaptan or polymer to seal the unreacted site in order to reduce the false signal caused by the specific adsorption. However, the local crowded surface assembled monolayers reduced the accessibility of telomerase primers and the activity of telomerase. In order to avoid this effect and control the orientation and density of primers, DNA tetrahedrons with cubic cone shape are designed and widely used to

immobilize DNA/RNA on electrode surface. Li et al. found that double stranded DNA at the apex of DNA tetrahedron can connect the precursor of telomerase [30]. Using biotin-labeled deoxyadenine nucleoside triphosphate (biotin-dATP) as the substrate, telomerase-catalyzed extension products carrying many biotin groups were generated on the electrode surface. After the capture of DNA, a large number of streptavidin-modified horseradish peroxidase (HRP) was immobilized on the electrode surface to catalyze the electrochemical reduction of TMB. With the help of K^+ ions, the G-tetrahedron formed by telomerase extension products can bind to hemagglutinin, which has horseradish peroxidase like properties and can catalyze the reaction between hydrogen peroxide and TMB. Based on this principle, Liu et al. constructed an electrochemical biosensor which can detect prostate specific antigen and telomerase activity [31].

In addition to the above enzymes-based signal amplification strategies, nucleases and DNases are often used in the construction of electrochemical biosensors. For example, the popular exonuclease III can hydrolyze DNA from the 3 'end of double stranded DNA but not single stranded DNA. Li et al. reported a highly sensitive electrochemical method for the analysis of telomerase activity by using magnetic separation and exonuclease III-assisted target cycle amplification strategy [32]. At the same time, DNA enzymes based on metal ions (e.g. copper and zinc) have also attracted the attention of scientists. For example, He et al. constructed an electrochemical biosensor for the detection of telomerase activity by exponential amplification using copper-dependent Dnase [33].

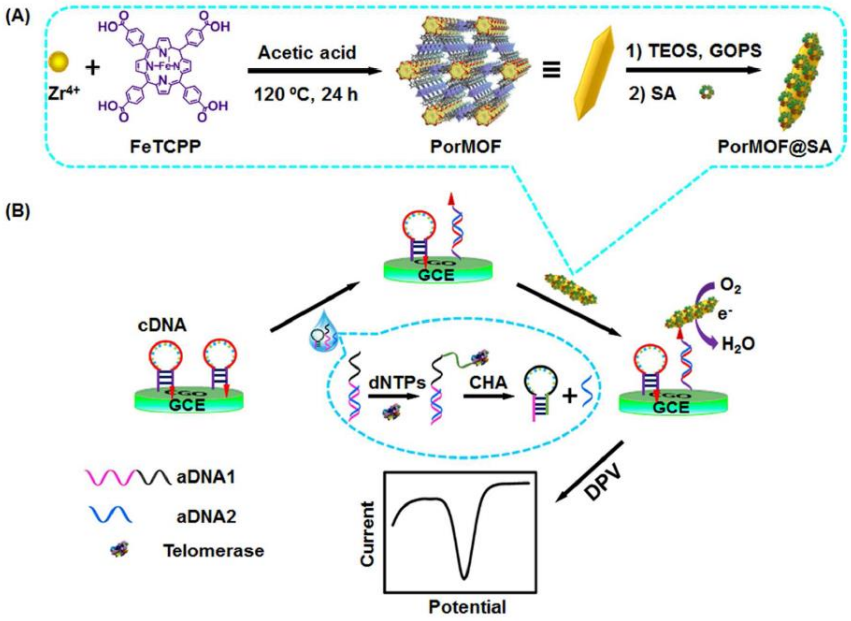

Figure 6. Schematic illustration of (A) preparation of nanoscaled PorMOF and (B) electrochemical detection strategy for telomerase activity via telomerase triggered conformation switch. Reprinted with permission from reference [34]. Copyright 2016 American Chemical Society.

Multifunctional porphyrin ligands have been widely used to synthesize MOFs with biomimetic catalytic activity. The high specific surface area and multi space porphyrin MOFs have a rigid structure, which not only enables the substrate to contact each porphyrin molecule, but also avoids the dimerization of the active center. Ling et al. have successfully synthesized nanosized porphyrin MOF

(PorMOF) and employed it to detect telomerase by the catalytic reduction of oxygen.[34] As shown in Fig. 6, a hexagonal needle-like MOFs with a length of 600 nm and a width of 100 nm were synthesized by hydrothermal reaction using iron porphyrins as ligands and zirconium ions as nodes, and further modified with streptavidin (PorMOF@SA). Biotin-modified hairpin capture DNA (cDNA) was immobilized on the electrode surface. Due to the steric hindrance, biotin can not interact with nanoprobes, which is called "off" state. Auxiliary DNA1 (aDNA1) and auxiliary DNA2 (aDNAs) formed a double chain structure. Under the catalysis of telomerase, aDNA1 was extended to form hairpin structure. The aDNA2 released from the double strand hybridized with the hairpin structure cDNA on the electrode surface, and turned into "open" state. Through the interaction of biotin and streptavidin, nanoprobes PorMOF@SA was introduced to the electrode surface to catalyze the electrochemical reduction of oxygen and improve the current intensity. The detection limit of this method for telomerase was 30 cell/mL, which could even evaluate the telomerase activity in a single HeLa cell.

Noble metal nanoparticles, such as gold, silver, platinum and palladium, are widely used in the construction of electrochemical biosensors due to their unique heterogeneous catalytic ability [35,36]. For example, gold nanorods (AuNRs) are widely used as electrochemical labels because of their excellent catalytic performance, strong adsorption capacity and good conductivity. Wang et al. reported the electrochemical detection of telomerase activity based on the AuNRs-catalytic oxidation of methylene blue and acetaminophen [37,38]. However, due to the disadvantage of easy aggregation, noble metal nanoparticles usually need to be encapsulated in organic or inorganic shells. For this consideration, Ling et al. synthesized metal organic frameworks (MOFs, UiO-66-NH_2)-encapsulated Pt NPs by "one pot method", and employed them to detect telomerase activity by the catalytic decomposition of sodium borohydride [39]. Recently, Pd NPs were modified in situ on the surface of as-prepared MOFs (MIL-101-NH_2) and the nanocomposites were used to detect telomerase activity by the catalytic oxidation of acetaminophen [40]. Moreover, Ling et al. reported the detection of telomerase by Pt NPs-decorated PorMOF [41]. Dong et al. constructed a ratiometric electrochemical sensing strategy for the detection of telomerase using Ce-containing MOF with catalytic activity and MB-modified hairpin DNA [42].

2.3 Immobilization-free methods

Although electrochemical biosensors have made great progress in the detection of telomerase, most of the electrochemical methods are heterogeneous and require the immobilization of precursors on electrode surface. Thus, there are some problems such as tedious process and long time. Because the recognition reaction takes place between the solution and the electrode interface, the steric hindrance of electrode surface and the decrease of the degree of freedom of precursor molecular configuration make the heterogeneous biosensors have low binding efficiency and poor enzymatic kinetics. Based on the difference of electroactive tags-labeled nucleotide sequence and the difference of diffusion between single nucleotide and negatively charged electrode surface, Liu et al. reported a homogeneous, immobilization-free and highly sensitive electrochemical method for detecting telomerase activity using T7 exonuclease-assisted target cycling amplification strategy (Fig. 7) [43]. In

this method, the methylene blue-modified hairpin DNA probe can hybridize with the telomerase extension product. With the help of T7 exonuclease, a small amount of telomerase extension products cause a large amount of hairpin DNA to be hydrolyzed. As a result, a large number of methylene blue-modified single nucleotides are produced. These released methylene blue produced high current on the electrode surface. The unhydrolyzed hairpin DNA has strong electrostatic repulsion force with the negatively charged electrode; thus, the current intensity is very weak. Similarly, the G-tetrahedron formed by telomerase extension products can capture methylene blue in solution, thus reducing the current of methylene blue on the electrode surface [44]. The telomerase precursor can block the pore size of mesoporous silicon loaded with methylene blue [45]. After telomerase catalysis, the extended precursor molecule formed a hairpin structure and broke away from the surface of mesoporous silicon, thus releasing a large amount of methylene blue and generating a strong current.

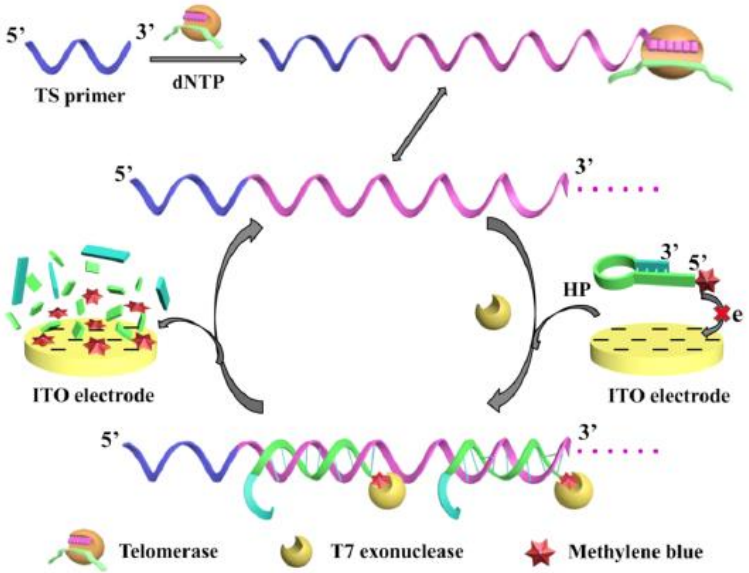

Figure 7. Principle of the homogeneous electrochemical strategy for the detection of telomerase activity based on T7 exonuclease-aided target recycling amplification. Reprinted with permission from reference [43]. Copyright 2015 American Chemical Society.

2.4 Other

Nanoparticles are composed of thousands of atoms, and these atoms can be electrochemically oxidized or reduced. Therefore, nanoparticles can be used as electroactive tags for signal amplification of disease markers such as DNA and proteins [46-48]. For example, after the quantum dots such as Cds, ZnS and PbS are disolved by nitric acid, a large number of heavy metal ions are released. These metal ions with different oxidation potentials can be quantified by anodic stripping voltammetry. Based on this fact, Li et al. reported an electrochemical biosensor for the detection of telomerase at the single cell level [49]. By using biotin-labeled deoxyadenine nucleoside triphosphate as the substrate, telomerase catalyzed extension products carrying many biotins were modified on the electrode surface. After the capture of DNA, streptavidin-modified quantum dots could be further immobilized on the electrode surface, and then the concentration of telomerase could be indirectly quantified by anodic stripping voltammetry (ASV).

Small molecules with electroactivity are usually used as the signal reporters to label biological molecules such as DNA or antibodies. Yi et al. constructed a DNA structure transformation based on ferrocene (Fc)-labeled DNA [50]. After telomerase catalyzed extension, the structure of the substrate chain changed to a hairpin structure, and the Fc-labeled DNA fragment separated from the electrode surface. The increase of electron transfer distance led to a significant decrease of redox current. However, the traditional electrochemical biosensors with single output signal usually have the disadvantages of instability and high background signal. Therefore, Meng et al. developed a ratiometric electrochemical sensing strategy for telomerase activity detection [51]. The hairpin DNA modified with methylene blue (MB) was immobilized on the electrode surface through the formation of Au-S bond, and the Fc-labeled DNA complementary to its stem was also immobilized as the substrate chain of telomerase. After telomerase catalysis, the derived product and hairpin DNA form a more stable double stranded DNA hybrid, making the hairpin structure open and methylene blue away from the electrode surface to decrease the corresponding oxidation current. In this process, the oxidation current of Fc as the reference signal remained unchanged since the distance between Fc and electrode remained unchanged. In addition to directly modifying recognition elements, porous or mesoporous nanomaterials are also used to load electroactive molecules and are widely used in biochemical analysis. For example, dopamine molecules were encapsulated in biotinylated liposomes. After telomerase-catalyzed reaction and DNA hybridization, dopamine was released from the liposome by adding methanol and then quantitatively detected by multiwalled carbon nanotube-modified glassy carbon electrode [52].

3. ELECTROCHEMILUMINESCENT DETECTION

Electrochemiluminescence (ECL) technique combining the advantages of chemiluminescence and electrochemical analysis shows low background, high sensitivity, high selectivity, simple instrument and low cost [53]. Ru $(bpy)_3^{2+}$ is the most commonly used ECL emitter and tripropylamine (TPA) is the most commonly used CO reactant. Luminol is often used in ECL detection due to its special photoelectric properties. In recent years, ECL has become an important analytical technique in the field of analysis and clinical practice. In 2009, Zhou et al. developed a new magnetic bead-based method for telomerase detection without PCR amplification [54]. This method does not need radioactive materials and high-purity telomerase samples. The magnetic beads coated with streptavidin were used as separation elements and immobilization matrix. A large number of ECL signal probes were modified on the AuNPs to amplify the signal. When telomerase was present, AuNPs modified with ECL signal probe were captured to generate detection signal. This ECL detection strategy based on magnetic beads and nanoparticles provides a reliable method for direct measurement of telomerase activity in cancer cells. Zhou et al. also proposed a method for telomerase detection, which can quickly, sensitively and accurately determine the telomerase activity of cancer cells [55]. This method only takes 30 min to complete ECL detection. Wu et al. constructed a label-free telomerase ECL sensor by using the electrode modified with TAPP/CCG (meso-tetra (4-N,N,N-tri-methylanilinium) porphyrin (TAPP) functionalized chemically converted graphene) [56]. Tween 20 was used to prevent the nonspecific adsorption and Ru $(bpy)_3^{2+}$ was used as the signal molecule. This ECL method has the

advantages of simple design and fast operation, and does not need PCR amplification process. Zhang et al. developed a visualized ECL microarray for determining telomerase activity [57]. AuNPs were modified with DNAzyme and luminol as the double catalytic amplification markers, which could enhance the luminescence signal in luminol-H_2O_2 system. At the same time, Zhang et al. also reported a novel ECL method based on bifunctional luminol-gold nanoparticles (L-Au NPs) to detect human telomerase (Fig. 8) [58]. After the telomerase-triggered prolongation reaction and the binding of L-Au NPs, the biosensor can be used to detect human telomerase in HL-60 cells and evalue cell apoptosis with high sensitivity.

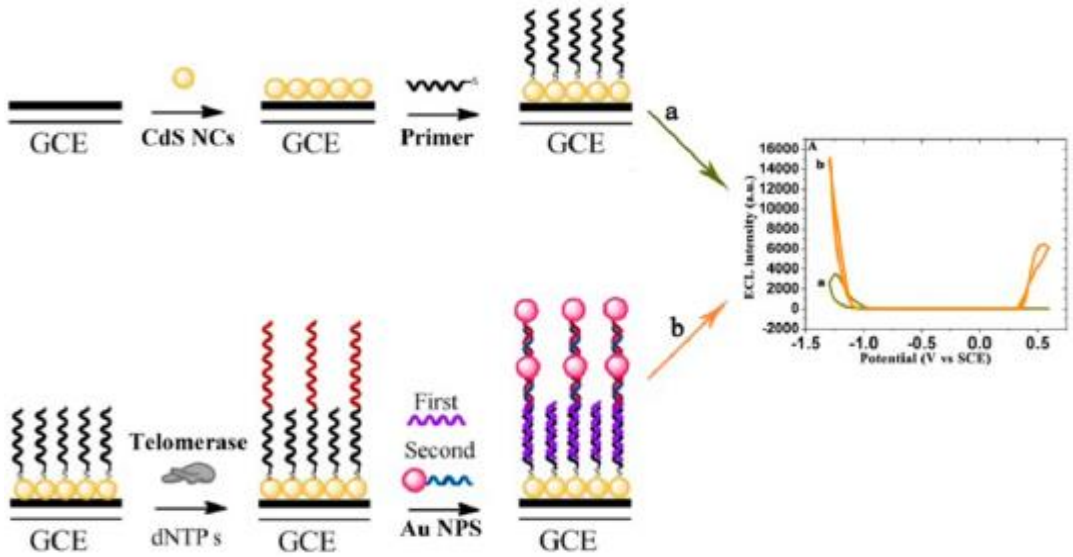

Figure 8. Schematic representation of dual-potential ECL for telomerase detection system. Reprinted with permission from reference [58]. Copyright 2014 American Chemical Society.

Lin et al. successfully developed a label-free, ultrasensitive ECL biosensor to detect telomerase activity in HeLa cells [59]. Firstly, thiolated telomerase substrate was immobilized on the surface of gold electrode by Au-S interaction, and telomerase was used to specifically prolong the DNA. Then, it was hybridized with complementary hybrid DNA to form double stranded DNA (dsDNA) fragments on the electrode surface. Meanwhile, Ru $(phen)_3^{2+}$ was inserted into the dsDNA groove as a signal molecule to enhance ECL signal. The linear detection range of the sensor is 5-5000 HeLa cells, and the minimum detection limit is 2 HeLa cells. This work provides a more convenient method for the diagnosis of telomerase-related cancer therapy.

"On-off" ECL biosensor has the advantages of high sensitivity, high stability and high linearity. Xiong et al. proposed an "on-off" ECL biosensor to detect telomerase activity (Fig. 9) [60]. Firstly, ruthenium polyethlenimine (Ru-PEI) complex was synthesized on zeolitic imidazole framework-8 (ZIF-8) to produce Ru-PEI@ZIF-8 as a signal molecule of ECL. Graphene was modified on the electrode to block the signal. The strategy of enzymatic DNA cyclic amplification and double stranded DNA shearing enzyme were introduced to release graphene and amplify the signal. This method provides a new way to improve the utilization rate of self-reinforced materials and broaden the application range of self-reinforced materials.

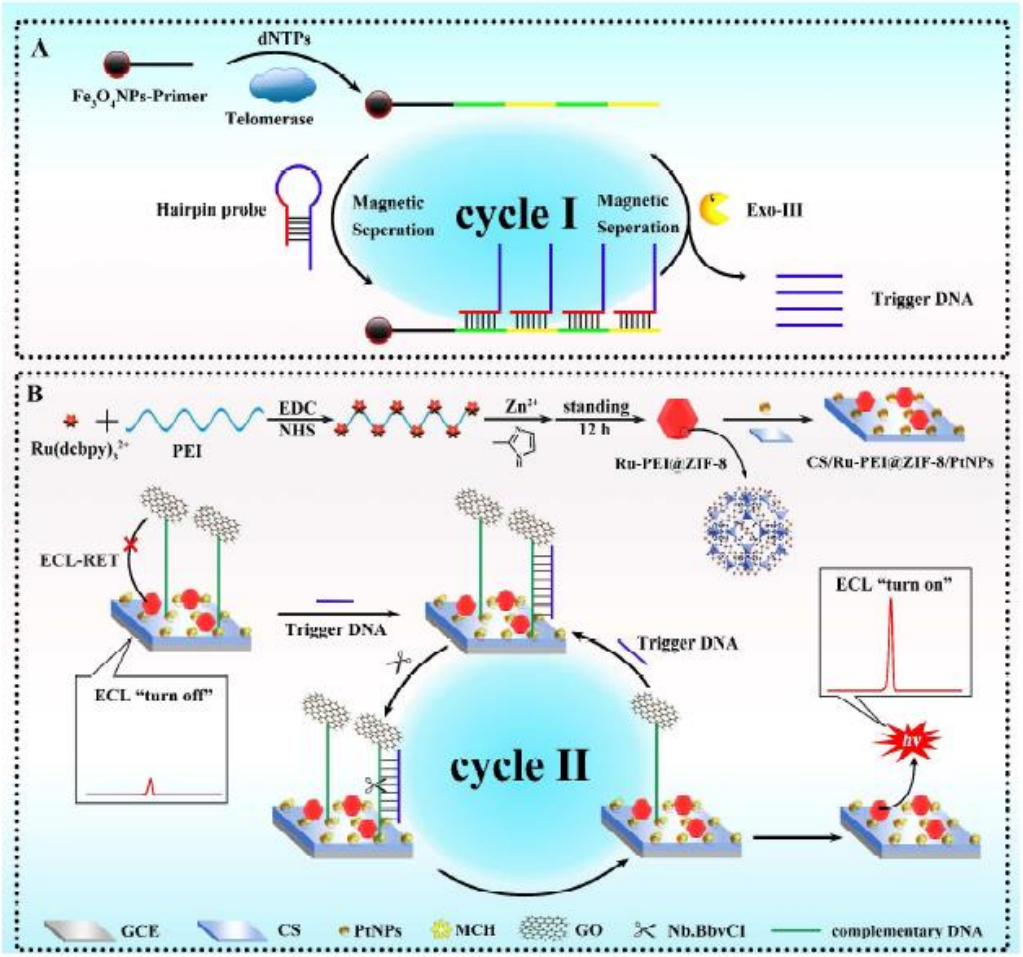

Figure 9. (A) The preparation of trigger DNA (B) the synthetization of Ru-PEI@ZIF-8 nanocomposite and the construction of proposed ECL biosensor. Reprinted with permission from reference [60]. Copyright 2017 American Chemical Society.

Zhang et al. also introduced a new strategy to detect telomerase activity based on the generation of reactive oxygen species (ROS) [61]. Firstly, polyluminol Pt NPs composite films were electrodeposited on ITO electrode, and aptamer DNA was modified on the electrode surface to capture HL-60 cancer cells with high specificity. Then, phorbol 12-myristate 13 acetate (PMA) was filled into mesoporous silica nanoparticles (MSNs), and the surface was sealed with aptamer and T-primer DNA. Aptamer can specifically recognize and connect HL-60 cancer cells, and T-primer DNA would be released from MSN@PMA surface after extended by telomerase. After that, PMA was released from MSN and induced the generation of ROS. In addition, polyluminol Pt NPs composite films can react with H_2O_2 to produce ECL signal. Zhao et al. designed an improved cascade ECL signal amplifier to detect telomerase activity [62]. The DNA strand extended by magnetic primers has repeated TTAGGG fragments, which can be combined with DNA probes modified with $[Ru(bpy)_3]^{2+}$ for the cascade assembly of ECL signal molecules. The sensitivity of this cascade ECL signal amplifier was 100, 50 and 100 for A549, MCF7 and HepG2 cell lines, respectively. In addition, the platform has good performance in the detection of telomerase in tumor cells and tissues, and has great potential for technological innovation in the field of telomerase activity detection.

4. PHOTOELECTROCHEMICAL DETECTION

Photoelectrochemistry (PEC) biosensors have also attracted wide attention due to their advantages of simple operation, simple device, good performance, low background signal, high sensitivity and strong reproducibility [53]. Recently, PEC biosensors have been used to detect kinds of biomolecules and biomarkers including telomerase. For example, Liu et al. developed a "signal on" PEC telomerase biosensor using bismuth@N,O-codoped-carbon core-shell nanohybrids (Bi@NOC NHS) [63]. The nanohybrids showed excellent PEC property under visible light irradiation. Thioflavin T (ThT, a fluorescent dye) can interact with the telomere-extension sequence to form G-quadruplex. In this work, Bi@NOC NHS was used as the photoactive material, ascorbic acid was used as the electron donor, and ThT was introduced to improve the PEC signal. The linear detection range of the PEC method for Hela cell was $5 \times 10^2 \sim 1 \times 10^6$ cells, and the detection limit is 60 cells.

Lei et al. reported a magnetically controlled PEC biosensor based on the electron receptor elimination strategy to detect telomerase activity with high sensitivity [64]. Firstly, telomerase primers (TS primer) were fixed on the surface of magnetic beads (MBs), and then the TS primers were amplified under the catalysis of telomerase to generate longer G-rich single stranded DNA. The G-rich single stranded DNA can bind with hemin to form G-quadriplex/hemin complexes. Based on this mechanism, the modified MBs can be used to capture hemin and reduce its concentration in electrolyte solution. By recording the attenuation of photocurrent, telomerase activity has been monitored with high sensitivity. Fan et al. reported a highly sensitive PEC strategy for telomerase detection with an innovative nanochannel system [65]. Firstly, telomerase primer sequence (TS) was immobilized in the nanoporous anodic alumina (NAA) nanochannels. It is then extended by telomerase in the presence of dNTPs. Then, the extended single strand DNA (ssDNA) and auxiliary single strand DNA (aDNA) were matched and complemented to form multiple DNAzymes. Because DNAzymes can mimic the catalytic function of HRP, biocatalytic precipitation occurs in the nanotubes to generate a PEC signal. Human telomerase RNA (HTR), an important biomarker for cancer diagnosis, is a template for repeated synthesis of telomerase DNA. In tumor cells, the content of HTR is 7 times higher than that of telomere DNA. Recently, Chu et al. proposed a novel PEC biosensor for detecting HTR based on the cascade catalytic hairpin assembly (CHA) and hyperbranched hybridization chain reaction (HB-HCR) (Fig. 10) [66]. The photocurrent response has a negative linear relationship with the logarithm of HTR concentration in the range of 200 fM ~ 20 nM.

In PEC bioanalysis, energy transfer (ET) between noble metal nanoparticles (NPs) and traditional inorganic quantum dots (QDs) is often involved. Zhang et al. reported a "signal on" cathodic PEC bioanalysis based on the energy transfer between Au NPs and polymer dot (Pdots), achieving the detection of telomerase activity in cells (Fig. 11) [67]. Pdots was modified on the ITO electrode to immobilize the capture probe DNA (cDNA). The cDNA hybridized with TS and the probe modified on Au NPs (AuNP-pDNA). After the binding of AuNP-pDNA, Pdots were quenched by the ET effect. In the presence of telomerase, the prolongation of TS resulted in the detachment of AuNP-pDNA from cDNA and the photocurrent of Pdots was restored again. This work reveals the application potential of Pdots and provides ideas for the development of advanced cathode PEC bioanalysis method.

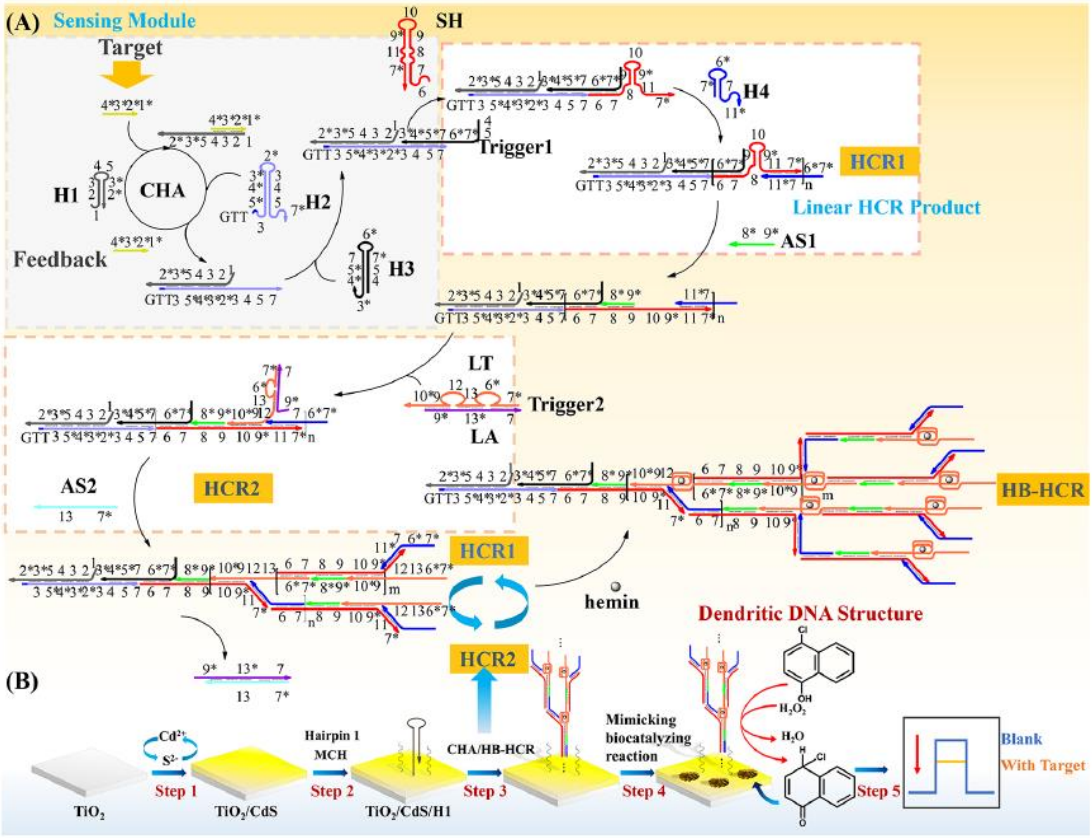

Figure 10. Schematic illustration of the cascaded CHA/HB-HCR amplification strategy (A) and the building and biosensing process of the PEC biosensor (B). Reprinted with permission from reference [66]. Copyright 2019 American Chemical Society.

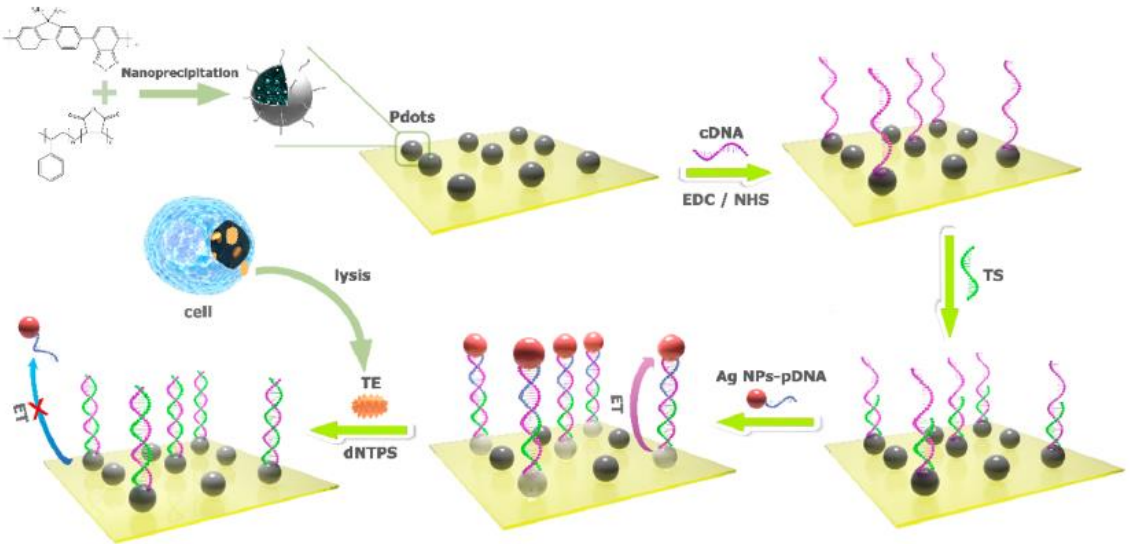

Figure 11. Signal-on ET-based photocathodic bioanalysis of TE activity in cell extracts. Reprinted with permission from reference [67]. Copyright 2019 American Chemical Society.

5. CONCLUSION

Telomerase has been the persistent pursuit of researchers in this field to realize simple and accurate evaluation of its activity. Although the high sensitivity, convenience and rapidity of EC, ECL, and PEC methods have been improved for telomerase detection, these methods can not be directly used in clinical diagnosis and monitoring of cancer progess probably due to their poor stability. Therefore, the development of simple, sensitive, stable and low-cost methods for real-time monitoring of telomerase activity is still urgently desired.

ACKOWLEDGMENTS
Partial support of this work by the Henan Key Laboratory of Research for Centraol Planins Ancient Ceramics (ZYGTCKF201908) and the Science & Technology Foundation of Henan Province (202102310476) was acknowledged.

References

1. L. Wang, F. Ma, B. Tang and C. Y. Zhang, *Chem. Sci.*, 8 (2017) 2495.
2. C. Wang, H. Yang, S. Wu, Y. Liu, W. Wei, Y. Zhang, M. Wei and S. Liu, *TrAC-Trend. Anal. Chem.*, 105 (2018) 404.
3. X. Zhang, X. Lou and F. Xia, *Theranostics*, 7 (2017) 1847.
4. N. Xia, D. Deng, X. Mu, A. Liu, J. Xie, D. Zhou, P. Yang, Y. Xing and L. Liu, *Sens. Actuat. B: Chem.*, 306 (2020) 127571.
5. L. L. Hou, C. Y. Duan and P. P. Ding, *Int. J. Electrochem. Sci.*, 14 (2019) 5661.
6. N. Xia and Y. Q. Hao, *Int. J. Electrochem. Sci.*, 14 (2019) 1843.
7. U. Eskiocak, D. Ozkan-Ariksoysal, M. Ozsoz and H. A. Oktem, *Anal Chem*, 79 (2007) 8807.
8. Z. Shao, Y. Liu, H. Xiao and G. Li, *Electrochem. Commun.*, 10 (2008) 1502.
9. N. Xia, X. Wang, J. Yu, Y. Wu, S. Cheng, Y. Xing and L. Liu, *Sens. Actuat. B: Chem.*, 239 (2017) 834.
10. N. Xia, Y. Huang, Z. Cui, S. Liu, D. Deng, L. Liu and J. Wang, *Sens. Actuat. B: Chem.*, 320 (2020) 128436.
11. W. Yang, X. Zhu, Q. Liu, Z. Lin, B. Qiu and G. Chen, *Chem. Commun.*, 47 (2011) 3129.
12. L. Cunci, M. M. Vargas, R. Cunci, R. Gomez-Moreno, I. Perez, A. Baerga-Ortiz, C. I. Gonzalez and C. R. Cabrera, *RSC Adv*, 4 (2014) 52357.
13. X. Liu, M. Wei, Y. Liu, B. Lv, W. Wei, Y. Zhang and S. Liu, *Anal. Chem.*, 88 (2016) 8107.
14. D. C. Diaz-Cartagena, G. Hernandez-Cancel, D. P. Bracho-Rincon, J. A. Gonzalez-Feliciano, L. Cunci, C. I. Gonzalez and C. R. Cabrera, *ACS Omega*, 4 (2019) 16724.
15. S. Sato and S. Takenaka, *Anal. Chem.*, 84 (2012) 1772.
16. Y. Li, B. Liu, X. Li and Q. Wei, *Biosens. Bioelectron.*, 25 (2010) 2543.
17. W. J. Wang, J. J. Li, K. Rui, P. P. Gai, J. R. Zhang and J. J. Zhu, *Anal. Chem.*, 87 (2015) 3019.
18. F. Meng, Y. Xu, W. Dong, Y. Tang and P. Miao, *Mikrochim. Acta*, 185 (2018) 398.
19. S. Takenaka, K. Yamashita, M. Takagi, Y. Uto and H. Kondo, *Anal. Chem.*, 72 (2000) 1334.
20. S. Sato, Y. Maeda, T. Nojima, H. Kondo and S. Takenaka, *Nucleic Acids Res. Suppl.*, (2003) 169.
21. S. Sato, T. Saeki, T. Tanaka, Y. Kanezaki, M. Hayakawa, K. Haraguchi, M. Kodama, T. Nishihara, K. Tominaga and S. Takenaka, *Appl. Biochem. Biotechnol.*, 174 (2014) 869.
22. S. Sato, H. Kondo, T. Nojima and S. Takenaka, *Anal. Chem.*, 77 (2005) 7304.

23. K. Mori, S. Sato, M. Kodama, M. Habu, O. Takahashi, T. Nishihara, K. Tominaga and S. Takenaka, *Clin. Chem.*, 59 (2013) 289.
24. M. Hayakawa, M. Kodama, S. Sato, K. Tomoeda-Mori, K. Haraguchi, M. Habu, S. Takenaka and K. Tominaga, *Br. J. Oral Maxillofac Surg.*, 54 (2016) 301.
25. M. Hayakawa, S. Sato, I. Diala, M. Kodama, K. Tomoeda-Mori, K. Haraguchi, K. Tominaga and S. Takenaka, *Electroanalysis*, 28 (2016) 503.
26. S. Sato, A. Kajima, H. Hamanaka and S. Takenaka, *J. Organomet. Chem.*, 897 (2019) 107.
27. G. Wang, H. Wang, S. Cao, W. Xiang, T. Li and M. Yang, *Mikrochim. Acta*, 186 (2019) 96.
28. L. Wu, J. Wang, J. Ren and X. Qu, *Adv. Funct. Mater.*, 24 (2014) 2727.
29. V. Pavlov, I. Willner, A. Dishon and M. Kotler, *Biosens. Bioelectron.*, 20 (2004) 1011.
30. Y. Li, Y. Wen, L. Wang, W. Liang, L. Xu, S. Ren, Z. Zou, X. Zuo, C. Fan, Q. Huang, G. Liu and N. Jia, *Biosens. Bioelectron.*, 67 (2015) 364.
31. J. Liu, C. Y. Lu, H. Zhou, J. J. Xu, Z. H. Wang and H. Y. Chen, *Chem. Commun.*, 49 (2013) 6602.
32. J. Li, J. Ma, Y. Zhang, Z. Zhang and K. Hu, *Bioelectrochemistry*, 130 (2019) 107341.
33. J. L. He, Y. Zhang, T. T. Mei, L. Tang, S. Y. Huang and Z. Cao, *Biosens. Bioelectron.*, 144 (2019) 111692.
34. P. Ling, J. Lei and H. Ju, *Anal. Chem.*, 88 (2016) 10680.
35. L. Hou, Y. Huang, W. Hou, Y. Yan, J. Liu and N. Xia, *Int. J. Biol. Macromol.*, 158 (2020) 580.
36. N. Xia, D. Deng, S. Yang, Y. Hao, L. Wang, Y. XLiu, C. An, Q. Han and L. Liu, *Sens. Actuat. B: Chem.*, 291 (2019) 113.
37. L. Wang, T. Meng, G. Yu, S. Wu, J. Sun, H. Jia, H. Wang, X. Yang and Y. Zhang, *Biosens. Bioelectron.*, 124-125 (2019) 53.
38. L. Wang, T. Meng, D. Zhao, H. Jia, S. An, X. Yang, H. Wang and Y. Zhang, *Biosens. Bioelectron.*, 148 (2020) 111834.
39. P. Ling, J. Lei, L. Jia and H. Ju, *Chem. Commun.*, 52 (2016) 1226.
40. L. Wang, T. Meng, L. Liang, J. Sun, S. Wu, H. Wang, X. Yang and Y. Zhang, *Sens. Actuat. B: Chem.*, 278 (2019) 133.
41. P. Ling, C. Qian, J. Yu and F. Gao, *Biosens. Bioelectron.*, 149 (2020) 111838.
42. P. Dong, L. Zhu, J. Huang, J. Ren and J. Lei, *Biosens. Bioelectron.*, 138 (2019) 111313.
43. X. Liu, W. Li, T. Hou, S. Dong, G. Yu and F. Li, *Anal. Chem.*, 87 (2015) 4030.
44. X. Liu, M. Wei, E. Xu, H. Yang, W. Wei, Y. Zhang and S. Liu, *Biosens. Bioelectron.*, 91 (2017) 347.
45. M. Lu, *Analyst*, 142 (2017) 3477.
46. N. Xia, C. Cheng, L. Liu, P. Peng, C. Liu and J. Chen, *Microchim. Acta*, 184 (2017) 4393.
47. N. Xia, L. Liu, Y. Chang, Y. Hao and X. Wang, *Electrochem. Commun.*, 74 (2017) 28.
48. N. Xia, X. Wang, B. Zhou, Y. Wu, W. Mao and L. Liu, *ACS Appl. Mater. Interfaces*, 8 (2016) 19303.
49. C. C. Li, J. Hu, M. Lu and C. Y. Zhang, *Biosens. Bioelectron.*, 122 (2018) 51.
50. Z. Yi, H. B. Wang, K. Chen, Q. Gao, H. Tang, R. Q. Yu and X. Chu, *Biosens. Bioelectron.*, 53 (2014) 310.
51. F. Meng, X. Chen, W. Cheng, W. Hu, Y. Tang and P. Miao, *ChemElectroChem*, 6 (2019) 2000.
52. M. Alizadeh-Ghodsi, A. Zavari-Nematabad, H. Hamishehkar, A. Akbarzadeh, T. Mahmoudi-Badiki, F. Zarghami, M. Pourhassan Moghaddam, E. Alipour and N. Zarghami, *Biosens. Bioelectron.*, 80 (2016) 426.
53. M. La, C. Chen, X. Xia, J. Zhang and B. Zhou, *Int. J. Electrochem. Sci.*, 14 (2019) 5547.
54. X. Zhou, D. Xing, D. Zhu and L. Jia, *Electrochem. Commun.*, 10 (2008) 564.
55. X. Zhou, D. Xing, D. Zhu and L. Jia, *Anal. Chem.*, 81 (2009) 255.
56. L. Wu, J. Wang, L. Feng, J. Ren, W. Wei and X. Qu, *Adv. Mater.*, 24 (2012) 2447.

57. H.-R. Zhang, Y.-Z. Wang, M.-S. Wu, Q.-M. Feng, H.-W. Shi, H.-Y. Chen and J.-J. Xu, *Chem. Commun.*, 50 (2014) 12575.
58. H.-R. Zhang, M.-S. Wu, J.-J. Xu and H.-Y. Chen, *Anal. Chem.*, 86 (2014) 3834.
59. Y. Lin, L. Yang, G. Yue, L. Chen, B. Qiu, L. Guo, Z. Lin and G. Chen, *Anal. Bioanal. Chem.*, 408 (2016) 7105.
60. C. Xiong, W. Liang, Y. Zheng, Y. Zhuo, Y. Chai and R. Yuan, *Anal. Chem.*, 89 (2017) 3222.
61. H. Zhang, B. Li, Z. Sun, H. Zhou and S. Zhang, *Chem. Sci.*, 8 (2017) 8025.
62. Z. Zhao, Q. Tan, X. Zhan, J. Lin, Z. Fan, K. Xiao, B. Li, Y. Liao and X. Huang, *Theranostics*, 8 (2018) 5625.
63. S. Liu, S. Zhao, W. Tu, X. Wang, X. Wang, J. Bao, Y. Wang, M. Han and Z. Dai, *Chem. Eur. J.*, 24 (2018) 3677.
64. J. Lei, B. Han, S. Lv, Y. Li, J. Tang, Y. Mao and J. Zhuang, *Electrochem. Commun.*, 92 (2018) 43.
65. G.-C. Fan, Y. Lu, L. Ma, Z.-L. Song, X. Luo and W.-W. Zhao, *Biosens. Bioelectron.*, 142 (2019) 111564.
66. Y. Chu, A.-P. Deng, W. Wang and J.-J. Zhu, *Anal. Chem.*, 91 (2019) 3619.
67. L. Zhang, X.-M. Shi, Y.-T. Xu, G.-C. Fan, Y.-Y. Liang, C. Wang and W.-W. Zhao, *Anal. Chem.*, 91 (2019) 6403.