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Mini review

Electrochemical Biosensors for the Detection of Cancer Biomarkers with Different Signal Amplification Strategies

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Early diagnosis of cancer is the key to successful treatment. Biomarkers for cancer diagnosis can significantly improve the level of early diagnosis and follow-up treatment. Biosensors play an important role in the detection of biomarkers because they are easy to use, portable and can be used for real-time analysis. In this paper, we introduced the types of electrochemical biosensors and reviewed the signal amplification strategies for electrochemical detection of cancer biomarkers.

Keywords: Electrochemical biosensors; cancer biomarkers; signal amplification; nucleic acid; enzyme; nanomaterials

1. INTRODUCTION

Cancer biomarkers are a kind of over-expressed biological components in patients, which are secreted by tumor tissues or produced by specific body reactions. Some cancer biomarkers do not exist in adult tissues, but only exist in embryonic tissues, or the content in tumor tissues is much higher than that in normal tissues. Cancer biomarkers, including proteins, nucleic acids, circulating tumor cells (CTCs), exosomes, small molecules and so on, play an important role in early detection, staging, progression monitoring and chemotherapy evaluation of cancers [1, 2].

Biosensors are a kind of devices with biological active units as recognition elements to transform biochemical reactions into quantitative physical and chemical signals, so as to detect and monitor life and chemical substances. Electrochemical biosensors include three elements (Fig. 1): biometric unit, converter (electrode) and detector (electrochemical workstation). Typical three-electrode electrochemical cells include chemically stable solid working electrode (conductive materials

such as platinum, gold or carbon), reference electrode and platinum wire auxiliary electrode. Electrochemical biosensors have the advantages of low cost, miniaturization and real-time monitoring with good selectivity, specificity and sensitivity [3-5]. It has broad application prospects in biomedical diagnosis, agriculture and food safety, environmental detection, import and export quarantine, national defense security and other fields. This paper introduced the types of electrochemical biosensors and reviewed the signal amplification strategies for the detection of cancer biomarkers.

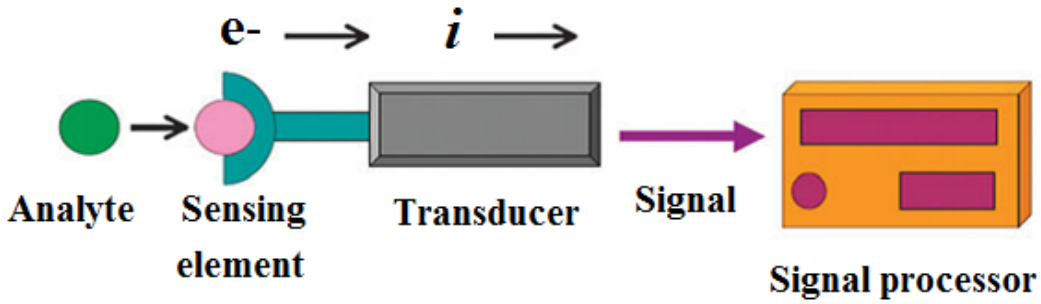

Figure 1. Schematic of a biosensor with electrochemical transducer.

2. TYPES OF ELECTROCHEMICAL BIOSENSORS

According to the signal transduction technology, electrochemical biosensors can be divided into voltammetric biosensor, impedance biosensor, conductance capacitance biosensor, potential biosensor and field effect transistor biosensor. Voltammetric or amperometric biosensor can measure the current generated by a redox reaction on the electrode, which is often limited by the diffusion speed of reactant molecules from the solution to the electrode surface. Electrochemical impedance is to detect the resistance and capacitance characteristics of electrode and obtain the impedance spectroscopy by changing the frequency in a certain range. Conductive sensor is mainly used to measure the conductivity of solution when the chemical reaction occurs, including the biological enzyme that changes the ionic strength of solution. Capacitive biosensor is based on the change of dielectric properties of electrode surface due to characteristic bonding, which has high sensitivity and shows non-specific adsorption. The potential biosensor is mainly used to measure the current inside cell, including two reference electrodes. The sensor can sense the potential changes generated by the reaction with charged ions across the ion selective membrane. Field effect transistor biosensor is also called semiconductor biosensor. Charged biomolecules can recognize and form complexes to produce ionic products. The change of surface charge density of ion sensitive film can lead to the change of membrane potential.

According to the target recognition unit, electrochemical biosensors can be divided into enzyme biosensor, immunosensor, aptasensor, DNA biosensor and cell sensor. The electrochemical enzyme biosensor uses enzyme-linked recognition element to recognize the target and catalyze the specific reaction to generate signal. Electrochemical immunosensor is based on antigen-antibody immune reaction. Electrochemical aptasensor employs an aptamer as the target identification unit. Electrochemical DNA biosensor refers to the single strand DNA (ssDNA) hybridized with complementary DNA chain with high efficiency and specificity. Electrochemical cell biosensor is an

electrochemical sensor that combines the nanomaterials modified by the biometric unit with proteins, glycans and receptors that are abnormally expressed on the cell surface.

According to the reaction mode, electrochemical biosensors can be divided into three types: direct detection, competition and sandwich. Direct detection is a method for measuring the target by directly combining the sensor surface recognition unit with the target for the electrochemical signal output. The competitive combination between interfering molecules and target is detected based on the sensor identification unit. Sandwich method includes three parts: target recognition unit, target molecule and signal output.

3. SIGNAL AMPLIFICATION STRATEGIES

In the fields of medical diagnosis, environmental monitoring and food safety, the development of rapid and sensitive detection methods has always been the focus of research [6]. Electrochemical biosensors have attracted much attention due to their low cost, high sensitivity and good selectivity. In this work, we highlights various signal amplification strategies for electrochemical detection of cancer biomarkers, including nucleic acid-based amplification, enzymes, nanomaterials, nanocomposites and so on.

3.1 Nucleic acid-based amplification

DNA amplification is mainly used to increase the concentration of target molecules by enzymes of exonuclease, endonuclease, polymerase and ligase [7-10]. They include DSN for specific hydrolysis of double strand DNA (dsDNA) or ssDNA in DNA/RNA, RNase for hydrolysis of RNA strand in DNA/RNA, Exo I for hydrolysis of phosphate diester bond in ssDNA from 3' − 5' terminal, Exo III for hydrolysis of phosphate diester bond in the end of dsDNA from 3 '− 5' terminal, DNase for specific endonuclease of ssDNA or dsDNA, S1 ribozyme hydrolyzing ssDNA, Nb.BbvCI for recognition and cleavage of the site in specific sequence of dsDNA, T4 ligase to connecting two ssDNA, Phi 29 DNA polymerase and so on.

3.1.1 Nuclease-assisted target amplification

Enzyme amplification technology is one of the most commonly used technologies in the field of biochemical analysis. For example, Exo III enzyme can hydrolyze the probe of dsDNA on the electrode. After releasing the target DNA, it continues to hybridize with the probe. A single target molecule can trigger a large number of probe to hydrolysis, which can be used for the construction of electrochemical sensing platform. For example, Xiong et al. developed an electrochemical proportional biosensor to detect target DNA based on Exo III-assisted target cycle amplification (Fig. 2) [11]. The hairpin probe on the electrode surface was labeled with methylene blue (MB) and ferrocene (Fc) at the 3'-end and the middle of the ring. The target DNA (T-DNA)-triggered cleavage reaction resulted in the decrease of MB oxidation current and the increase of Fc oxidation current. The linear concentration range for T-DNA detection was 0.01 ~ 0.8 pM, and the detection limit was 4.16 fM. Moreover, Hao et

al. constructed a proportional electrochemical miRNA biosensor by DSN-based cyclic amplification and energy resonance transfer [12]. Wang et al. detected DNA in circulating tumor cells based on multiple signal amplification of ribonuclease and terminal transferase [13].

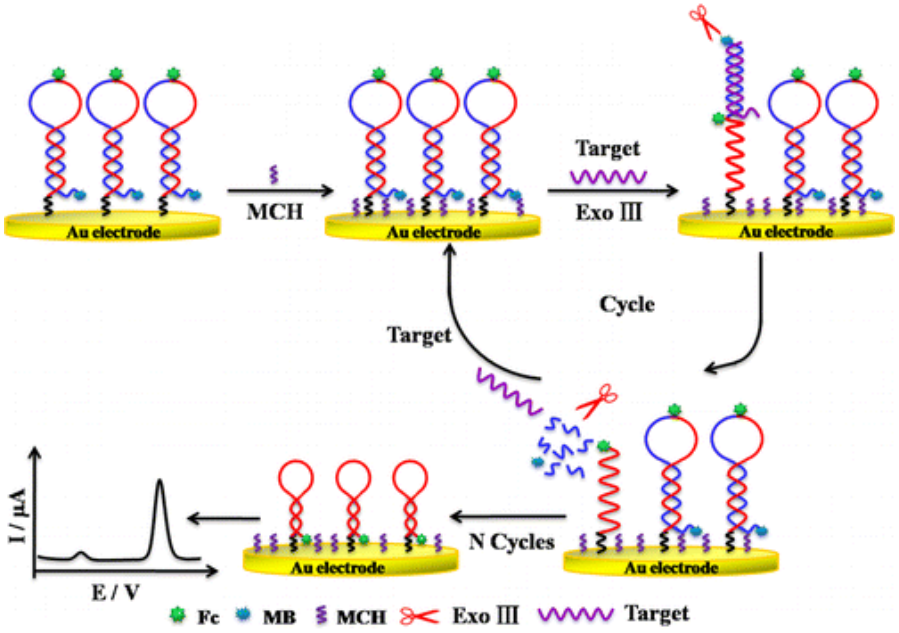

Figure 2. Schematic illustration of the electrochemical biosensor for target DNA detection. Reprinted with permission from reference [11]. Copyright 2015 American Chemical Society.

3.1.2 Polymerase chain reaction

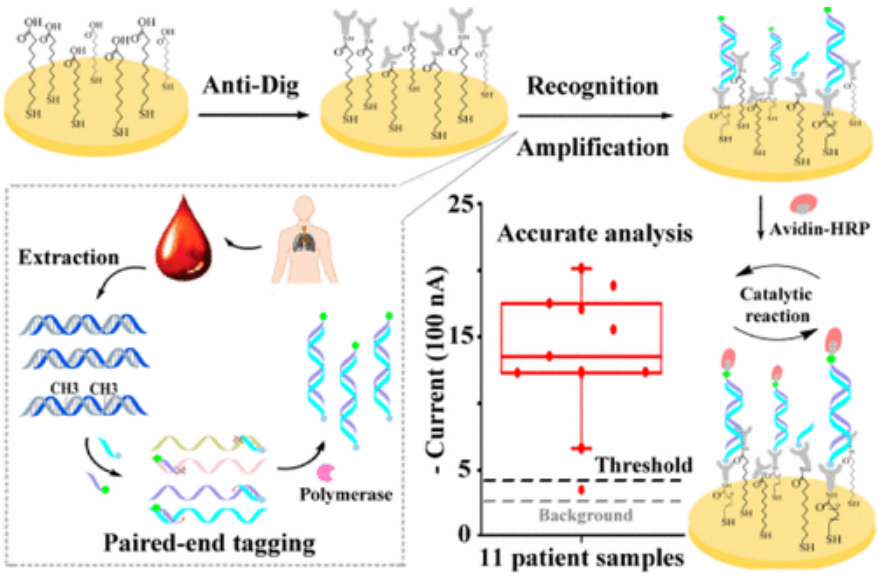

Figure 2. Schematic illustration of paired-end tagging and amplifications for accurate electrochemistry analysis of circulating methylated DNA from clinical plasma. Reprinted with permission from reference [15]. Copyright 2017 American Chemical Society.

Polymerase chain reaction (PCR) is a molecular biological technology with polymerase to generate more DNA fragments, thus increasing the amounts of DNA. It was first proposed by Mullis in 1985 [14]. Zhu et al. detected DNA methylation based on terminal-labeling and PCR technology (Fig. 3) [15]. The methylated DNA hybridized with Dig-labeled pre-primer and biotin-labeled post-primer. The DNA polymerase promoted the formation of DNA antigen which can specifically bind with anti-Dig modified on the electrode. Through the catalytic reaction of HRP-conjugated dsDNA, the methylation level of 1% could be detected.

3.1.3 Rolling circle amplification

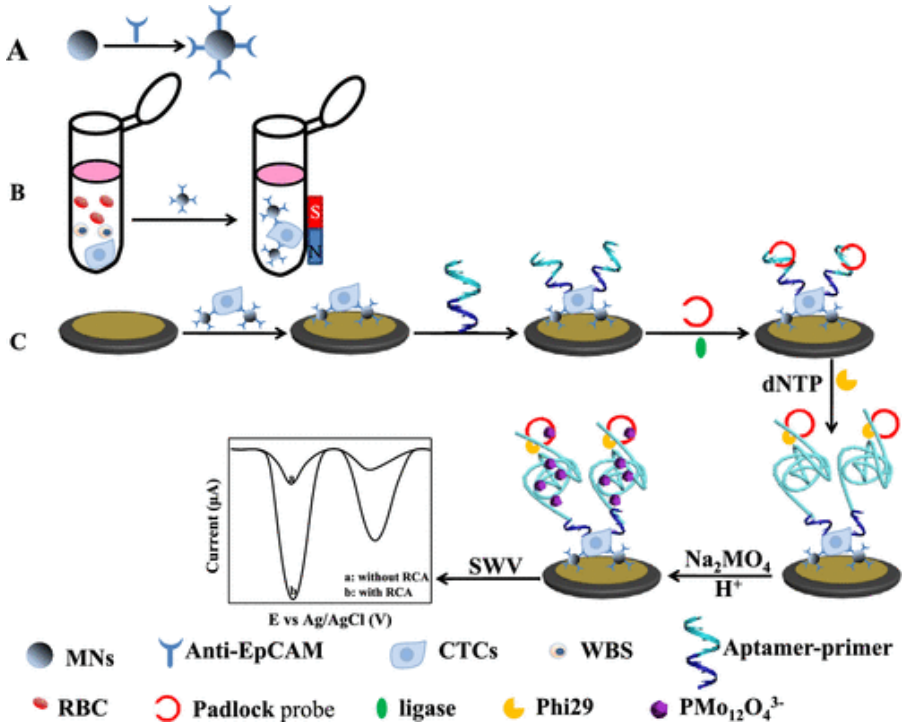

Figure 4. Illustration of CTC measurement in whole blood based on MN isolation and RCA signal amplification. Reprinted with permission from reference [16]. Copyright 2015 American Chemical Society.

Rolling circle amplification (RCA) is a target amplification method with circular DNA as the template to transform short DNA primer into long ssDNA. For example, Yang's team reported a magnetic aptasensor for the detection of CTCs based on RCA (Fig. 4) [16]. EpCAM modified-magnetic beads were used to capture CTCs. ALP was used as the signal marker to catalyze the generation of a large number of electroactive p-aminophenol molecules. In addition, Zhang et al. found that miRNA-21 can open the hairpin probe by hybridization, thus initiating the ligase reaction and RCA amplification in the presence of primer, ligase and polymerase, thus producing guanine-rich DNA sequence [17]. MiRNA-21 was detected by in situ synthesis of platinum nanoparticles. Wang et al. constructed a nuclease-triggered RCA-assisted electrochemical DNA biosensor [18]. Fc-labeled tracer DNA and capture probe are modified on the electrode surface. The arm of ternary complex chain

(padlock, primer, block DNA) was attached on the electrode through hybridization to form m-DNA walker. After binding to the targeted DNA, RCA polymerization is triggered to generate DNA enzyme. In the presence of Mn^{2+}, Fc molecule is released by enzyme digestion. The linear range of DNA detection is 1.0 fM − 1.0 nM with a detection limit of 0.28 fM. Zhu et al. found that the target DNA can hybridize with the probe on the electrode surface to form a sandwich structure with the cyclization mixture [19]. The ssDNA and phi 29 DNA polymerase started the RCA amplification to form long ssDNA. With the signal amplification of DNA-modified AuNPs, ALP was captured to catalyze the hydrolysis of phosphate, thus achieving the detection of target DNA.

3.1.4 Ligase chain reaction

Ligase chain reaction (LCR) is a new DNA amplification and detection technology in vitro, which is mostly used for the connection of two dsDNA strands. If any ssDNA in dsDNA can not be hybridized to form dsDNA due to gene mutation, it will lose the ability to be recognized and connected by ligase. For example, Wee and co-workers employed ligase chain reaction to detect single-base mutation of DNA [20]. Four DNA strands are targeted by ligase. When the DNA was completely matched, a long strand was formed in the presence of ligase, which can hybridize with the capture probe modified on the electrode. Methylene blue or HRP can interact with dsDNA for the electrochemical signal output. When there is a single-base mutation in the DNA fragment, the probe could not be hybridized and recognized by the enzyme to form a long strand; thus, a weak electrochemical signal was obtained. The method is often used for the analysis of DNA mutation. Recently, Zhong and co-workers reported a DNA probe-aided LCR-based electrochemical method (cmDNA-eLCR) for the detection of RNA mutation by using bovine serum albumin as carrier (Fig. 5) [21]. HRP-H_2O_2-TMB sensing system was used for signal output. The method has a wide linear range (10^{-15} ~ 10^{-10} M) and a low detection limit (0.6 fM).

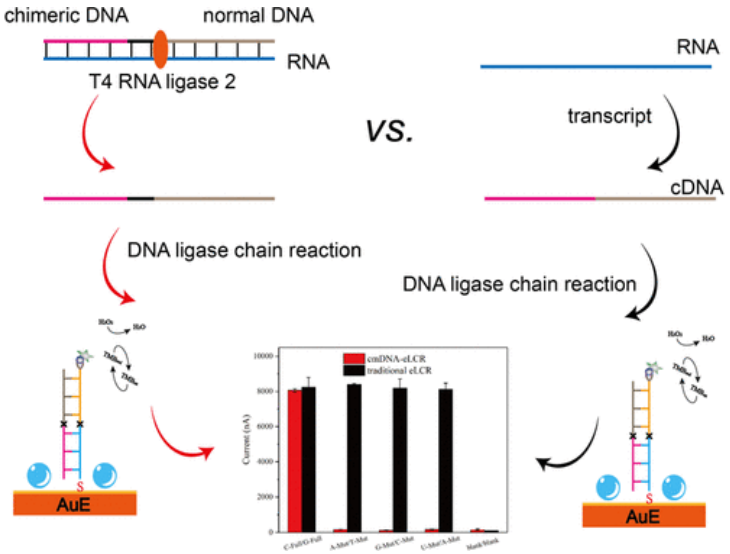

Figure 5. Schematic illustration of the developed cmDNA-eLCR. Reprinted with permission from reference [21]. Copyright 2021 American Chemical Society.

3.1.5 Strand displacement amplification

Strand displacement amplification (SDA) is a method of enzyme catalyzed isothermal amplification of DNA in vitro. Endonuclease opens the gap of target dsDNA at the recognition site. Polymerase extends the 3 'end of the gap and replaces the next DNA strand. After combining with primer, the dsDNA is amplified under the condition of polymerase. For example, Chen et al. used the targeted molecule to open the electrode hairpin DNA probe and synthesize the dsDNA under the action of polymerase, releasing the targeted molecule into the next cycle. The synthesized dsDNA released targeted molecular analogues under the action of cleavage enzyme, and then continued to synthesize dsDNA under the action of polymerase. The enzyme continued to cleave and release targeted molecular analogues, so as to repeat the cycle. The synthesized dsDNA and two hairpin DNA strands expand to produce long C-base-rich chains through HCR, which can be detected through the formation of silver nanoclusters [22]. Zhu et al. constructed an electrochemical DNA sensor with $[Ru(NH_3)_6]^{3+}$ as the electrochemical label by combining isothermal chain replacement polymerase reaction with single target multi-trigger hybridization chain reaction [23]. The linear range for DNA detection was 0.1 fM − 10 pM, and the detection limit was 0.02 fM. Recently, Zhou and co-workers constructed a surface-enhanced Raman scattering (SERS)-electrochemistry dual-mode for miRNA detection by using three-dimensional popcorn-like gold nanofilms as active substrates (Fig. 6) [24]. The detection limits of the SERS and electrochemical methods are 0.12 fM and 2.2 fM, respectively.

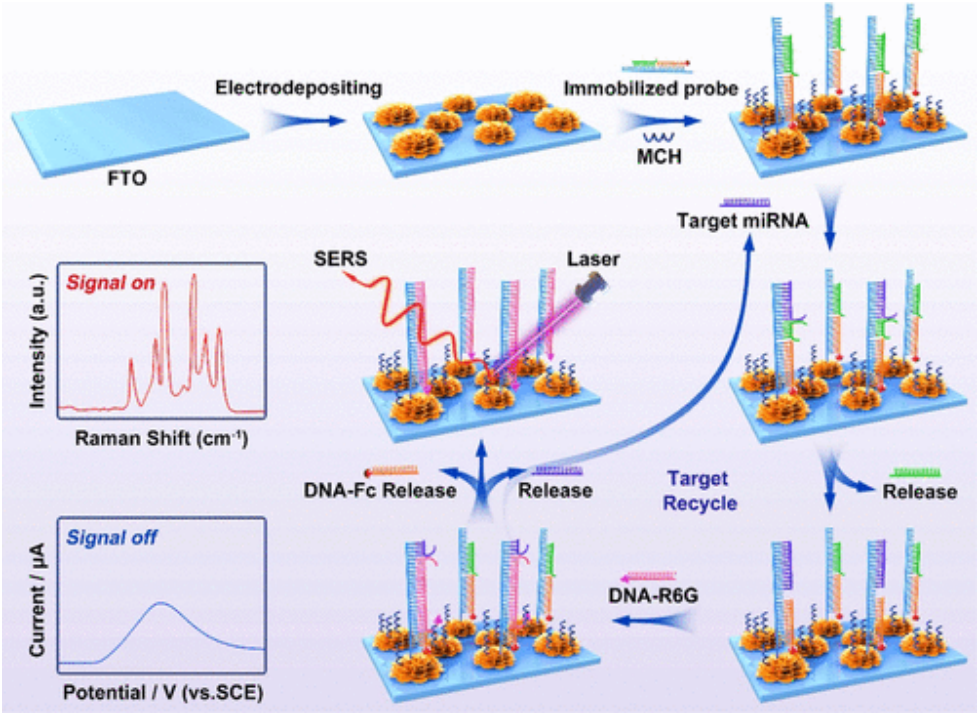

Figure 6. Schematic illustration of the proposed strategy for enzyme-free target recycling amplification detection of miRNA. Reprinted with permission from reference [24]. Copyright 2021 American Chemical Society.

3.1.6 Hybrid chain reaction

Hybrid chain reaction (HCR) can promote the formation of long dsDNA hybrid with ssDNA as the promoter to hybridize with hairpin chains H1 and H2, which was originally proposed by Piece and Dirks in 2004 [25]. Based on HCR, Ge and others reported the detection of miRNA with SA-conjugated HRP to reduce H_2O_2. Tetrahedral nanoprobes were used to hybridize with target molecules, then expanding the double chains with biotin-modified H1 and H2 hairpin chains. The detection limits of this method for DNA and miRNA were 100 aM and 10 aM [26]. Liu et al. found that the modified DNA hairpin probe on the electrode could be opened by hybridization with miRNA, thus making the second hairpin probe attached on the electrode to release the target miRNA into the next cycle [27]. CdTe QDs were captured by the electrode through the product, other two DNA chains and the dsDNA generated by HCR amplification. The detection limit for miRNA-21 detection is 33 aM. By the hybridization of target molecule and capture probe, the hairpin structure was opened, which allowed for the attachment of DNA-modified AuNPs, the HCR amplification and the capture of ALP. The captured ALP catalyzed the hydrolysis of p-aminobenzene to p-aminobenzene for silver nanoparticle deposition, thus realizing double signal amplification for miRNA-21 detection [28]. After the hybridization of target molecule and capture probe on electrode surface, Zhu et al. found that DNA-modified AuNPs can be captured and then trigger the HCR amplification. $[Ru(NH_3)_6]^{3+}$ was attached on the resulting electrode as the signal label. The linear range for miRNA-21 detection was 1 fM ~ 1 nM, and the detection limit was 0.12 fM [29]. Recently, Miao's group developed an electrochemical method for in vitro miRNA analysis by combining SDA with dumbbell HCA (DHCR) (Fig. 7) [30]. The target triggered isothermal SDA, producing a large number of ssDNA products to assist in the formation of three-way junctions on the electrode surface. At the same time, dumbbell DNA fuel chains (DHP1and DHP2) were designed for HCA. The double signal amplification significantly improved the sensitivity.

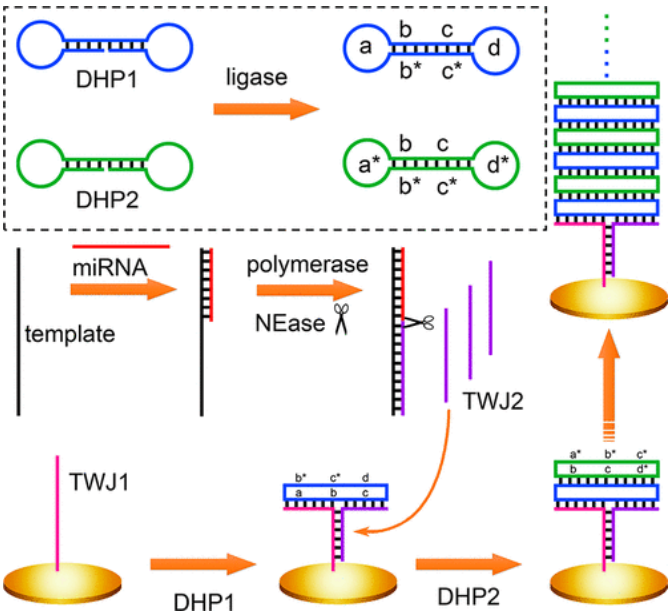

Figure 7. Illustration of the DHCR based biosensor for the exosomal miRNA assay. Reprinted with permission from reference [30]. Copyright 2010 American Chemical Society.

3.1.7 Catalytic hairpin assembly

In catalytic hairpin assembly (CHA), hairpin DNA is opened by using short DNA as catalyst, which was then hybridized with other hairpin DNA to form long dsDNA. Cheng et al. reported the detection of miRNA-21 using H1 hairpin probe-modified porous silicon sphere loaded with methylene blue (MB) (Fig. 8) [31]. After hybridizing with H1 hairpin probe, the targeted molecule breaks away from the surface of porous silicon sphere, thus opening the MB molecular channel. The resulting dsDNA hybridized with H2 hairpin to release miRNA-21. The target molecule entered the next cycle to perform CHA amplification. The generated double chain product H1/H2 hybridized with the capture probe on the electrode, further starting the hairpin H3 and H4 for HCR amplification to form long double chain H3/H4, which can capture MB molecules to achieve target detection. The linear range of miRNA-21 was 0.1 fM − 5.0 pM, and the detection limit was 0.037 fM. Based on the CHA-RCA signal amplification, Wang et al. reported the detect of miRNA-21 by controlling the potential to regulate CHA efficiency on the electrode. The linear range for miRNA-21 detection was 15 fM − 250 pM, and the detection limit was 13.5 fM [32].

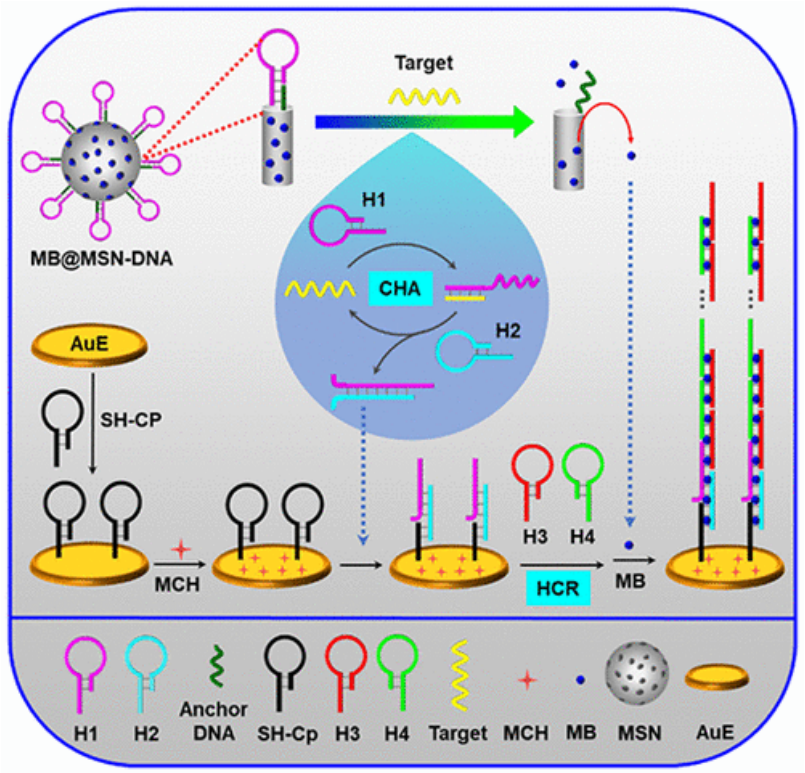

Figure 8. Schematic diagram of mesoporous silica containers and programmed catalytic hairpin assembly hybridization chain reaction based electrochemical sensing platform for miRNA ultrasensitive detection with low background. Reprinted with permission from reference [31]. Copyright 2019 American Chemical Society.

3.1.8 Non-enzymatic target amplification

Based on the two Fc-modified DNA strands, Zhang et al. prepared a bipedal DNA probe with high space utilization on the electrode surface through the proximity binding mechanism. In the presence of target miRNA-21 and hairpin DNA, the DNA probe was disintegrated, and the two signal strands and miRNA-21 were released from the electrode surface at the same time. MiRNA-21 continued to the next round of hybridization, opening more DNA probes on the electrode to achieve the target circular amplification (Fig. 9) [33]. The linear concentration range of miRNA-21 was 1 fM − 10 nM, and the detection limit was 0.31 fM. Hu et al. found that miRNA-21 can open two hairpin DNA probes in a target cycle, and the product can be captured by the electrode. Using $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ as the signal tag, an electrochemical biosensor for miRNA-21 detection was constructed. In the absence of miRNA-21, the hairpin DNA interacted with the capture probe to produce a weak electrochemical signal. The linear range of this method was 0.1 fM ~ 5 pM with a detection limit of 56.3 aM [34].

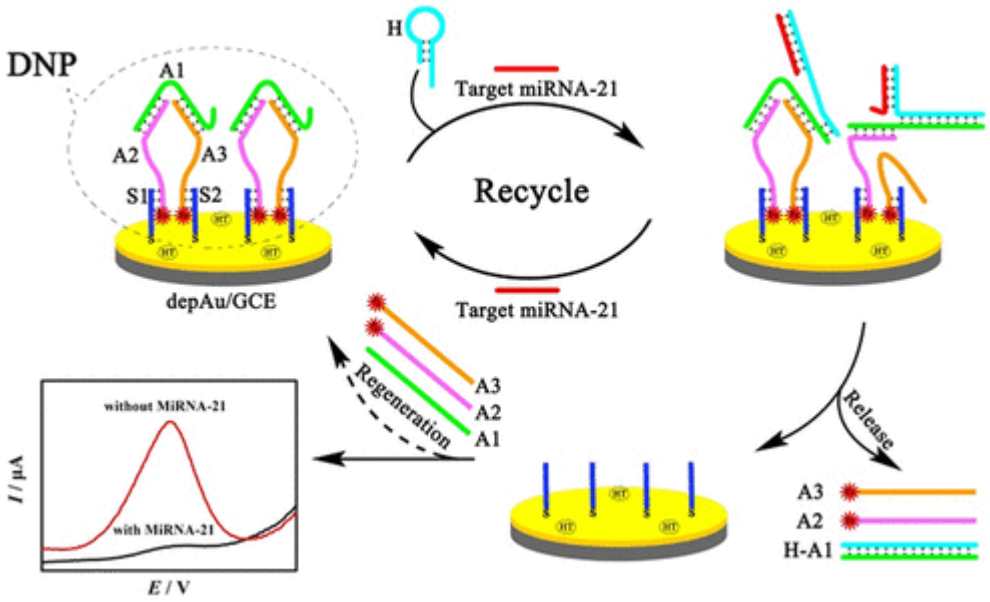

Figure 9. Schematic illustration of the biosensor based on the 2D DNA nanoprobe (DNP) and enzyme-free-target-recycling amplification for the miRNA-21 assay. Reprinted with permission from reference [33]. Copyright 2018 American Chemical Society.

3.2 Enzymes

Enzyme is a special type of protein with catalytic function, which has high efficiency, specificity and specificity. It can be used as a biological recognition unit to amplify the detection signal. Horseradish peroxidase (HRP), alkaline phosphatase (ALP) and glucose oxidase (GOx) are the commonly used enzymes to amplify the electrochemical signals [35].

Enzyme can directly catalyze a specific chemical reaction on the electrode or in solution. For example, Zhang et al. used GOx to catalyze the reduction of chloroaurate, leading to the deposition of

AuNPs on the electrode to detect IgG [36]. Wang et al. constructed an electrochemical biosensor for AFP detection in serum based on the dual signal amplification strategy of HRP and Fe_3O_4 nanoparticles to catalyze H_2O_2 reduction [37].

Because of their large surface area, good conductivity and biocompatibility, nanocomposites combined with enzyme-functionalized nanoprobes can be used to amplify the electrochemical signal, thus improving the detection sensitivity. First, the signal amplification can be achieved by combining enzyme catalysis with nanocomposite-modified electrode. For example, Huang et al. reported the detection of DNA where AuNPs were loaded with probe molecules and conjugated with GOx. Glassy carbon electrode was modified with molybdenum disulfide/multi-walled carbon nanotube composites [38]. By using HRP-loaded carbon nanotubes as signal amplification labels, p185 BCRABL (a fusion transcription gene of human acute lymphoblastic leukemia) was detected with a detection limit of 83 fM [39].

Ribozymes can amplify the detection signal. DNA walker can be released by the hybridization of target with short DNA to protect the long DNA. The released long strand can hybridize with signal probe to form Pb^{2+}-specific enzyme on the electrode. MB molecules were released after hydrolysis in the presence of Pb^{2+} [40]. Based on the specific binding of concanavalin to polysaccharide on cell surface, Cheng et al. reported the detection of acute leukemia cells (CCRF-CEM) by using reduced grapheme and polyamide dendrimer-modified electrode and aptamer/HRP-modified gold nanoparticles (Fig. 10) [41].

Biomimetic enzyme-labeled nanoprobes can achieve signal amplification. For example, Chang et al. reported the determination of AFP using platinum nanoparticles and hemin/G-quadruplex-modified polymer microspheres to catalyze H_2O_2 reduction [42]. Antibody and enzyme-labeled nanomaterials have also used to amplify the detection signal. In Chen's work, Au/Ag nanoparticles modified with secondary antibody and HRP was used as signal amplification promoters to catalyze the reaction of H_2O_2 and hydroquinone [43], thus allowing for the detection of CEA at antibody-fixed N-doped grapheme-modified electrode.

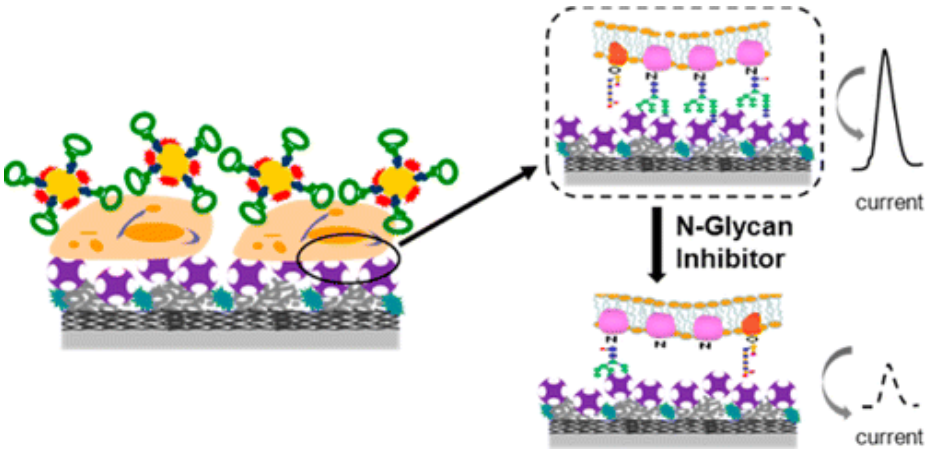

Figure 10. Schematic illustration of the electrochemical aptamer biosensor for dynamic evaluation of cell surface N-glycan expression based on multivalent recognition and dual signal amplification. Reprinted with permission from reference [41]. Copyright 2014 American Chemical Society.

3.3 Nanomaterials

Nanomaterials have good catalytic activity, conductivity and electrochemical activity. Their applications in the field of biosensing can significantly improve the detection sensitivity. Nanomaterials used for amplifying electrochemical detection signals include metal nanoclusters (MNPs), quantum dots (QDs), nanoparticles, core-shell nanospheres, carbon nanotubes, metal disulfide, metal disulfide, black phosphorus, and magnetic nanoparticles (MNPs).

3.3.1 Nanoparticles

NPs have unique physicochemical properties with small volume and particle size of 10 − 100 nm. Nanoparticles can be loaded with electrochemical signal molecules to amplify the detection signal [44]. Miao et al. reported the detection of Ag^+ by using AuNPs to load electroactive $[Ru(NH_3)_6]^{3+}$ and combining the Nt.CviPII cleavage technology. The detection limit is as low as 470 fM [45]. Qiu et al. reported the detection of p53 protein by using Fc, DNA and SA-modified AuNPs as signal probes. The target probe opened the hairpin probe by hybridization, exposing the terminal biotin group to interact with SA on the surface of AuNPs to generate electrochemical signal. EcoRI cleaved the unhybrid hairpin probe and the residual single strand on the electrode hybridized with the DNA probe modified on the AuNPs, making Fc close to the electrode [46].

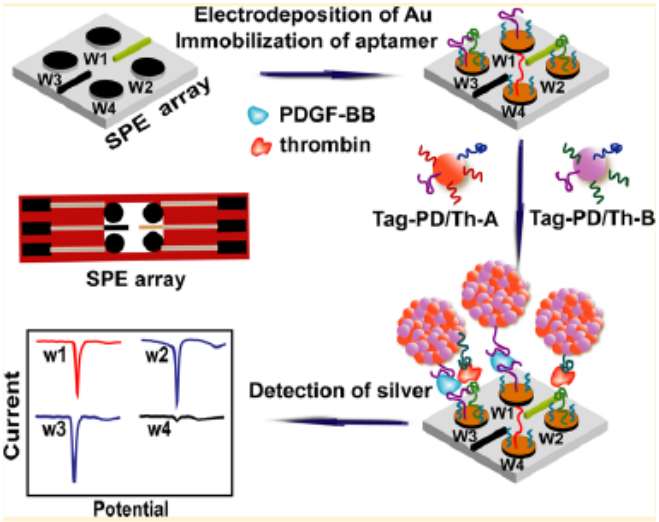

Figure 11. Schematic illustration of the strategy for the electrochemical assay of protein with signal amplification through AgNPs aggregate induced by in situ hybridization on SPE Array. Reprinted with permission from [52]. Copyright 2014 American Chemical Society.

Nanoparticles with enzyme-like catalytic properties have also been used to amplify the detection signal. For example, Kwon et al. constructed a sandwich biosensor using PtNPs-based hydrazine oxidation reaction to detect DNA [47]. AgNPs have low oxidation potential, which can be used as electrochemical signal markers [48-50]. For example, carbon nanotubes coated with SA-conjugated AgNPs are used as the signal probes by combining with biotin-labeled antibody. Immune

complexes catalyzed silver deposition for the signal-amplified detection of CEA and AFP [51]. Song et al. reported the detection of platelet-derived growth factor (PDGF-BB) through the hybridization-inducing *in situ* assembly of DNA-AgNPs (Figure 11) [52]. Nanoparticles can catalyze the reduction and deposition of metal ions on electrode surface, thus achieving the amplification detection. Duangkaew et al. used the double signal amplification of gold deposition and silver deposition to detect p16 protein markers in patients with cervical cancer [53]. The electrocatalyst deposited on the surface of nanoparticles can electrolyze the substrate in solution. In addition, Zhang et al. used the deposition strategy to obtain platinum nanoparticles for PSA detection [54].

3.3.2 Metal nanoclusters

Metal nanoclusters (MNCs) are composed of several to hundreds of metal atoms, including precious metal clusters and copper clusters. Compared with nanoparticles, MNCs have unique optical, electrical and chemical properties due to their smaller size. The metal nanoclusters include gold, platinum, silver and other noble metal nanoclusters and copper nanoclusters. Among them, silver nanoclusters (AgNCs) has been widely used as the electrochemical signal markers to amplify the detection signal. For example, Peng et al. reported the detection of Dam MTase by using restriction endonuclease and HCR to generate the template for AgNC synthesis at glassy carbon electrode modified with reduced graphene oxide/gold nanoparticle hybrid [55]. Yang et al. developed a method for miRNA-199a detection based on in situ synthesis of AgNCs [56]. As shown in Fig. 12, after the hybridization of miRNA-199a and DNA chain, the target-assisted enzyme polymerization and cleavage reaction was initiated to produce a large number of intermediate products. HCR was further triggered on the electrode surface after introducing the self-assembled DNA probe, and a large number of sequences as synthesis templates of AgNCs are generated in the amplified double chain. In situ synthesis of AgNCs enhanced the electrochemical signal, and the detection limit was 0.64 fM.

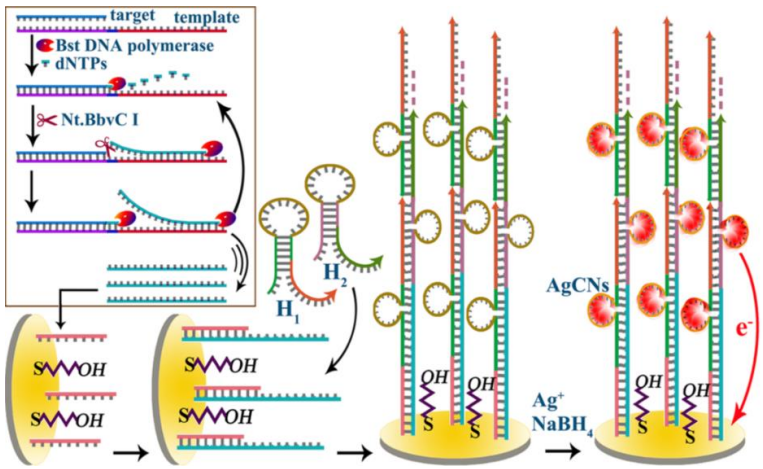

Figure 12. Schematic illustration of ultrasensitive and label-free electrochemical detection of miRNA-199a based on in situ generated AgNCs by coupling TAPNR with HCR amplifications. Reprinted with permission from reference [56]. Copyright 2015 American Chemical Society.

3.3.3 Quantum dots

QDs are one-dimensional spherical semiconductor nanomaterials with particle size of 2 − 20 nm, which have narrow stripping voltammetric signals that are easy to be distinguished [57]. For example, Li et al. detected the activity of telomerase by introducing a large number of QDs to achieve signal amplification [58]. Based on LCR amplification strategy by T4 ligase, Zhu et al. used magnetic nanoparticles as carriers and CdS as well as PbS QDs as signal probes to simultaneously detect miRNA-155 and miRNA-27b in serum [59]. Wu and co-workers developed a QDs-based dual signal amplification immunosensor for CTC detection (Fig. 13) [60]. In this study, graphene-modified electrode was used to accelerate the electron transfer, and silicon nanoparticles coated with two types of QDs were used as the signal markers.

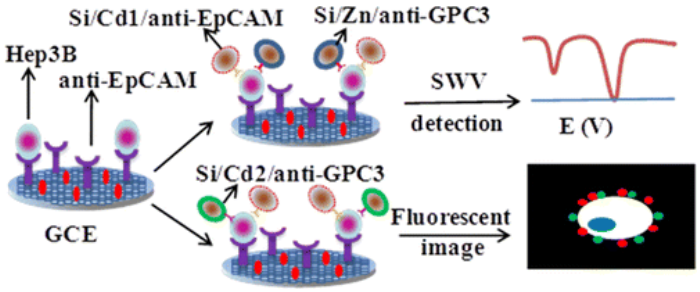

Figure 13. The fluorescent and electrochemical detection procedures of circulating tumor cells. Reprinted with permission from reference [60]. Copyright 2013 American Chemical Society.

3.3.4 Core-shell nanospheres

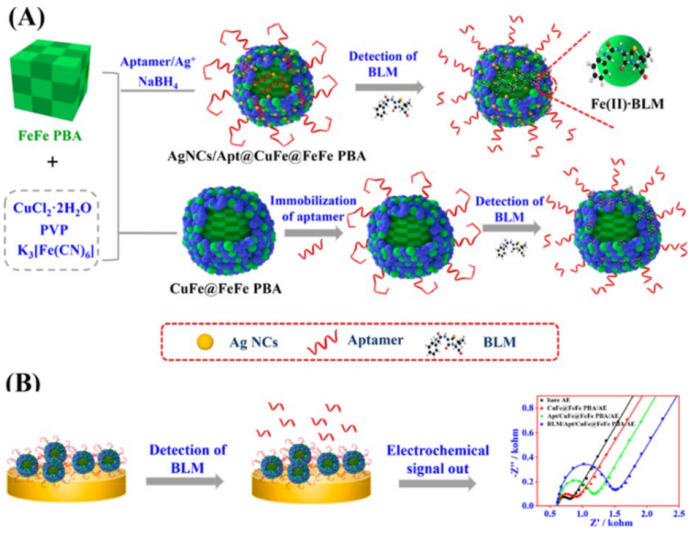

Figure 14. Schematic diagram of (A) the fabrication of BLM aptasensors based on AgNCs/Apt@CuFe@FeFe PBA and CuFe@FeFe PBA and (B) BLM detection and electrochemical signal out. Reprinted with permission from reference [61]. Copyright 2018 American Chemical Society.

Copper/iron Prussian blue (CuFe PB) and iron/iron Prussian blue (FeFe Pb) have $Cu^{I/II}$ and $Fe^{II/III}$ redox pairs, which can coordinate with bleomycin (BLM) for its detection. Zhou et al. synthesized bimetallic core-shell nanospheres with FeFe PB as the core for BLM detection based on AgNC synthesis and BLM-targeted aptamer (Fig. 14) [61].

3.3.5 Carbon nanotubes

Carbon nanotube (CNTs) are one-dimensional materials which have been widely used in bioassays due to its large surface area, good conductivity, rich surface group and biocompatibility [62-64]. It can be used as the carrier of electrochemical signal markers such as nanoparticles, ferrocene, biological enzymes and so on. For example, Rahman and Rhee developed an electrochemical immunosensor for PSA detection by using multiwall carbon nanotubes (MWCNTs)/AuNPs to load large numbers of HPR molecules (Fig. 15) [65]. The signal was detected by the HPR-catalyzed precipitation of 4-chloro-1-naphthol on the electrode. Li et al. synthesized magnetic multi-walled CNTs to load AuNPs and lead ions for the detection of AFP in liver cancer cells through its synergistic catalysis of H_2O_2 hydrolysis [66]. Yang et al. used DNA and Fc-modified single-walled CNTs as the signal probes to amplify the electrochemical signal by catalyzing the reduction of H_2O_2 [67]. Xiang et al. constructed an electrochemical immunosensor for the detection of CEA by using antibody-modified single-walled CNTs to load ALP as the signal probe. The detection limit of the method was improved by 2 − 4 orders of magnitude [68].

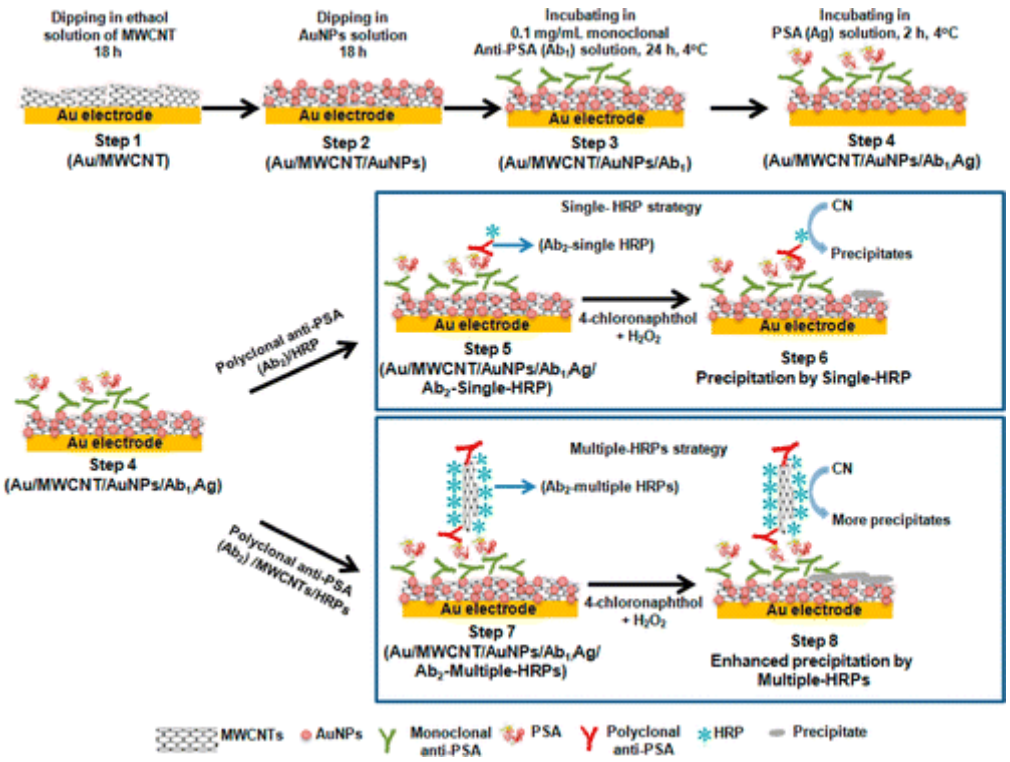

Figure 15. Schematic illustrations of the single-HRP and multiple-HRP strategy-based PSA immunosensor. Reprinted with permission from reference [65]. Copyright 2018 American Chemical Society.

3.3.6 Graphene oxide

Graphene is a two-dimensional carbon nanomaterial with large surface area, good mechanical strength and electrical conductivity. Using graphene as electrode material, the sensitivity of electrochemical detection can be improved by its good conductivity, strong loading capacity and the stabilization of bioactive molecules [69]. Yang et al. synthesized polyxanthuric acid/GO component with xanthuric acid monomer as precursors. The carboxyl groups on the surface interacted with the amino groups of ssDNA probe to achieve DNA detection using $[Fe(CN)_6]^{3-/4-}$ as the signal medium (Fig. 16) [70]. The linear concentration range was $10^{-14} - 10^{-8}$ M, and the detection limit was 4.2×10^{-15} M. Based on the double signal amplification of graphene and AuNPs for catalyzing the hydrolysis of H_2O_2, Wang et al. developed an electrochemical biosensor for polypeptide antigen detection by using graphene modified with antibody-labeled AuNPs as the signal labels [71]. Lu et al. constructed an electrochemical sensor for CEA detection using ultra-thin gold platinum nanowire-modified thionine/reduced GO composite as the signal probe [72].

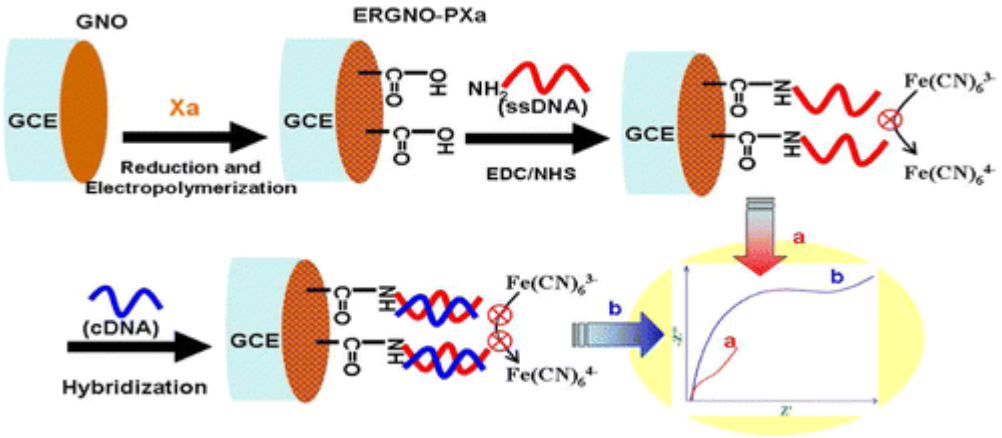

Figure 16. Schematic of synchronous electrosynthesis of PXa-ERGNO for DNA EIS detection. Reprinted with permission from reference [70]. Copyright 2013 American Chemical Society.

3.3.7 Metal disulfide

Molybdenum disulfide (MoS_2) nanosheet is a layered two-dimensional nanomaterial formed by van der Waals force of molybdenum metal layer, which shows good photoelectric properties [76]. For example, MoS_2 nanosheet was modified with AuNPs and $[Ru(NH_3)_6]^{3+}$ was used as signal molecule to detect miRNA-21 [73]. Duan et al. constructed an electrochemical aptasensor for PSA detection by modifying chitosan-stabilized AuNPs with two-dimensional MoS_2 QDs and g-C_3N_4 nanosheets. The detection limit of PSA was 0.71 pg/mL by impedance measurement [74].

3.3.8 Black phosphorus

Black phosphorus (BP) is prepared by mechanical stripping of massive black phosphorus in 2014, which has high fluidity, anisotropic and catalytic properties [75]. BP has unique electronic

properties, such as high fluidity, layer-dependent band gap, anisotropy and excellent catalytic performance [76]. BP exists in the formation of QDs, nanoparticles and nanosheets. Liu et al. synthesized the BP film anchored by AuNPs [77]. The circulating tumor cells were enriched and separated by anti-EpCAM-modified magnetic particles. CTC was detected by using BP/AuNPs/aptamer conjugate as signal probe. Mayorga Martinez et al. constructed an electrochemical sensor for rabbit IgG detection by the BP nanoparticles-catalytic reduction of H_2O_2 (Fig. 17) [78]. The linear range was 2 − 100 ng/mL, and the detection limit was 0.98 ng/mL.

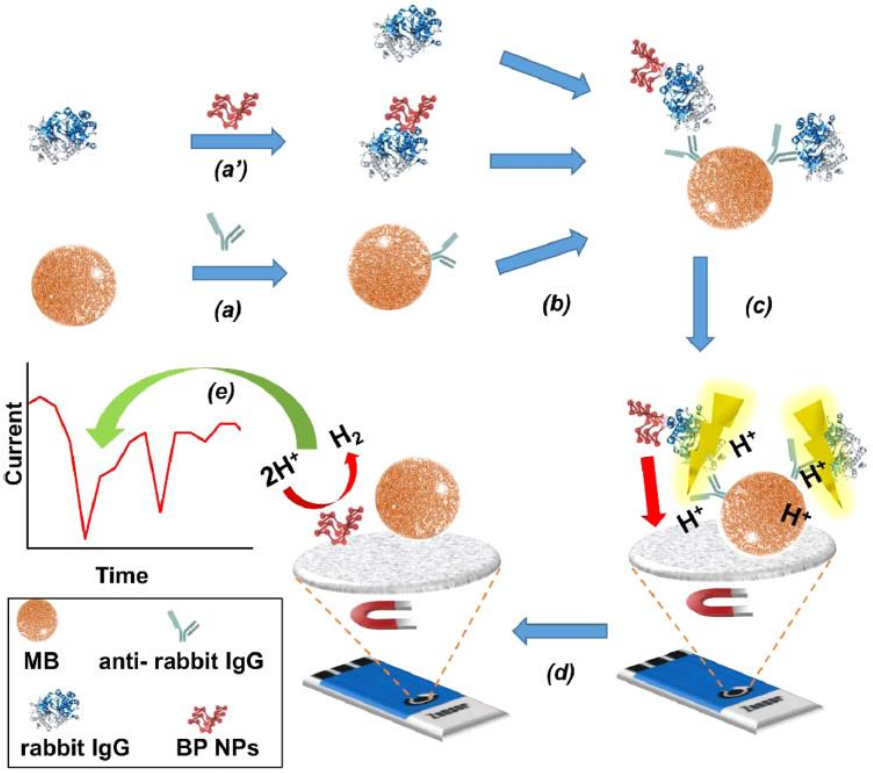

Figure 17. Schematic of the competitive magneto-immunoassay for protein using BP NPs as a tag and HER electrocatalysis (proton reduction) by impact electrochemistry (spikes count) as a detection technique. Reprinted with permission from reference [78]. Copyright 2016 American Chemical Society.

3.3.9 Magnetic nanoparticles

Magnetic nanoparticles (MNPs) have a large specific surface area, easy to functionalization and magnetic separation. It has been widely used in cell separation, enzyme fixation and cancer diagnosis [79]. Moreover, MNPs have natural peroxidase-like activity. For example, it has been suggested that Fe_3O_4 magnetic nanoparticles have higher binding ability than HRP for the substrate, and their enzyme activity is 40 times higher than HRP at the same concentration [80].

3.4 Nanocomposites

Nanocomposites have good conductivity, catalytic activity and biocompatibility, and can be used as electrode materials. Nanocomposite particles have strong catalytic performance, which can greatly improve the sensitivity of electrochemical detection. For example, Yan et al. reported the detection of hepatitis B surface antigen (HBeAg) using N-doped graphene QDs loaded with Au/Pd/Cu ternary nanoparticles to catalyze H_2O_2 reduction [81]. Nanomaterial-modified electrode combined with target amplification, QDs and nanoparticles can amplify the electrochemical signal. For example, Bao et al. used nanomaterial-modified electrode combined with CHA and HCR amplification to detect human serum miRNA-16 [82]. Jie et al. used carbon nanotube/AuNPs hybrid as signal amplification platform and DNA-modified CdSe-CdS quantum dots as signal probe to detect DNA [83]. Zhang et al. reported the detection of thrombin using aptamer and PtNPs-modified dendrimer-stabilized reduced graphene as electrode materials and heme/G-quadruplex-modified silica/multi-walled carbon nanotubes as signal probes to catalyze H_2O_2 reduction [84].

3.5 Others

Electrochemical-Chemical (EC) or Electrochemical-Chemical-Chemical (ECC) redox cycling are two efficient electrochemical signal amplification methods which have been widely used for the design of electrochemical biosensors [85-92]. For example, Akanda et al. developed an immunosensor based on the EC strategy of ALP-catalyzed hydrolysis of L-ascorbic acid phosphate (AAP) (Fig. 18) [85]. The produced L-ascorbic acid (AA) can be oxidized by the oxidized mediator and then regenerated by TCEP.

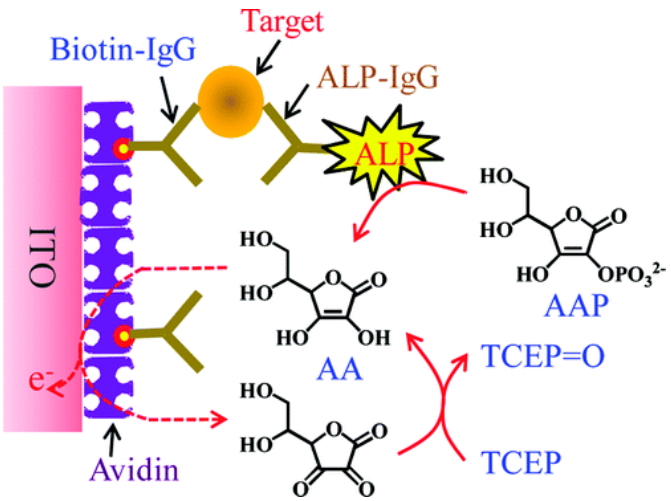

Figure 18. Schematic representation of an electrochemical immunosensor using the generation of AA by ALP and the redox cycling of AA by TCEP. Reprinted with permission from reference [85]. Copyright 2011 American Chemical Society.

MOFs are a type of porous materials assembled by metal/metal clusters and organic ligands. Metal nanoparticles and metal clusters-loaded MOFs can be used to catalyze and amplify the detection

signal [93]. Chen et al. reported the detection of PSA using three-dimensional porous Pt/Cu-MOFs. The antigen-antibody complex inhibited the catalytic reduction of H_2O_2 [94]. Dai et al. constructed an electrochemical immunosensor for PSA detection based on antibody-modified Co-MOF, in which the aminoimidazole composite framework component (Pd-NH_2-ZIF-67) was used as the carrier of PdNPs to catalyze the reduction of H_2O_2 [95]. Guo et al. reported the detection of CEA using silver clusters-loaded aptamer-modified Zr-MOF (Fig. 19) [96]. The linear range was 0.01 − 10 ng/mL.

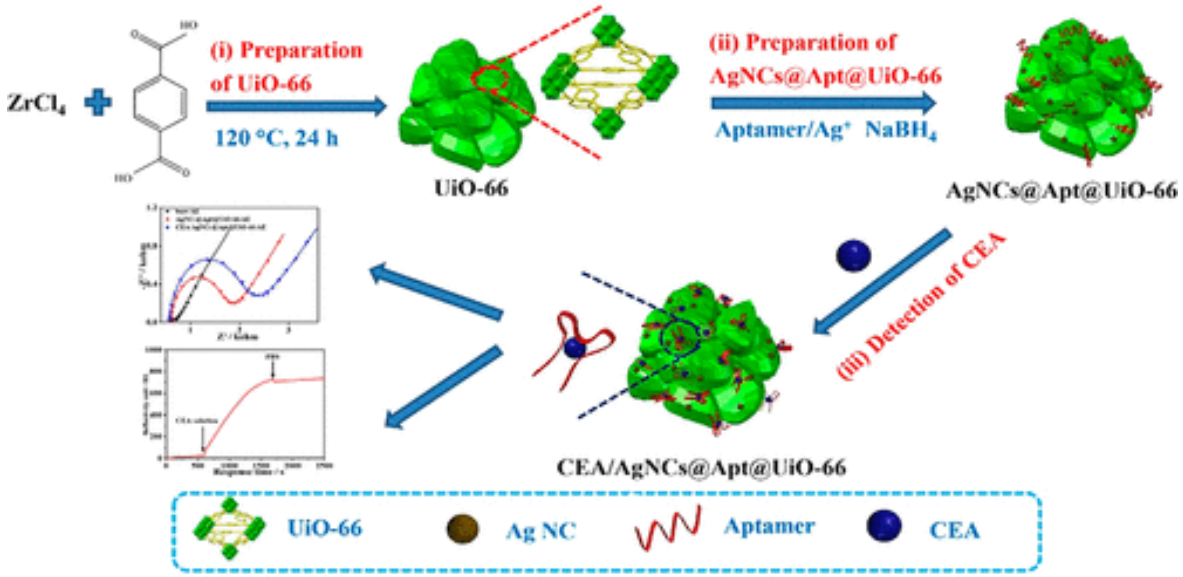

Figure 19. Schematic diagram of the AgNCs@Apt@UiO-66-based aptasensor for detecting CEA, including (i) preparation of UiO-66, (ii)preparation of AgNCs@Apt@UiO-66, and (iii) detection of CEA. Reprinted with permission from reference [96]. Copyright 2017 American Chemical Society.

4. CONCLUSION

In this paper, the methods for detecting cancer biomarkers by electrochemical signal amplification are reviewed. Although there are numerous electrochemical methods for early diagnosis of cancers by signal amplification, researchers still need to work more carefully to propose upgrading and reasonable strategies. Therefore, the teamwork among nanotechnologists, biotechnologists, clinicians and engineers to transform current diagnostic technologies into customized point-of-care diagnostic equipment will bring more encouraging work.

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