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Mini review

Recent Advances in Nanomaterials-Based Electrochemical Biosensors for MicroRNAs Detection

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MicroRNAs (miRNAs), a class of non-protein-coding and small RNAs, play a critical role in post-transcriptional regulation of gene expression, thus having been regarded as important biomarkers for many diseases. In the past decade, electrochemical biosensors have been employed widely for the highly sensitive and multiplexed detection of miRNAs. In particular, integrating into nanomaterials further improve the performances of the developed assays. This review mainly focuses on the achievement in the use of nanomaterials in electrochemical biosensors for facile and sensitive miRNAs detection, which will be useful toward understanding the methodology of assays and proposing more effective and practical electrochemical biosensors for miRNAs detection.

Keywords: Metal–organic frameworks; electrochemical biosensors; signal labels

1. INTRODUCTION

MicroRNAs (miRNAs) are a group of small non-coding, single-stranded RNAs (about 18–24 bases), which widely exist in intra and extra cellularly in plants, animals and humans [1]. According to previous reports, miRNAs play central roles in regulation of gene expressions in various biological processes, such as cell differentiation, proliferation and control of developmental timing, organ development and apoptosis. Furthermore, the expression level of miRNAs is closely relationship with the occurrence and development of diseases, including cancers, diabetes and heart diseases [2, 3]. MiRNAs have already been regarded as promising biomarkers for the prognosis, diagnosis and

treatment of different cancers. For instance, the specific miRNA-21 sequence, which is always observed to be over-expressed in the tumor tissues, especially in prostate, lung cancers and hepatocellular carcinoma, is an important biomarker for cancer diagnosis [4, 5]. However, designing of sensitive methods for miRNAs detection is very difficult due to their low abundance, extremely short lengths (17 ~ 28 nt) and the heterogeneity in the G-C contents. Therefore, there is an increasing need for fast, reliable, and cost-effective assays for miRNAs detection.

In early period of miRNAs analysis, reverse-transcription polymerase chain reaction (RTPCR), Northern blot and quantitative polymerase chain reaction (qPCR) are mainly used [6]. Nonetheless, tedious laboratory procedures, time consuming, and the lack of sensitivity have limited its practice application. In recent years, with the flourishing development of biotechnology and nanotechnology, many innovative detection techniques with different signal amplification strategy have been designed and applied for miRNAs detection, such as fluorescence (FL), electrochemiluminescence (ECL), surface-enhanced Raman spectroscopy (SERS), and mass spectrometry (MS) [7-10]. Among those methods, electrochemical assays show highly attractive potential in miRNAs detection due to the inexpensive instruments, sensitivity and low cost, especially for point-of-care diagnostics and multiplexed platforms [11, 12]. Because of their outstanding electrochemical properties, good biocompatibility, chemical stability and large surface area, nanomaterials including metal, metal oxide, carbon-based nanomaterials, are employed for miRNAs detection. Introduction of nanomaterials into electrochemical sensors will greatly improve sensitivity and selectivity in comparison with conventional sensors. In this review, we mainly provide an overview on electrochemical sensors for miRNAs detection based on nanomaterials in recent years, in which nanomaterials can be used as electrode materials, electrocatalysts, electroactive labels, separators and as carriers for signal amplification units.

2. NANOMATERIALS-BASED BIOSENSORS

2.1 Nanomaterials as electrode materials

The introduction of nanomaterials into the electrode as substrates could not only efficiently increase the specific biosensing surface and amounts of probe molecules, but also make immobilization of functional probes easier and more stable [13]. The performances of those sensors could be obviously enhanced. Due to their large surface area, easy functionalization and electrical conductivity, gold nanomaterials are always used as the electrode materials for sensitive detection of miRNAs [14-16]. For instance, in the work reported by Tang's group, gold nanoparticles (AuNPs) were first electrodeposited on the pretreated Au electrode in electrolyte solution containing $HAuCl_4$ (Figure 1A) [17]. The capture DNA probes were immobilized on the surface of the AuNPs/Au electrode through the strong Au-S interactions. The hybridization between the target miRNAs and the capture probes induced the change of drain-source current (I_{DS}) using organic electrochemical transistors. Furthermore, the performance of gold nanomaterials in biosensing was greatly depended on their size and morphology, and anisotropic and hierarchical gold nanostructures have attracted more research interests due to their higher surface areas and special shapes. In 2016, Wang's group reported

on a one-step and template-free synthesis method for making hierarchical flower-like gold nanostructures (HFGNs) on indium tin oxide (ITO) substrates for sensitive electrochemical sensing of miRNAs [18]. Shape-controlled gold nanostructures were electrodeposited on ITO without any templates assisted. The sizes and morphologies of gold nanostructures could be finely tuned by altering electrodeposition conditions, such as deposition potential, $HAuCl_4$ concentration, deposition time, and composition of electrolyte. Four typical gold nanostructures were chosen to investigate the dependence of detection performances on sizes and morphologies of gold nanostructures. The results showed that the HFGNs possess larger surface area and more suitable for electrochemical DNA/miRNA sensing.

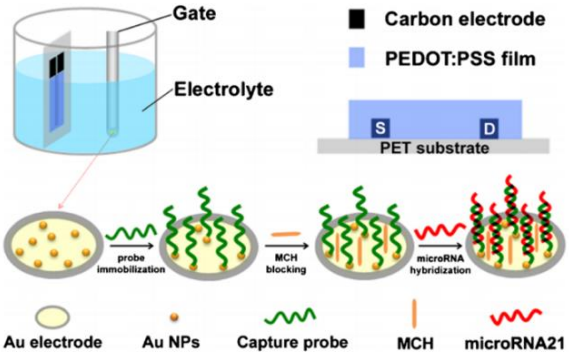

Figure 1. Schemes demonstrating the principle of microRNA21 biosensor based on OECTs. Reprinted with permission from [17]. Copyright 2018 Springer.

Graphene and graphene-like two-dimensional lamellar nanomaterials (such as molybdenum disulfide (MoS_2) and molybdenum carbide) are also widely used in the construction of electrochemical sensors for biomolecules because of the excellent electroconductivity and easy modification with abundant molecules and nanomaterials [19, 20]. As an-atom-thick planar sheet of sp^2-hybridized carbon atoms, graphene has attracted much attention in materials science and biotechnology from 2004. Graphene oxide (GO) and reduced graphene oxide (RGO) have many functional groups such as epoxy, hydroxyl and carboxyl on their basal plane and edges. They display substantially different electrochemical properties from that of pristine graphene, which is attributed to the edges and defects on carbon lattice and a variety of oxygenated groups. Pham's group reported an immunosensor for detection of miRNAs based on a conducting polymer/RGO-modified electrode (Figure 2A) [21]. The NH_2-modified DNA probes were covalently grafted immobilized on the surface of the modified electrode through EDC/NHS reaction and hybridized with miRNAs into dsDNA/RNA, increasing the faradic current. To further improve the properties of those two-dimensional nanomaterials, metal NPs can be decorated on the surface via in-situ growth or subsequent assembly [22-24]. Wang's group synthesized AuNPs-functionalized MoS_2 nanosheet with thionine (Thi) as reducing reagent (Figure 2B) [25]. Meanwhile, Thi on the surface of nanosheets could also be used as the signaling molecule to monitor DNA-RNA hybridization because of the influence of the formed dsDNA/RNA on the electron transfer.

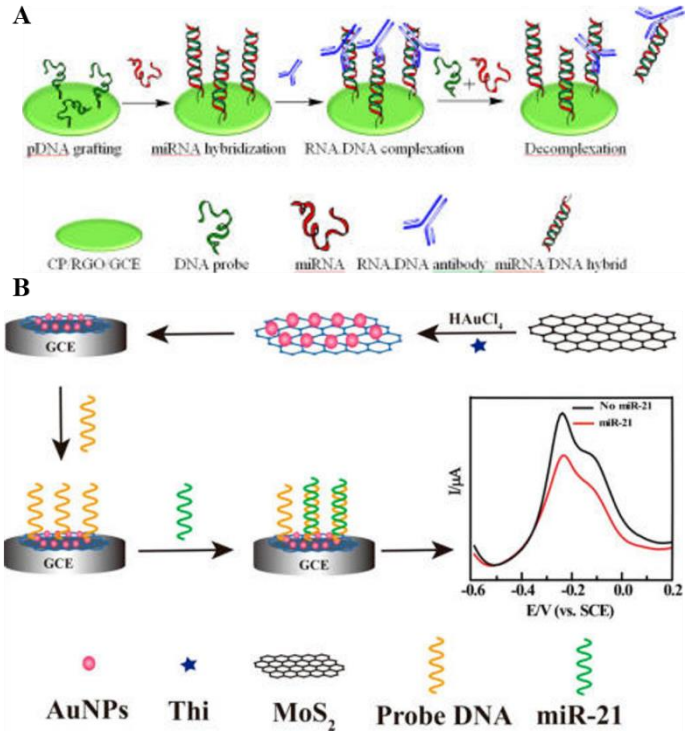

Figure 2. (A) Illustration of antibodies directed to RNA/DNA hybrids for miRNA detection based on graphene-composite electrodes. Reprinted with permission from [21]. Copyright 2013 American Chemical Society. (B) Schematic fabrication of electrochemical platform for miR-21 detection based on MoS_2 nanosheet modified with thionine and gold nanoparticles (MoS_2-Thi-AuNPs). Reprinted with permission from [25]. Copyright 2017 American Chemical Society.

As a typical one-dimensional nanomaterial, carbon nanotubes (CNTs) including multi-walled carbon nanotubes (MWCNTs) and single-walled carbon nanotubes (SWCNTs) have prosperous applications in biosensors due to their large surface area ratio size, excellent electron transfer ability, and easy of immobilization. In Mulchandani's group, SWCNTs were used as substrates to construct CNTs-FET transducer using glass carbon electrode functionalized with Carnation Italian ringspot virusp19 protein that has the highest affinity for 21−23 nt dsRNA (Figure 3A) [26].

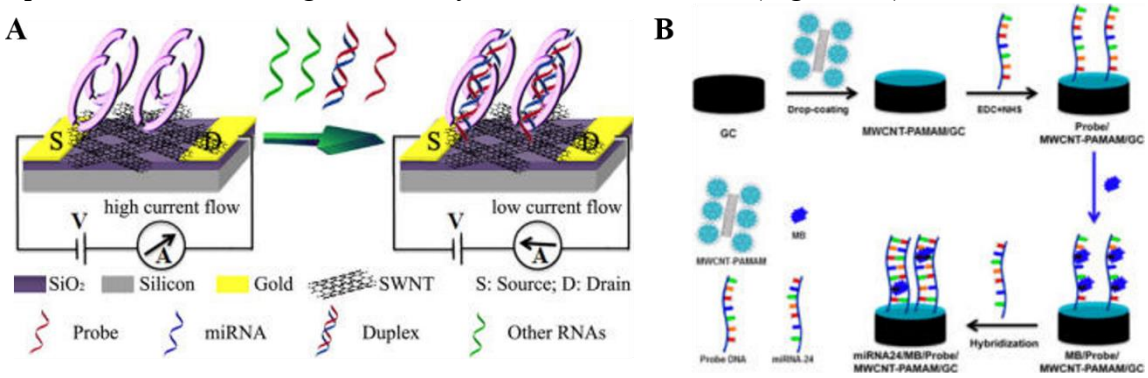

Figure 3. (A) Schematic of miRNA detection principle by the p-19 functionalized CNTs-FET nanobiosensor. Reprinted with permission from [26]. Copyright 2013 American Chemical Society. (B) Schemes demonstrating the principle of miRNA24 electrochemical biosensor based on the MWCNT-PAMAM hybrids. Reprinted with permission from [27]. Copyright 2015 American Chemical Society.

The interaction of the dsRNA to the protein generated a change in conductance of the CNTs. Like graphene, other nanomaterials or molecules can also be employed to decorate CNTs. In 2015, Tang's group employed the hybrid of polyamidoamine (PAMAM) dendrimer and MWCNT as the electrode substrate for miRNAs detection (Figure 3B) [27]. The use of MWCNT-PAMAM hybrids can enhance the number of captured DNA molecules on the surface and improve the sensitivity by measuring MB reduction signal.

2.2 Nanomaterials as electrocatalysts

In the early stage of biosensors, natural enzymes (such as horseradish peroxidase (HRP), alkaline phosphatase (ALP) or enzyme-like molecules are always used to amplify the recognition signal for sensitive detection. However, the intrinsic drawbacks including susceptibility to digestion by proteases and lose of the catalytic activity under extreme pH and high temperature hugely block the practical applications of enzymes. Thanks to the nanotechnology, many interesting NPs have been successfully designed, prepared and demonstrated to exhibit enzyme-like activities [28, 29]. Compared with natural enzymes, nanozymes possess various distinguish advantages, such as robustness to harsh environments, tunable catalytic activities, easy functionalization and mass production, which also can be employed as catalysts/electrocatalysts for developing electrochemical biosensors [30]. Zhang's group demonstrated that DNA-templated silver nanoclusters (AgNCs) possessed enzyme-mimic property for catalyzing H_2O_2 reduction and further used AgNCs for developing miRNAs biosensor (Figure 4A) [31]. In this work, Ag-NCs were *in situ* synthesized by using the recognition oligonucleotide probe as template. After the molecular beacon (MB) probe was immobilized on the surface of the electrode and hybridized with the Ag-NCs-labelled recognition probe, Ag-NCs were brought to the electrode surface and generated a detection signal, in response to H_2O_2 reduction. This method showed a linear relationship in the range from 10 nM to 100 fM and the limit of detection (LOD) was 67 fM. PdNPs and PtNPs also possess excellent electrocatalytic activity towards H_2O_2 to produce an enormous amplified electrochemical signal [32-34]. Yuan's group developed an electrochemical biosensor for sensitive detection of miRNA-155 by integrating PtNPs with catalyzed hairpin assembly target recycling and cascade electrocatalysis for signal amplification [34]. As shown in Figure 4B, porous TiO_2 nanosphere was first synthesized and subsequently decorated with PtNPs, DNA S1 and cytochrome *c* (Cyt *c*). After the catalyzed hairpin assembly target recycling, a large amount of alcohol oxidase (AOx) were brought close to TiO_2 nanosphere and numerous methylene blue (MB) were intercalated into the dsDNA. In the presence of ethanol, the cascade catalysis amplification reaction was initiated, resulting in the catalytic oxidation of ethanol to acetaldehyde by AOx and the generation of H_2O_2 by using dissolved O_2. The reduction of H_2O_2 was collaboratively catalyzed by Cyt c and PtNPs, and this method showed an extremely high sensitivity with a LOD of 0.35 fM for miRNA-155.

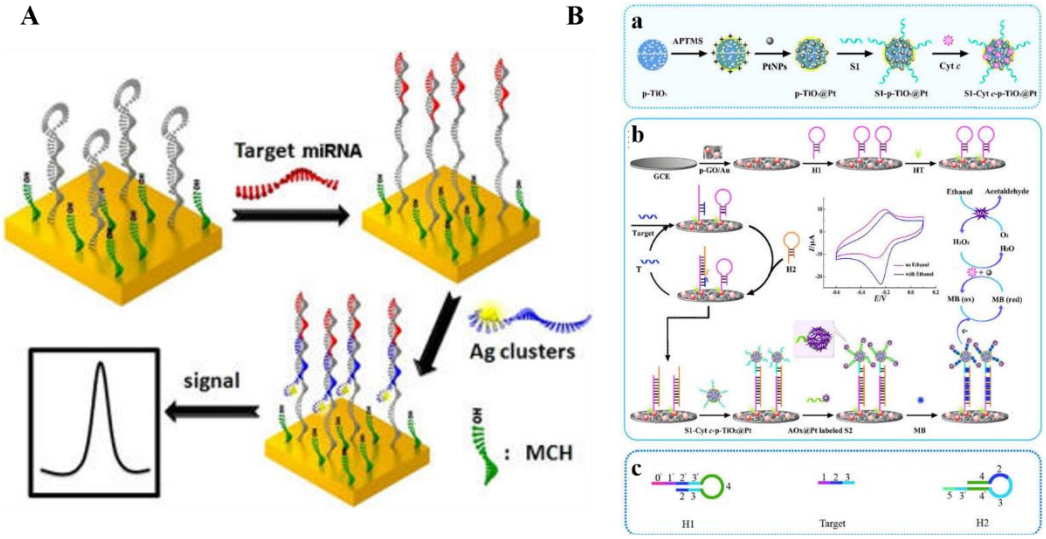

Figure 4. (A) Illustration of electrochemical detection of miRNA using oligonucleotide encapsulated Ag-NCs Reprinted with permission from [31]. Copyright 2012 American Chemical Society. (B) (a) The preparation process of the biobarcode conjugate labels. S1 and Cyt *c* are attached to the p-TiO_2@PtNPs. (b) Schematic representation of the proposed strategy for miRNA-155 detection. (c) Structures of H1, target, and H2. Reprinted with permission from [34]. Copyright 2015 American Chemical Society.

Metal oxide nanoparticles with electrocatalytic activity have also increasingly attracted intensive attention in biosensors and diagnoses. In 2006, OsO_2 nanoparticles were prepared and used to label periodate-treated miRNA by Gao and co-workers (Figure 5A) [35]. OsO_2 nanoparticles can reduce the oxidation overpotential and catalyze the oxidation of hydrazine. Then, Gao's group reported a highly sensitive miRNAs biosensor by employing ruthenium oxide nanoparticle (RuO_2 NP) as catalysts for initiating the polymerization of 3,30-dimethoxybenzidine (DB) with dsDNA/miRNA as the template for the deposition of an insulating poly(3,30-dimethoxybenzidine) (PDB) film (Figure 5B) [36]. As described in this work, after the extraction, the enriched target miRNAs were further labeled with RuO_2 NPs and then hybridized with the capture probes (CPs) on a gold electrode. In the presence of a mixture of DB/H_2O_2 in pH 5.0 acetate buffer, the produced insulating PDB film could significantly increase Rct, linearly related with the concentration of the target miRNAs ranging from 6.0 fM to 2.0 pM. They also used RuO_2 NPs to catalyze the polymerization of aniline into redox-active material polyaniline for miRNAs detection with peptide nucleic acid as the recognition probe [37]. Recently, Cu and Co doped CeO_2 nanospheres (CuCo-CeO_2 NSs) were reported to exhibit excellent electrocatalytic ability towards H_2O_2 decomposition and were utilized to develop dual-mode biosensor for miRNAs (Figure 5C) [38]. In this study, CuCo-CeO_2 NSs with redox property could generate an enhanced differential pulse-voltammetry (DPV) signal in the presence of H_2O_2. On the other hand, similar to RuO_2 NPs, CuCo-CeO_2 NSs can catalyze the oxidation of 3,3-diaminobenzidine (DAB) into nonconductive insoluble precipitates (IPs), resulting in an amplified electrochemical-impedimetric spectroscopy (EIS) signal. In addition, magnetic nanoparticles possess high electrocatalytic activity towards electroactive molecules such as toluidine blue and ruthenium hexaammine(III), and have been used as electrocatalysts for miRNAs detection [39-41].

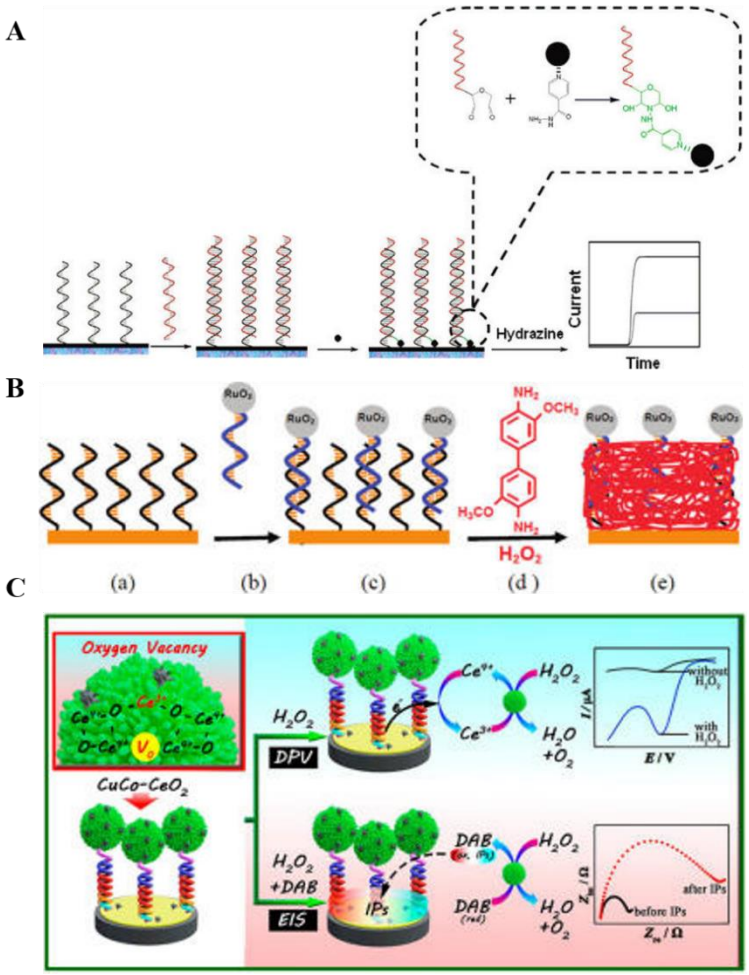

Figure 5. (A) Schematic illustration of miRNA assay using electrocatalytic OsO_2 nanoparticles. Reprinted with permission from [35]. Copyright 2006 American Chemical Society. (B) Schematic illustration of the miRNA biosensor based on RuO_2 NP-catalyzed miRNA-templated deposition of a thin PBD insulating film. Reprinted with permission from [36]. Copyright 2011 American Chemical Society. (C) Schematic illustration of dual-mode sensing principle for miRNA-141 determination. Reprinted with permission from [38]. Copyright 2019 American Chemical Society.

2.4 Nanomaterials as electroactive labels

Metal NPs or NCs, especially Ag and Cu, composed of several to tens of thousands of metal ions, are widely applied to instruct optical probes for fluorescent and colorimetric assays [42-44]. Nevertheless, colorimetric assays are easily influenced by high concentration salts and coexistences in samples, and the fluorescence of NCs sometimes cannot last long time. Interestingly, those NPs can be used in electrochemical sensors by the determination of metal ions released from the dissolution of NPs, avoiding the above problems. Sometimes, NPs with redox properties can be directly detected as electroactive labels by the differential pulse voltammetry (DPV) after acid dissolution. Due to the solid-state Ag/AgCl reaction, AgNPs and AgNCs can provide sharp silver stripping peaks [45-49]. For example, Miao's group developed electrochemical biosensors for detection of miRNAs based on AgNPs [50]. As shown in Figure 6A, tetrahedral DNA nanostructures were first prepared and

immobilized on the electrode. miRNA-21 could open the loop of tetrahedron A and facilitate the hybridization between the stem part and the AgNPs-labeled signal probe with the aid of Klenow fragment [51]. This method had high sensitivity with the low LOD down to 0.4 fM. Yuan's group described a label-free and sensitive assay for electrochemical detection of miRNA-199a based on electroactive AgNCs (Figure 6B) [52]. In this paper, after the subsequent initiation of the targetassisted polymerization nicking reaction (TAPNR) amplification and the hybridization chain reaction (HCR) amplification, numerous C-rich loop DNA templates in dsDNA polymer occurred on the surface of electrode and were further used to *in situ* synthesize AgNCs in the presence of $AgNO_3$ and sodium borohydride, resulting in an amplified DPV response. Similar to Ag nanomaterials, CuNPs and CuNCs can also be employed for electrochemical biosensors for miRNAs detection with the acid dissolution [53]. For example, target miRNAs could hybridize with the hairpin probe DNA and amino-tagged DNA, making the electrode functionalized with amino group (Figure 6C). Through the specific reaction between amino and carboxyl groups, apoferritin-encapsulated Cu nanoparticles (Cu-apoferritin) were bound to the electrode. An electrochemical oxidation peak was observed after Cu was released from apoferritin by adjusting the pH [54]. Recently, *in situ* synthesis of CuNPs using dsNDA as templates were integrating with two cycles with the aid of T7 exonuclease (exo) for miRNA-triggering cascade signal amplification by Miao's group (Figure 6D) [55]. The ultrasensitive electrochemical biosensor displayed a wide linear range from 10^{-16} to 10^{-13} M with a LOD as low as 4.5×10^{-17} M.

In contrast, AuNPs have more stable electrochemical properties and weak electroactivity. However, when coating with electrochemically active molecules (such as ferrocene, thionine and doxorubicin), AuNPs can possess excellent electroactivity [56-58]. For example, Xia and Co-workers developed a label-free and sensitive strategy for miRNAs detection based on the formation of boronate ester bonds and the dual-amplification of gold nanoparticles (Figure 7) [59]. They first investigated the reaction between the phenylboronic acid and cis-diol at the end of miRNAs, and utilized this specific interaction to grasp 4-mercaptophenylboronic acid (MBA)-capped AuNPs (MBA-AuNPs). Then electrochemically active dopamine (DA)-capped AuNPs (DA-AuNPs) were added and then attached by the anchored MBA-AuNPs via the interaction of boronic acids and DA, leading to numerous electrochemically active DA around the electrode. The electron transfer between DA and the electrode was facilitated by the net-work structure of AuNPs.

Like metal NPs, semiconductor quantum dots (QDs) have also been widely used as electroactive labels for the multiple analysis of DNA, protein and miRNAs, relying on the different composition of QDs [60-63]. For example, Liu's group developed an enzyme-free bioassay for miRNA-21 detection based on CdTe QDs and target-triggered hybridization chain reaction (Figure 7A) [64]. The attached QDs were dissolved with HNO_3 to release Cd^{2+}, which is determined by anodic stripping voltammetry for quantifying the concentration of microRNA-21. Besides, nanomaterials without electroactive properties can load various redox species for electrochemical detection of miRNAs, such as magnetic nanoparticles, MWCNTs, and graphene oxide [65-67]. For instance, Zhu's group prepared Cd^{2+}-loading titanium phosphate NPs as electrochemical signal reporters for miRNAs detection (Figure 7B) [68].

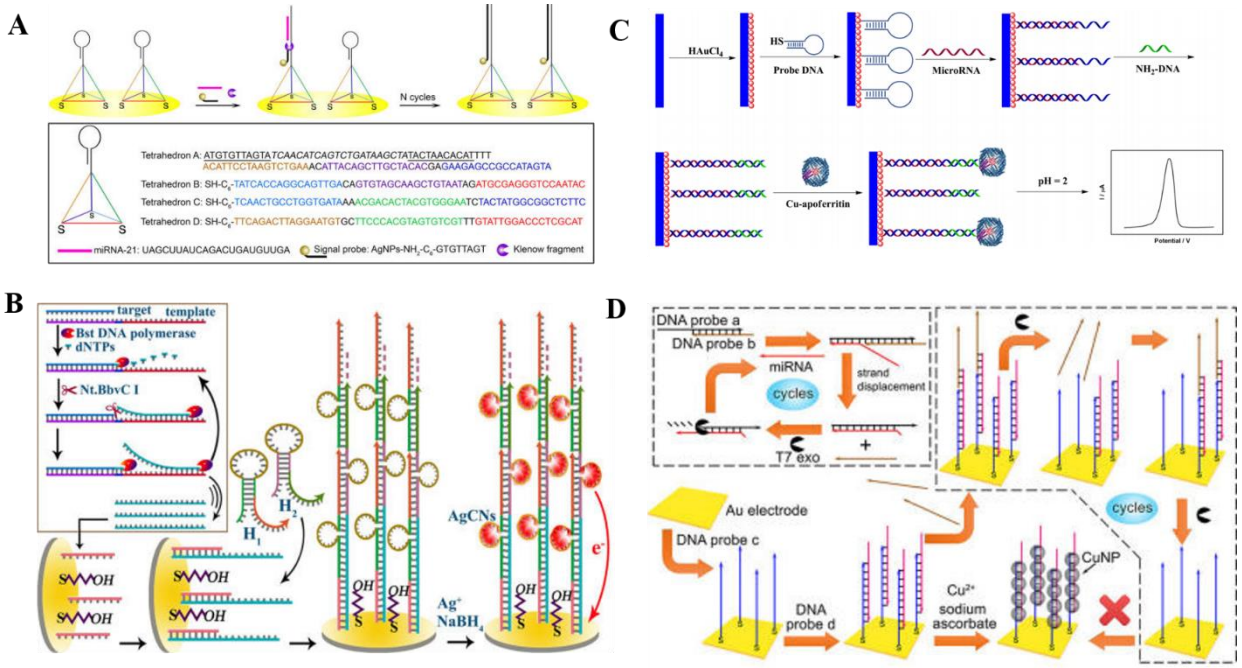

Figure 6. (A) Schematic illustration of the tetrahedral DNA nanostructure-based microRNA biosensor. Reprinted with permission from [51]. Copyright 2015 American Chemical Society. (B) Schematic illustration of ultrasensitive and label-free electrochemical detection of miRNA-199a based on in situ generated AgNCs by coupling TAPNR with HCR Amplifications. Reprinted with permission from [52]. Copyright 2015 American Chemical Society. (C) Schematic illustration of the biosensor fabrication process for miRNA-159a determination. Reprinted with permission from [54]. Copyright 2014 Springer. (D) Illustration of electrochemical biosensor for miRNA analysis based on T7 exo and DNA-templated CuNPs. Reprinted with permission from [55]. Copyright 2018 American Chemical Society.

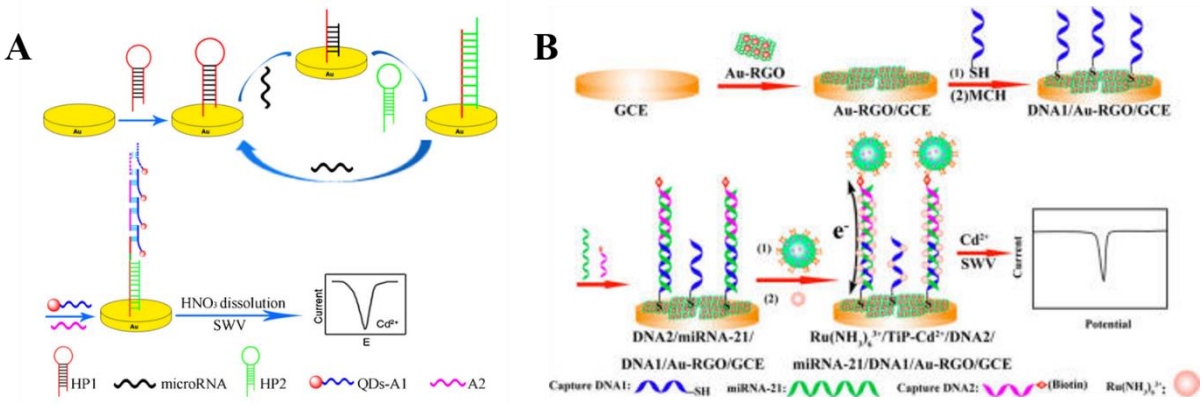

Figure 7. (A) Scheme of the electrochemical biosensor based on CdTe QDs and target triggered hybridization chain reaction amplification strategy. Reprinted with permission from [64]. Copyright 2016 Springer. (B) Schematic illustration of the stepwise sensor construction process based on Cd^{2+}-loading titanium phosphate NPs. Reprinted with permission from [68]. Copyright 2015 American Chemical Society.

Mesoporous silica nanoparticles (MSNs) have also been applied to develop electrochemical method for the sensitive detection of miRNAs, in which an electroactive reporter molecule was

entrapped into the pores and blocked by ssDNA as capture probe through the electrostatic interaction between negative ssDNA and positive MSNs. When the capture probe hybridized with target DNA/miRNA, the capture probes left away from the silica, and the entrapped signal molecule was released, making the massive signal amplification. Li's group designed an ultrasensitive ratiometric electrochemical sensor for miRNA detection by using MSNs to load the electroactive probes (Figure 8A) [69]. In this research, $[Ru(NH_3)_6]^{3+}$ was entrapped in the pores of MSNs and ssDNA complementary to the target miRNA. Once the target miRNA was captured and hybridized into dsDNA/RNA, $[Ru(NH_3)_6]^{3+}$ was released and electroreduced into $[Ru(NH_3)_6]^{2+}$, which was then chemically oxidized back to $[Ru(NH_3)_6]^{2+}$ by $[Fe(CN)_6]^{3-}$. The consumed $[Fe(CN)_6]^{3-}$ and liberated $[Ru(NH_3)_6]^{3+}$ produced an significant ratiometric signal. They also developed a biofuel cell (BFC)-based self-powered biosensors for miRNAs detection by employing MSNs to encapsulate the cathodic electron acceptor $[Fe(CN)_6]^{3-}$ [70]. Further, Wang's group combined electroactive cargo-loaded MSNs with λ-exonuclease (λ-Exo) cleavage reaction-assisted target recycling for a low background biosensing [71]. As shown in Figure 8B, target thymidine kinase 1 (TK1) mRNA could trigger the release of methylene blue (MB) and produce a lot of trigger DNA to initiate the hybridization chain reaction in the surface of the electrode. The released MB could intercalate into the dsDNA polymers, generating a significant electrochemical response.

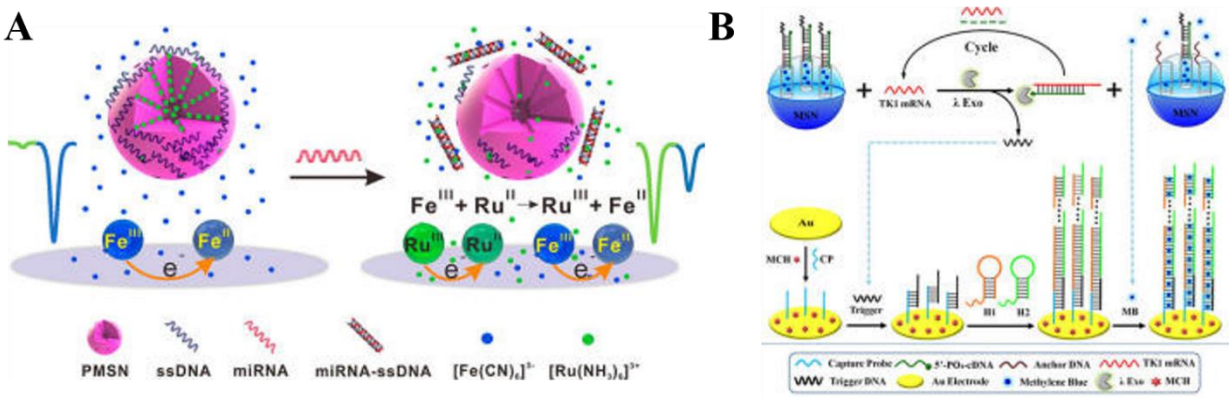

Figure 8. (A) Schematic principle of the ratiometric homogeneous electrochemical miRNA biosensor. Reprinted with permission from [69]. Copyright 2017 American Chemical Society. (B) Low background cascade signal amplification electrochemical sensing platform for mRNA quantification by target-activated HCR and electroactive cargo release. Reprinted with permission from [71]. Copyright 2018 American Chemical Society.

Magnetic micro- or nanoparticles (MNPs) have been broadly used to pre-concentrate target of interest from the samples in sensing, because MNPs can be easily manipulated with a magnetic electrode within a few seconds [72-74]. For instance, Wang and co-workers have developed multiplexed electrochemical assays of miRNAs with AuNP-coated magnetic microbeads (AuNP-MMBs) (Figure 9A) [75]. In this paper, AuNP-MMBs were first modified with two thiolated hairpin oligonucleotide (ODN) probes. Target miRNA-182 and miRNA-381 could hybridize with the hairpin probes on AuNP-MMBs, inducing the conformational change. The diblock ODN-modified AuNPs

could further hybridize with the corresponding miRNAs. Then, the hybrid of AuNP-MMBs and diblock ODN-modified-AuNPs were collected onto a magnetic electrode for electrochemical detection. By measuring the oxidation peak currents of MB and Fc moieties residing on the diblock ODNs, miRNA-182 and miRNA-381 were simultaneously detected.

Trau's group introduced an amplification-free electrochemical detection on screen-printed gold electrodes based on enhanced affinity between polyadenylated miRNAs and bare gold electrode (Figure 9B) [76]. Streptavidin-coated MMBs were first functionalized by biotin-labeled capture probes and further hybridized with miRNA targets. The captured miRNAs were magnetically purified and released from the capture probes through heating. Next, miRNAs were extended on 3' ends using poly A polymerase enzyme. The poly(A) extension endowed miRNAs with high adsorption efficiency onto gold electrode surfaces, leading to a change of Faradaic current signal.

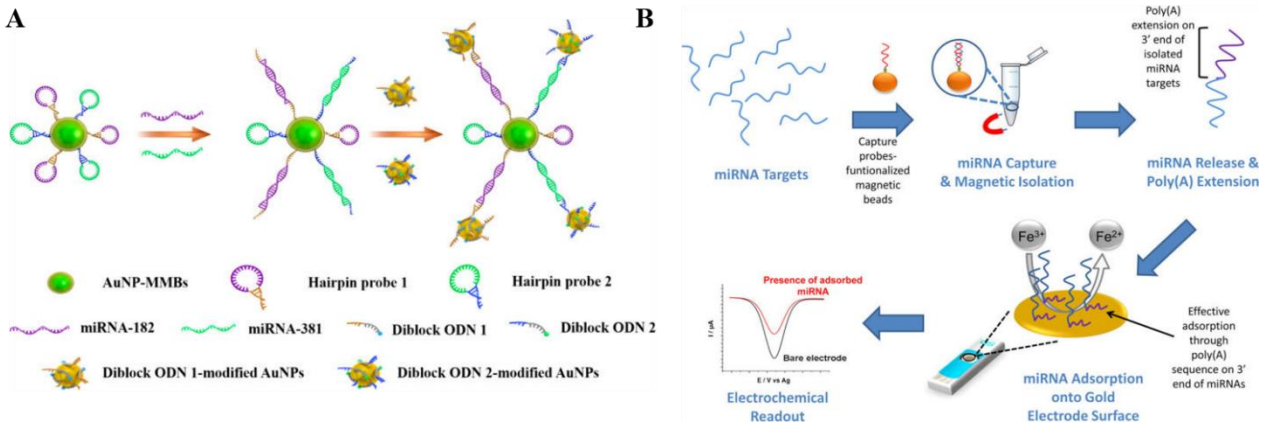

Figure 9. (A) Schematic showing the simultaneous electrochemical detection of miRNA-182 and miRNA-381 via the conjugates of AuNP-MMBs and diblock ODN-modified AuNPs. Reprinted with permission from [75]. Copyright 2017 American Chemical Society. (B) Schematic representation of assay based on an amplification-free miRNA assay based on elevated affinity interaction between polyadenylated miRNA and bare gold electrode. Reprinted with permission from [76]. Copyright 2016 American Chemical Society.

2.4 Nanomaterials as electroactive labels

Nowadays, due to the high surface area and ease of functionalization, nanomaterials have been widely employed to carry complementary binding probes and signal amplification units, such as enzymes and DNAzymes. Therefore, the detection sensitivity is significantly improved. Recently, AuNPs have been received much attention due to its well properties of easy preparation, low cost and good biocompatibility. Liu's group reported a label-free electrochemical genosensor for miRNAs detection with the triple signal amplification of AuNPs, alkaline phosphatase (ALP) and p-aminophenol (p-AP) redox cycling [77]. After miRNAs were captured by DNA probes in the surface of the electrode, 3-aminophenylboronic acid (APBA)/biotin-modified multifunctional AuNPs could be immobilized through the formation of boronate ester covalent bonds. Then, numerous streptavidin-conjugated alkaline phosphatase (SA–ALP) molecules were captured via the biotin–streptavidin interactions. ALP could converse the substrate of 4-aminophenylphosphate (p-APP) to p-AP. The

produced p-AP could be electrooxidized on the electrode and cycled by a chemical reducing reagent, causing an increase in the anodic current. Besides, AuNPs can also carry hemin/G-quadruplet DNAenzyme and horseradish peroxidase (HRP), which can catalyze the decomposition of H_2O_2 by oxidation of hydroquinone [78-80].

4. CONCLUSION

MiRNAs play a significant role in control of the cell cycle and exist in the developmental and physiological processes of human body. Since the discovery of miRNAs in 1990s and the first electrochemical biosensor for miRNAs in 2006, many elaborate detection methods and instruments have been designed by worldwide research groups, which help us deep know the relationship between human healthy and miRNAs. In this review, we have summarized the rapid progress of nanomaterials-based miRNAs detection that appeared in recent years. Different elegant sensing strategies were also summarized. Thanks to the huge progress of nanotechnology, nanomaterials with unique physical and chemical advantages have been prepared and fully exploited for electrochemical miRNA biosensors. However, more convenient, rapid, and efficient miRNAs biosensensors are still necessary and urgent. There is a huge gap between research in lab and putting methods into practice.

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