A Combinational Approach for More Efficient miRNA Biosensing

Cheolho Lee¹,*

¹Department of Chemical and Biological Engineering, Seokyeong University, Seoul, Korea

Abstract: MicroRNAs, short single-stranded noncoding RNAs ranging in length from 18 ~ 24 bp, are found in all kingdoms of eukaryotes and even viruses. It was found that miRNAs are involved in a variety of biological processes, and their intracellular aberrant expression is related to diseases and abnormalities in the immune system. Since then, it has been considered essential to develop an efficient miRNA detection system. In this review, the limitations of traditional scheme-based miRNA detection methods are compared and analyzed. In particular, nucleic acid amplification-based miRNA detection methods and nanomaterial-based miRNA detection methods, which are widely used as a biosensing platform because of various features and advantages, such as high sensitivity, specificity, and simplicity, are analyzed. Based on this analysis, the latest examples of a combination of the advantages of nucleic acid amplification and those of nanomaterials are examined to suggest the characteristics of the next-generation miRNA biosensing.

Keywords: miRNA detection system, biosensing platform, next-generation miRNA biosensing, miRNAs, nanomaterial-based miRNA, nucleic acid amplification.

1. INTRODUCTION

MicroRNAs are short single-stranded noncoding RNAs ranging from 18 ~ 24 bp in length. Since miRNA was first reported by Professor Victor Ambros and his research team at Harvard University in 1993, about 15,000 types have been found so far in all eukaryotic cells and viruses [1]. Together with ribozymes having enzymatic activity, they fall in the 4th group and are genetically well conserved [2-4]. It is known that miRNAs control the flow of multidimensional genetic information between the nucleus and cytoplasm and are involved in a variety of biological phenomena, including early development, cell differentiation, cell proliferation, cell death, developmental timing, immune response, insulin secretion, and neurotransmitter synthesis [5-10].

It has already been revealed that the aberrant expression of miRNAs is related to human diseases, genetic disorders, and immune system abnormalities [11-18]. Particularly, many studies have proved that miRNAs play a role in the occurrence, progress, and metastasis of most tumors [12, 19-21]. For instance, miR-143 or miR-145 has low expression in more than 80% of cancer cells [22], whereas miR-21 or miR-31 has over expression in more than 80% of cancer cells [23, 24]. As such, miRNAs are an excellent biomarker to evaluate cancer diagnosis, prognosis and classification, development, staging and progress, and reactions to medications [25-28].

In the meantime, miRNAs are known to work as oncomiRs or tsmiRs [20, 29-31]. Most miRNAs present the features of one of them. However, just as miR-125b, they show the features of oncomiR or tsmiR, depending on the cancer type [28]. It means that miRNAs have the potential as molecular therapeutic agents [32]. In reality, a molecular therapeutic agent targeting miRNAs contributed to creating the new term ‘theragnosis,’ implying diagnosis and treatment.

It is essential to develop an efficient detection system for miRNAs and their target sequences not only in the basic research of life science but in the diagnosis and treatment of diseases [33]. However, miRNAs are small and feature a diverse copy number in cells from 50,000 copies to several thousand in a small fraction (about 1% of total RNAs), exhibiting sequence similarity within miRNA family members [31, 34-36]. Such features are obstacles to the development of an efficient miRNA detection system. In particular, they can become obstacles to in situ detection or in vivo imaging because of background signals and the problem of noise.

Up to now, there have been many reviews on miRNA biosensing [37-44]. Since this area evolves fast, it is necessary to update the existing knowledge. To meet the requirements of the next-generation miRNA biosensing, it is necessary to carefully consider the combinational cases with the advantages of each one.

In this review, the advantages and disadvantages of traditional methods and miRNA detection methods in current use are compared and analyzed. In particular, nucleic acid amplification-based miRNA detection methods and nanomaterial-based miRNA detection methods, which are widely used as biosensing platforms because of various features and advantages, such as high sensitivity, specificity, and simplicity, are also analyzed. Based on this analysis, the latest examples of a combination of advantages of nucleic acid amplifi-
Table 1. Advantages and disadvantages of conventional miRNA detection methods.

| Methods          | Advantages                                                                 | Disadvantages                                                                 | Sensitivity  | Refs. |
|------------------|----------------------------------------------------------------------------|-------------------------------------------------------------------------------|--------------|-------|
| **Hybridization-Based Methods** |                                                                             |                                                                               |              |       |
| Northern blotting | • Low-tech  
• Cheap  
• Semi-quantitative  
• Novel and previously unidentified miRNAs are often identified | • Hazardous labeling  
• Low sensitivity  
• Time-consuming  
• Low-throughput  
• Requiring a large amount of total RNA  
• Requiring equipment | nM ~ fM       | [49, 50]|
| Microarrays      | • High throughput  
• Less expensive  
• miRNA profiling possible  
• Multiplexed miRNA analysis possible | • Selective performance  
• Lower specificity than qRT-PCR  
• Requiring equipment  
• Requiring further validation to quantify the expression more accurately | fM ~ pM       | [59, 60]|
| In situ hybridization | • able to visualize miRNA levels in cell or tissue | • Low throughput  
• Closed-ended* | -             | [48, 58]|
| **Amplification-based** |                                                                             |                                                                               |              |       |
| qRT-PCR          | • Sensitivity  
• Moderate throughput  
• Easy to perform  
• Quantification | • Low throughput  
• Costly  
• Cannot identify novel miRNAs  
• Requiring equipment | nM ~ fM       | [46, 55, 56]|

Note: *Open-ended methods enable the detection of new miRNA genes, whereas closed-ended methods assay a predetermined set of miRNAs.

2. CONVENTIONAL miRNA DETECTION METHODS

Conventional techniques used for miRNA detection are classified into nucleic acid hybridization-based detection techniques, such as northern blotting and microarray, or amplification-based detection techniques, such as PCR and qRT-PCR [45-47]. As summarized in Table 1, these techniques have their own advantages and disadvantages. They are used together to supplement each one's disadvantages (rechecking of the expression of miRNAs found by the microarray technique) [45, 48].

miRNAs are small and have high sequence homology among miRNA family members, and their content is very low in cells or tissues, which leads to reduced detection sensitivity and specificity. Up to now, a number of reports have been shown various improvements in the Northern blotting technique [49-51]. Most of them primarily differ in the labeling and design of the probes used to detect miRNA. Digoxigenin (DIG)-labeling system has been widely used due to several advantages, such as high sensitivity, short exposure time, long shelf life, and increased safety. Ramkissoon et al. [52] reported the nonisotopic detection of microRNA using digoxigenin-labeled RNA probes and demonstrated that the DIG-labeled RNA probe was equally as sensitive as 32P-labeled probes in detecting miRNA quantities.

On the other hand, Chen et al. [53] reported a novel microRNA quantification method using stem-loop RT followed by TaqMan PCR analysis. Like standard TaqMan gene expression assays, TaqMan miRNA analysis exhibited a dynamic range of 7 orders of magnitude. After that, modified versions of the TaqMan assay [54, 55] and SYBR Green-based qRT-PCR miRNA assays [54, 56] have also been developed.

In situ hybridization (ISH) is one of the potent tools for providing miRNA spatial expression profiles in histological sections [48]. Since Politz et al. [57] reported the intracellular localization of miRNA-206 during differentiation in a single rat myogenic cells by combining an LNA-based ISH system with high-resolution imaging microscopy, various modified versions were developed to improve the detection efficiency of the system. Lu and Tsourkas [58] developed an LNA-based ISH system with enzyme-labeled fluorescence, which exhibited 40 times brighter signals than probes directly labeled with fluorophores. The advantages and disadvantages of conventional miRNA detection methods are summarized in Table 1 [59, 60].

3. miRNA DETECTION BASED ON NUCLEIC ACID AMPLIFICATION

There are limitations in detecting miRNAs with high sensitivity by traditional methods like PCR since miRNAs are small and have high sequence homology, and their content is low in cells or tissues. For the highly sensitive and efficient detection of miRNAs, it is necessary to make a smart...
probe design and establish a highly efficient signal readout strategy considering the features of miRNAs. Recently, various signal amplification-based miRNA detection methods have been developed. These are classified into enzyme-based methods, including IEA, RCA, DSN, and LCR, and enzyme-free methods, including HCR and CHA [61]. This section analyzes their principles, strengths, and weaknesses based on examples.

3.1. Isothermal Exponential Amplification

IEA features good signal amplification efficiency, high sensitivity, and cost-effectiveness, making real-time assays possible in some cases. This method does not need special enzymes like nicking endonuclease or complicated equipment [62]. However, it has some weaknesses, such as complex probe design, high cost for the use of multiple enzymes, and high errors made by complex order of reactions [63-65].

Li et al. [66] developed an ultrasensitive biosensor with one-step loop-mediated isothermal amplification (LAMP) using Bst DNA polymerase. It exerts multiple enzymatic activities, such as DNA polymerase activity, RNA polymerase activity (using a DNA template), and strand displacement activity. Taking advantage of these activities, the loop-stem-like extension products are generated through several cycles of strand extension and detected using SYBR Green I. The system shows ultrasensitivity with LOD at the amol level. Duan et al. [64] also reported an ultrasensitive biosensor for detecting microRNAs at the single-cell level based on a quadratic enzymatic amplification strategy. The biosensor exhibited ultrasensitivity for miRNA-21 with detection limits of 10 fM at 37°C and 1 nM at 4°C. However, the method is costly due to the requirement of three enzymes for the amplification process, and the requirement of laborious steps for miRNA isolation is still troublesome.

Recently, Ma et al. [67] reported a simple, rapid, and sensitive fluorescent method for label-free detection of low-abundance microRNAs based on isothermal helicase-dependent amplification. The target miRNA may specifically hybridize to the 3′-terminus of the linear probe to form a DNA-miRNA heteroduplex, protecting the probe from exonuclease I digestion. The remaining probes may be subsequently amplified by helicase-dependent amplification, generating an ultrahigh fluorescence signal within 30 min. The assay was found to be very sensitive with a low detection limit of 12.8 fM and a large dynamical range from 100 fM to 10 nM.

3.2. Exponential Amplification Reaction

EXPAR is an IEA technique in combination with nicking, endonuclease reaction, and polymerase strand extension. It features high sensitivity, high amplification efficiency, and rapid amplification kinetics [62, 63].

Wang et al. [68] and Xu et al. [69] designed a new template for EXPAR based on the conventional EXPAR, which contains two nicking endonuclease recognition sites through the addition of the sequence complementary to the G-quadruplex DNAzyme. Following polymerization using the new template, the EXPAR products included replicated DNA strands complementary to the target miRNA and the signal molecule of horseradish peroxidase (HRP)-mimicking G-quadruplex DNAzyme. The DNAzyme self-assembled into a G-quadruplex/hemin complex, which produced the color and chemiluminescence signal by their own catalytic reaction. The system could detect the target miRNA quantitatively in a large dynamic range from 1 fM to 100 nM.

3.3. Rolling Cycle Amplification

RCA is one of the most widely used IEA techniques. It features good signal amplification efficiency and combinations with various signal techniques, such as fluorescence, electrochemistry, and SERS. Furthermore, in RCA, the short sequence of miRNAs can be used as an ideal template for the early ligation reaction necessary for RCA, and in the initial step of RCA reaction, it can be used as a primer directly. However, RCA depends on strong strand displacement polymerase activity of Phi 29 DNA polymerase and 3′→5′ exoribonuclease activity, so template-independent ligation can lead to nonspecific amplification, which can reduce a background problem and specificity [70, 71].

Since a miRNA detection system based on padlock probes and rolling circle amplification was reported by Jonstrup et al. [72], some modified RCA-based procedures with improved specificity and efficiency have been developed [73]. Wen et al. [74] developed an integrated RCA for the sensitive detection of miRNA by combining nicking enzyme reaction and DNAzyme signal amplification. The RCA reaction was first triggered by hybridizing the target miRNA with a padlock DNA template designed with the specific recognition sequence of a nicking endonuclease. The long RCA products further initiated the nicking endonuclease signal amplification, generating a large amount of cleaved DNA products. Finally, hemin was added to form DNAzymes, catalyzing the oxidation reaction of colorless 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS±) into green ABTS± as a readout signal. The signal provided simple colorimetric detection of the target miRNA. Moreover, the system showed remarkable sensitivity with a detection limit as low as 2 aM.

Liu et al. [75] reported the linear rolling circle reaction (LRCA) for the sensitive detection of miRNA using the specifically circularized padlock probe; the probe first induced the linear rolling circle reaction (LRCA) under isothermal conditions. Next, multiple padlock probes hybridized to multiple sites of the LRCA DNA product and nicking endonuclease recognized the sites and cut DNA products in the double-strand hybrids, resulting in multiple short DNA products as new primers to initiate multiple RCA. Therefore, LRCA and cleavage were repeated continuously in cycles, resulting in exponentially amplified products. The system was ultrasound sensitive with a detection limit of 0.24 zmol.

Zhang et al. [76] developed a homogeneous and label-free method for the sensitive detection of miRNA by combining nicking endonuclease-based strand displacement amplification (SDA) with hyperbranched RCA (HRCA).
The system showed a large dynamic range of 5 orders of magnitude from 0.1 pM to 10 nM with a detection limit of 0.18 pM.

3.4. Nuclease-assisted Amplification

Reactions of IEA and RCA are performed in multiple steps, making them complex and creating both side effects and background problems. To solve these problems, a variety of signal amplification methods based on nuclease-assisted amplification (NAA) were developed. NAA makes use of enzyme-specific reactions so that it is fundamentally different from the methods based on primer extensions, such as RCA and EXPAR. Therefore, NAA features good sensitivity, high specificity, and fewer side effects.

Duplex-specific nuclease (DSN) is a specific endonuclease that can only cleave double-stranded DNA and DNA in DNA-RNA hybrid duplexes and differentiate between one-nucleotide variations in DNA [77]. The DSN-based miRNA detection system is simple and convenient. DSN’s selective cleavage of the DNA strand in DNA/RNA hybrids allows a target miRNA to recycle continuously and consequently amplify the signal. In fact, Yin et al. [78] and Zhao et al. [79] developed a simple and efficient one-step multiplexed fluorescence detection of microRNAs based on DSN signal amplification and RNA amplification strategy based on the combination of 10–23 DNAzyme, the G-quadruplex hydrogen peroxidase, and strand displacement, respectively.

Meanwhile, TaqMan probes, molecular beacons, and nanoparticles have been used in DSN-based signal amplification for the simultaneously multiplexed detection of multiple miRNAs. Yin et al. [78] designed multiple TaqMan probes complementary to different target miRNAs to perform the DSN-assisted amplification reaction, and Lin et al. [80] synthesized a 2-OMe-RNA modified molecular beacon probe and developed a target recycling amplification method based on DSN. Recently, Ma et al. [67] developed a simple fluorescence method for the sensitive detection of microRNAs based on DSN-assisted target recycling and pyrene eximer switching. The system was extremely sensitive, with a large dynamic range from 1 fM to 10 nM and a detection limit of 0.58 fM.

3.5. Ligase Chain Reaction

LCR amplifies two oligonucleotides pairs with complementary sequences to target miRNA with a thermostable ligase. It has a high specificity to identify a single nucleotide difference of miRNAs because of the thermal stability of the ligase [81].

Zhang et al. [82] demonstrated that multiple miRNAs could be simultaneously detected in one LCR reaction by simply coding the DNA probes with different lengths of oligo(dA) for different miRNA targets. Yuan et al. [83] developed a simple and homogeneous miRNA method by integrating LCR and λ exonuclease-assisted CCP biosensing. λ exonuclease was introduced to minimize background signals by degrading the excessive fluorescein-labeled probes in LCR. Accordingly, upon the addition of CCP, efficient fluorescence resonance energy transfer from CCP to fluorescein in LCR products occurred. The method was sensitive enough to detect 0.1 fM target microRNA and specific enough to discriminate a one-base difference of microRNAs. Although LCR-based miRNA detection is highly sensitive and specific, it requires many DNA probes and tedious electrophoresis separation, leading to multiple steps, high cost, and long analysis time.

3.6. Enzyme-free Amplification

The label-free and enzyme-free strategies have been usually preferred for miRNA detection due to their features of high sensitivity and specificity and kinetics-controlled reaction. In particular, since the method can amplify the signal without enzymes, there is no need to take into account the limited factors of enzyme-coupled methods, such as pH, temperature, and the selection of a proper buffer [84, 85]. Hybridization chain reaction (HCR) and catalytic hairpin assembly (CHA) are representatives widely used in signal amplification for miRNA detection [86]. However, HCR requires a complex immobilization process, which influences the activity of the immobilized biomolecules and thereby lowers the binding efficiency [87], and CHA shows a high background signal caused by the nonspecific CHA products [88-91].

Therefore, HCR and CHA are usually coupled with other signal amplification strategies to further enhance the signal response. Wu et al. [92] developed an electrochemical biosensor based on HCR and CHA. The biosensor showed remarkable sensitivity with a wide dynamic range of 6 orders of magnitude and a detection limit of 3.3 fM. Zhang et al. [93] developed a universal ECL biosensor for the detection of miRNA-155 by using hydrogen peroxide (H₂O₂) and hemin as co-reactant and catalyzer, respectively. The biosensor possessed remarkable sensitivity with a detection limit of 1.67 fM and a wide dynamic range from 5 fM to 50 pM. Yan et al. [94] reported a simple and versatile colorimetric strategy for the ultrasensitive and specific determination of microRNAs based on molecular beacon-initiated SDA and CHA. The biosensor showed high sensitivity and selectivity in a dynamic response range from 5 fM to 5 nM with a detection limit as low as 1.7 fM. The characteristics of nucleic acid amplification-based miRNA detection methods are summarized in Table 2 [95].

4. NANOMATERIAL-BASED miRNA DETECTION METHOD

The ideal method for reducing side effects and errors in chemical reactions occurred in multiple steps, as shown in miRNA detection, and for increasing specificity and sensitivity is to reduce the order of reactions. From this perspective, a nanomaterial, which needs no combination with other materials and can transmit signals without any amplification, is the best candidate as a biosensing platform.
Table 2. Characteristics of nucleic acid amplification-based miRNA detection methods.

| Methods       | Advantages                                                                 | Disadvantages                                           | LOD          | Refs.          |
|---------------|----------------------------------------------------------------------------|--------------------------------------------------------|--------------|----------------|
|               | Enzyme-based methods                                                      |                                                        |              |                |
| General IEA   | • Good efficiency of signal amplification                                 | • Complicated probe design                              | aM−fM        | [63-65]        |
|               | • Good sensitivity even low at the single-cell level                       | • Multiple enzymes used                                  |              |                |
|               | • Real-time assay possible in some cases                                   |                                                        |              |                |
| LAMP          | • Easy                                                                     | • Requiring a cDNA copy and RNase H                     | fM           | [64, 66, 67]   |
|               | • Quick                                                                    | • Multiple enzymes used                                  |              |                |
|               | • Highly specificity                                                      |                                                        |              |                |
|               | • Highly sensitivity                                                      |                                                        |              |                |
| EXPAR         | • High-speed                                                               | • Multiple enzymes used                                  | fM           | [63, 68, 69]   |
|               | • Cost-effective                                                          |                                                        |              |                |
|               | • Highly sensitive and specific                                            |                                                        |              |                |
|               | • Differentiating miRNAs of one family                                     |                                                        |              |                |
|               | • Requiring low amounts of the substrate                                  |                                                        |              |                |
| DSNSA         | • Sequence-specific function                                               | • Special enzymes needed                                | fM           | [67, 77, 78]   |
|               | • Multiplex and easy detection                                             |                                                        |              |                |
|               | • Excellent specificity and sensitivity                                    |                                                        |              |                |
| RCA           | • Simple                                                                   | • Complicated probe design                              | aM−fM        | [70, 71, 95]   |
|               | • Specific                                                                 | • Nonspecific amplification                             |              |                |
|               | • Sensitive                                                                | • Background                                            |              |                |
|               | • Good efficiency of signal amplification                                 | • More condition optimization needed                    |              |                |
|               | • In situ detection is possible in some cases                              |                                                        |              |                |
| LCR           | • Highly sensitive                                                         | • Two probes needed                                     | fM           | [81-83]        |
|               | • Not requiring thermal cycler                                             | • Two enzymes needed                                    |              |                |
|               | • Simultaneous and multiplex detection possible                            |                                                        |              |                |
|               | Enzyme-free methods                                                       |                                                        |              |                |
| HCR           | • Programmable amplification cycles                                       | • Complex immobilization                                | fM           | [87]           |
|               | • Sequence-specific function                                               |                                                        |              |                |
|               | • HCR amplifiers can be implemented                                       |                                                        |              |                |
|               | • Simultaneous                                                            |                                                        |              |                |
| CHA           | • Simple and nimble                                                        | • A big background signal, caused by the nonspecific CHA products | fM           | [88-89]        |
|               | • Excellent sensitivity                                                    |                                                        |              |                |

Abbreviations: IEA; Isothermal exponential amplification; LAMP, Loop-mediated isothermal amplification; EXPAR, Isothermal EXPonential Amplification Reaction; DSNSA, Duplex-specific nuclease signal amplification; RCA, Rolling circle amplification; LCR, Ligase chain reaction; HCR, Hybridization chain reaction; CHA, catalytic hairpin assembl.

4.1. Metal Nanoparticles-based Method

Metal nanoparticles (NPs) feature high sensitivity and efficiency, a large surface-to-volume ratio, and size dependency. Therefore, they are widely used for miRNA detection [96, 97].

AuNPs feature high stability, conductivity, biocompatibility, and size-related electronic, magnetic, and optical properties. Due to their advantages, AuNPs have widely been used in biosensor, including SPR imaging and AuNP-based bio-barcode assay [98]. Recently, in combination with magnetic particles and MWCNTs, various platforms with high miRNA detection efficiency have been developed [97-99]. Labib et al. [99] developed a three-mode electrochemical sensor based on gold nanoparticle-modified screen-printed carbon electrode (GNPs-SPGE). The sensor showed ultrasensitivity for miRNA-32 and miRNA-12 within a very large dynamic range from 10 aM to 1 μM and a detection limit of as low as 5 aM (90 molecules of miRNA per 30 μL of the sample) without PCR amplification. Cardoso et al. [97] developed a simple and ultrasensitive electrochemical biosensor for the detection of miRNA-155, a cancer biomarker, based on Au-screen printed electrode (Au-SPE). The biosensor was assembled in two stages; the immobilization of the anti-miRNA-155 that was thiol-modified with an Au-SPE, followed by blocking the areas of nonspecific binding with mercaptosuccinic acid. The biosensor showed a large dynamic range from 10 aM to 1.0 nM with a low detection limit of 5.7 aM in real human serum samples.

Recently, other metallic nanoparticles, such as silver and copper, have also been preferentially used in biosensor. Hosseini et al. [100] developed a new biosensor for miRNA-155 detection based on the fluorescence quenching of oligonucleotide-templated silver nanoclusters (DNA-AgNCs). The
biosensor showed high sensitivity with a linear range of 0.2 nM to 30 nM and a detection limit of 0.1 nM. Chi et al. [101] reported a new strategy for direct detection of miRNA based on the enzymatically engineered primer extension poly-thymine (EPEPT) and CuNPs. The system showed a high sensitivity for miRNA-21 with a linear range from 1 pM to 1 nM and a detection limit of 100 fM.

Borghei et al. [102] developed a new strategy for sequence-specific miRNA-155 detection based on the fluorescence shifting of oligonucleotide-templated copper nanoclusters (DNA-CuNCs). The sensor showed a high sensitivity for miRNA-21 with a dynamic range from 5.0 pM to 10 nM and a detection limit of 2.2 pM.

4.2. Pure Quantum Dots

Quantum dots (QDs), as highly efficient fluorophores, feature broad excitation spectra, narrow emission bandwidth, high quantum yield, and photostability [103, 104]. The method of miRNA optical detection using a single platform like QDs is simple, fast, cost-effective, and features an excellent readout. Therefore, they are widely used as the platform of electrochemical and fluorometric biosensors [105-107].

Lv et al. [107] developed a rapid and facile resonance light scattering (RLS) technique for the detection of miRNA-122. The cDNA sequence probe with partially complementary sequences to miRNA-122, P1, and P2, was coupled to the QDs surface to form the functional QDs-P1 and QDs-P2 conjugates, respectively. The hybridization of each probe-functionalized QDs with the target miRNA changed the RLS signal. The RLS intensity was proportional to the concentration of miRNA-122. The sensor showed a linear range of 0.16 – 4.80 nM with a detection limit of 9.4 pM.

Similarly, Chen et al. [105] developed an intelligent and label-free resonance light scattering (RLS) sensor for the one-spot simultaneous detection of alpha-fetoprotein (AFP) and miRNA-122. Artificially constructed dsDNA and methyl violet (MV) were used to analyze the RLS signal. The signal was proportionally changed to the concentration of AFP and miRNA-122. The sensor was highly sensitive and selective to detect AFP and miRNA-122; the detection limit of AFP and miRNA-122 was 0.94 μg/L and 98 pM, respectively, and the linear range was from 5 to 100 μg/L and from 200 pM to 10 nM, respectively.

4.3. Doped Quantum Dots

The limited extinction pattern due to a wide bandgap of the outer layer ZnO of QDs and their cytotoxicity become an obstacle to the expansion of a biosensing platform. Moreover, the efficacy of a biosensor using a QDs-based probe is influenced by nonspecific fluorescence and light scattering of specimens. To overcome these problems, a variety of doped QDs with elements or organic molecules have been developed.

Graphene quantum dots (GQDs), the first doped QDs, are 0-D nanomaterials obtained from graphene and carbon dots. GQDs have excellent photoelectricity, water solubility, and chemical inertness, as well as a large surface area and good surface grafting ability due to their π-π conjugation network [108]. Furthermore, they are resistant to photobleaching, biocompatible, and cost-effective [109, 110]. Owning to their unique properties, GQDs are widely used in fluorescent bioimaging, photonics, and electronics [111]. Zhang et al. [112] developed a novel biosensor platform for the detection of miRNAs based on GQDs and pyrene-functionalized molecular beacon probes (py-MBs). The developed biosensor based on the platform could selectively detect the target miRNAs with a linear range from 0.1 nM to 200 nM and discriminate even a single nucleotide between different kinds of miRNAs.

Recently, it has been reported that the intrinsic properties of carbon materials, such as PL stability and electrical activity, can be effectively tuned by doping with heteroatoms, consequently producing new phenomena and unexpected characteristics [113]. In 2016, Zhang et al. [114] demonstrated that boron-doped graphene quantum dots (BGQDs) with an atomic boron percentage of 0.67-2.26% were synthesized by electrolytic exfoliation of B doped graphene rods, and their 60 days PL stability and resistance was improved by 51% and decreased by 11%, respectively, compared to that of the pure GQDs, when 1.29% (atomic percentage) of boron was doped. Moreover, they developed a simple and specific electrochemiluminescence (ECL) sensor for the detection of miRNAs-20a using the BGQDs as a platform. The sensor could sensitively detect miRNAs-20a with a linear range of 0.1 – 10 nM and a detection limit of 0.1 pM.

Carbon quantum dots (CQDs) are fluorescent graphite nanocrystals with a size of 1-10 nm. CQDs have low toxicity, excellent water solubility, and environmental friendliness, as well as the unique properties of pure QDs, such as size-dependent luminescence emission, excellent photostability, and chemical inertness [115, 116]. Due to such favorable attributes, CQDs have been considerably paid attention as an alternative material for pure QDs. In fact, Khakbaz and Mahani [117] developed a CQDs-based FRET biosensor for miRNA-9-1 detection. The FAM-labeled single-stranded DNA, as a sensing element, was adsorbed on CQDs by π-π interaction. In the presence of the complementary miRNA, the FRET did not occur and the fluorescence was recovered. Although CQDs have attracted attention due to their particular properties, their insufficient optical characteristics and surface chemical structure limit the range of their practical applications. Therefore, many researchers have focused on CQDs surface modifications with different functionalities to expand the range of applications [108, 118].

Doping is an excellent method to modify the properties of CQDs [119]. Nitrogen-doped CQDs (N-CQDs) have been widely used in the biomedical field due to their photoluminescence, photostability, and photoelectric physical properties [120]. Particularly, the amino groups on the surface of N-CQDs can not only improve their fluorescence but also facilitate subsequent modification and application [119]. In fact, Liu et al. [121] developed a novel electrochemilumines-
A Combinational Approach for More Efficient miRNA Biosensing

Fig. (1). Schematic diagram of the designed nanoprobe for the detection of miRNA-21 based on the PRET system [128]. (Reprinted from Lv, J.; Miao, Y.; Yan, G. Detection of tumor marker miRNA21 based on phosphorescent resonance energy transfer of Mn–ZnS QDs. RSC Advances, 2017, 7(65), 41063-41069. doi: 10.1039/C7RA04521B). (A higher resolution / colour version of this figure is available in the electronic copy of the article).

4.4. Hybrid Nanomaterials

Currently, for more efficient miRNA detection, various hybrid nanomaterials, such as gold nanorods, WO3-graphene composites, MgO nanoflower, GO–AuNPs hybrids, and MoS2 microcubes are used.

Cheng et al. [38] designed a distance-dependent electrochemiluminescence resonance energy transfer (ERET) system based on CdTe nanocrystals and Au nanoclusters (AuNCs) for the detection of microRNA. The AuNCs were functionalized with hairpin DNA through Au–S bonding. The DNA–AuNCs composite was incorporated into the carboxylated CdTe nanocrystals on a glass carbon electrode. The strong interaction between CdTe nanocrystals and AuNCs led to the electrochemiluminescence (ECL) quenching of CdTe nanocrystals. The sensor showed high selectivity with a large linear range of 6 orders of magnitude and excellent sequence specificity.

Li et al. [129] developed a simple and ultrasensitive electrochemical biosensor for miRNA detection. The oligonucleotide captured probes were immobilized onto a glass carbon (GC) electrode modified with multiwalled carbon nanotube (MWCNT)-polyamidoamine (PAMAM). The sensor showed ultrasensitivity with a detection limit of 0.5 fM and a dynamic range of 3 orders of magnitude.

Meanwhile, Azimzadeh et al. [130] developed an electrochemical nanobiosensor for circulating miRNA-155 detection, based on thiolated probe-functionalized gold nanorods...
(GNRs) and graphene oxide (GO) sheets. The reduction signal was measured by a differential pulse voltammetry (DPV) method. The sensor showed remarkable sensitivity with a wide linear range from 2 fM to 8 pM and a detection limit of 0.6 fM and was able to discriminate single- or three-base mismatched nucleotides between complementary and non-complementary miRNA.

Recently, Kamal et al. [131] have developed a nonenzymatic, amplification-free, and sensitive platform for the detection of miRNA based on a new class of electrocatalytically active superparamagnetic gold-loaded nanoporous iron oxide nanocubes (Au@NPFe$_2$O$_3$NC). The biosensor exhibited an excellent detection sensitivity down to 100 fM and specificity for miRNA-21 in cell lines and tissue samples derived from patients with oesophageal squamous cell carcinoma (ESCC).

Li et al. [132] developed a simple, label-free, and sensitive electrochemical biosensor for the detection of miRNA-24. The synthetic DNA probes complementary to miRNA-24 were immobilized onto the surface of MWCNT-modified glass carbon electrodes by covalent cross-linking. The probes were hybridized with different concentrations of miRNA-24. The formed hybrids on the electrode surface were evaluated by differential pulse voltammetry. The proposed biosensor exhibited good sensitivity for miRNA-24 with a low detection limit of 1 pM.

Meanwhile, organometal halide perovskites, such as CH$_3$NH$_3$PbI$_3$, are utilized to improve the light absorbency of pure QDs. CH$_3$NH$_3$PbI$_3$ can absorb visible light in the wavelength range of 400 ~ 800nm [133] and long-range exciton diffusion length [134, 135]. Since the first report in 2009, a new kind of light-harvesting material, CH$_3$NH$_3$PbI$_3$, has been applied in various fields due to its strength [136].

Pang et al. [137] developed an ultrasensitive photoelectrochemical (PEC) aptasensor based on CH$_3$NH$_3$PbI$_3$-QDs. CH$_3$NH$_3$PbI$_3$-QDs, functionalized ZnO nanosheets (ZnONSs) were employed as a light harvester. Owing to the synergetic effect between CH$_3$NH$_3$PbI$_3$-QDs and ZnONSs, ZnO@CH$_3$NH$_3$PbI$_3$ could provide an increasing PEC signal. The optimized aptasensor showed ultrasensitivity for miR-155 with a wide detection range of 0.01 ~ 20 fM and a detection limit of 5 aM. The characteristics of miRNA detection methods based on different nanomaterials are summarized in Table 3 [137-139].

### Table 3. The characteristics of miRNA detection methods based on different nanomaterials.

| Nanomaterial       | Target                  | Signal       | LOD           | Dynamic Range  | Refs. |
|--------------------|-------------------------|--------------|---------------|----------------|-------|
| **Metal NPs**      |                         |              |               |                |       |
| AuNPs              | -                       | SPR imaging  | Attomole      | -              | [138] |
| GNPs-SPGE          | miRNA-32 and miRNA-12   | Electrochemical | 5 aM          | 10 aM to 1 μM  | [99]  |
| Au-SPE             | miRNA-155               | Electrochemical | 5.7 aM        | 10 aM ~ 1.0 nM | [97]  |
| AgNCs              | miRNA-155               | Fluorescence shifting | 0.1 nM | 0.2 nM ~ 30 nM | [100] |
| CuNPs/EPEPT        | miRNA-21                | Fluorescence | 100 fM        | 1 pM ~ 1 aM    | [101] |
| **QDs**            |                         |              |               |                |       |
| Dox-CdTe           | miRNA-200b and miRNA-429| ECL          | 5.5 fM        | 10 fM ~ 1.2 pM | [105] |
| Dual QDs           | miRNA-122               | RLS          | 9.4 pM        | 0.16 ~ 4.80 nM | [107] |
| QDs                | miRNA-122               | RLS          | 98 pM         | 200 pM ~ 10 nM | [37]  |
|                    | AFP                     | -            | 0.94 μL/L     | 5 ~ 100 μL/L   |       |
| **dQDs**           |                         |              |               |                |       |
| GQDs               | miRNA-155               | PEC          | 0.14 fM       | 1 fM ~ 100 pM  | [139] |
| CQDs               | miRNA-9-1               | FRET         | -             | -              | [117] |
| BGQDs              | miRNA-20a               | ECL          | 0.1 pM        | 0.1 ~ 10 nM    | [114] |
| Mn–ZnS RTP QDs     | miRNA-21                | PRET         | 1.60 nM       | 8 ~ 80 nM      | [128] |
| **Hybrids**        |                         |              |               |                |       |
| AuNP@Fe$_2$O$_3$NC | miRNA-21                | Electrochemical | 100 fM      | -              | [131] |
| CuO-CuWO$_3$       | miRNA-319a              | PEC          | 0.47 fM       | 1 fM to 0.1 nM | [140] |
| MWCNT/AuNCs        | miRNA-155               | Fluorescence quenching | 33.4 fM | -              | [141] |
| MWCNT/glass carbon | miRNA-24                | DPV          | 1 pM          | -              | [132] |

(Table 3 contd...)
### 5. COMBINATIONAL STRATEGY FOR MORE EFFICIENT miRNA DETECTION

Most studies on miRNA detection methods, which have been conducted since 2015, have used the strategy of combining nucleic acid amplification and nanomaterials to improve the detection efficiency. Since nanomaterials feature unique photoelectric properties, side effects, and surface effects, they are widely used as a versatile platform in miRNA detection [39, 40, 44, 84, 121, 140, 142]. For instance, the combination of QD-based biosensors with signal amplification techniques makes it possible to identify the target miRNA at a single-particle level with the detection limit of the atomolar scale [143, 144].

#### 5.1. Nanoparticles-based Biosensor

Degliangeli et al. [39] developed an ultrasensitive biosensor based on the combination of gold nanoparticles with DSN cleavage-mediated target recycling. PEGylated gold nanoparticles (AuNPs) were conjugated with DNA probes by simple coupling between their amino groups and the 3’-thiols of the DNA probes. The fluorescence signal of DNA-probe strands were immobilized on PEGylated-AuNPs. In the presence of target miRNA, DNA–RNA heteroduplexes were formed and then enzymatically hydrolysed by the duplex-specific nuclease (DSN) endonuclease, which produced a fluorescence signal. The biosensor showed ultrasensitivity for miRNA-203 with a detection limit of as little as 0.2 FM. More recently, Bo et al. [145] have reported a biosensing scheme for the determination of miRNA-21 with triple signal amplification based on target-triggered cyclic duplex specific nuclease digestion and bridge DNA–gold nanoparticles. Electrochemical signals were recorded to present the initial levels of miRNA. This method showed ultrasensitivity with a wide linear range of 10 aM to 10 nM and a detection limit of 6.8 aM.

Meanwhile, it has been well-known that magnetic nanoparticles can be assembled super structurally, which enables approaches to biosensing by combining specially tailored properties of superstructures. Furthermore, they have favorable features, such as low background signal, easy manipulation, cost-efficiency, and potential for bioresponsive multiplexing [143, 146].

In fact, Tian et al. [143] developed a rapid and sensitive miRNA detection method based on the combination of multilayer core-satellite magnetic superstructure and duplex-specific nuclease (DSN-assisted target recycling. In the presence of target miRNA, the core-satellite magnetic superstructures released their “satellites” into the suspension through DSN-assisted cleavage. Target miRNAs preserved in the cleavage reaction triggered more cleavage and released “satellites” repeatedly. For single-plex detection of miRNA-let-7b, the sensor could detect the target miRNA within 70 min with a linear detection range of 10 fM ~ 10 nM and a detection limit of 4.8 fM. Multiplexed detection was also possible by releasing nanoparticles of different sizes in the presence of different miRNAs.

More recently, Tian et al. [146] proposed a new strategy for the rapid and more sensitive miRNA-let-7b detection. Based on the combination of magnetic nanoparticles (MNPs), on-particle rolling circle amplification (RCA), and DSN-assisted target recycling, the long ssDNA produced by RCA was hydrolyzed by DSN in the presence of target miRNA, consequently releasing MNPs. The released MNPs were quantified using an optomagnetic sensor. The proposed system showed the sensitivity of miRNA-let-7b with a wide linear detection range of approximately 5 orders of magnitude and a detection limit of 1 fM. Moreover, it discriminated against single-nucleotide mismatches and detected target miRNA in cell extracts and serum.

Meanwhile, Li et al. [140] proposed a new strategy for sensitive and specific detection of miRNA-319a, based on the combination of a magnetic particle, CuO-CuWO4, and rolling circle amplification followed by nicking endonuclease triggered exponential amplification. The developed strategy greatly amplified the detection signal by the addition of a trace amount of target miRNA-319a. The system showed high sensitivity with a very wide linear range from 1 fM to 0.1 nM and a detection limit of 0.47 fM.

#### 5.2. Quantum Dot-based Nansensor

Zhang and Zhang [144] developed a rapid, highly sensitive, and specific miRNA assay based on the two-stage exponential amplification reaction (EXPAR) and a single-quantum-dot (QD)-based nanosensor. They used a two-step amplification reaction of exponential and linear amplification similar to a general isothermal amplification [147]. The schematic diagram for the miRNA assay based on the two-stage EXPAR and single-QD-based nanosensor is represented in Fig. (2). The assay was highly sensitive and specific with a detection limit of 0.1 aM and could even discriminate...
single-nucleotide differences between miRNA family members. Moreover, in combination with the specific templates, the assay method could be applied for multiplex miRNA assays by simply using the same set of capture and reporter probes.

Meanwhile, quantum dots have been used for miRNA detection in combination with NAA other than IEA [121, 148, 149].

Yuan et al. [148] developed a simple and novel fluorescence biosensing strategy based on the combination of target-triggered DNA nano assembly on quantum dots (QDs) with DNAzyme-modulated double quenching of QDs. In the presence of miRNA-21, the target triggered catalytic hairpin assembly (CHA) amplification and powered highly efficient DNA nano assembly on the surface of QDs; the biosensor showed a high sensitivity for miRNA-21 with a detection limit down to 37 fM and a very large linear range of 100 fM to 10 nM.

Liu et al. [121] developed an ultrasensitive electrochemiluminescence (ECL) biosensor for the detection of miRNA based on the nicking enzyme Nb.BbvCl-mediated signal amplification (NESA). The ECL intensity increased with the concentration of the target miRNA and the sensitivity of the biosensor was promoted because of the efficiently amplified signal. The biosensor showed ultrasensitivity for miRNA-21 detection with a remarkably wide dynamic range from 10 aM to 40 nM and a detection limit of 0.16 nM.

Yang et al. [149] developed a selective room-temperature phosphorescence (RTP) detection method for miRNA, based on a duplex-specific nuclease (DSN)-assisted signal amplification and phosphorescence resonance energy transfer (PRET) between poly-diallyldimethylammonium chloride (PDDAC)-modified quantum dots (QDs@PDDAC) and 6-carboxy-X-rhodamine-modified oligonucleotide (ROX-ssDNA) being complementary to miRNA sequences. The schematic diagrams of the designed nanoprobe for the detection of miRNA-21 are based on the PRET system [128]. The system showed a high sensitivity for miRNA-21 with a dynamic range of 0.25 ~ 40 nM and a detection limit of 0.16 nM.

5.3. Carbon Nanomaterials-based Biosensor

Graphene oxide (GO), a 2-D planar structured nanomaterial, has excellent electronic, thermal, and mechanical properties, as well as enables multiplexed detection of target miRNAs by labelling a probe with a different fluorophore. In particular, because of its extraordinary and distance-dependent fluorescence-quenching properties, it has been paid attention as an ideal platform for biological target detection.

Dong et al. [40] reported a simple, highly sensitive, selective, and multiplexed miRNA detection method based on the graphene oxide (GO) fluorescence quenching and isothermal strand-displacement polymerase reaction (ISDPR). The biosensor showed a linear range of 4 orders of magnitude and a detection limit of 2.1 fM.

Jing et al. [150] developed a new graphene oxide enhanced fluorescence anisotropy biosensor for miRNA-21 detection, based on graphene oxide (GO) amplified fluorescence anisotropy (FA) and target catalyzed signal cyclic am-
plification strategy. In the system, a GO/capture DNA (cDNA)/Linker DNA/probe DNA (pDNA) (DNA-GO) composite probe was firstly constructed, wherein target catalyzed signal cyclic amplification was used to assist GO to amplify the FA signal. In the presence of miRNA-21, pDNA was released from Linker DNA through a toehold-mediated strand displacement (TMSD) reaction. Fuel DNA was then hybridized with Linker DNA to form a double-stranded DNA (dsDNA), which was detached from the surface of GO, and the FA of the fluorophore on Linker DNA was reduced. Additionally, miRNA-21 was released after the formation of dsDNA, which triggered the next cycle, resulting in a further reduction of FA values. The biosensor showed high sensitivity with a linear range of 10 ~ 330 nM and a limit of detection of 1.09 nM.

Tian et al. [44] developed an ultrasensitive protocol for electrochemical detection of miRNA through carbon nanotube (CNT) enhanced label-free detection based on hairpin probe triggered solid-phase rolling-circle amplification (RCA). They purposely designed a solid-phase RCA strategy, using CNTs as the solid substrate, integrated with a hairpin structured probe to recognize the target miRNA. In the presence of miRNA, the stem-loop structure of the probe was unfolded and triggered the CNT-based RCA process. Due to the efficient blocking effect originating from the polymeric RCA products, the label-free assay of miRNA exhibited an ultrasensitive value with a detection limit of 1.2 fM. Furthermore, the protocol showed an excellent specificity for resolving lung cancer-related miRNA-let-7 family members, which have only one nucleotide variation.

5.4. Chacogenides-based Biosensor

Transition metal dichalcogenides (TMDCs) is a 2-D nanomaterial with a layered sandwich structure. TMDCs feature a large surface area, high electronic conductivity, eminent catalytic properties, intercalatable morphology, and fast heterogeneous electron transfer [151]. Therefore, they draw attention as a nanomaterial to complement the disadvantages of gapless graphene in the area of electrochemical biosensors [61, 142, 152, 153].

Xi et al. [142] reported a new strategy for simple, sensitive, and selective detection of miRNA by combining WS₂ nanosheet-based fluorescence quenching with duplex-specific nuclease signal amplification (DSNSA). The assay exhibited high sensitivity and selectivity with a detection limit of 300 fM and discriminated even single-base differences between the miRNA family members.

Xiao et al. [152] reported a novel design of nanoprobe for highly sensitive and selective detection of miRNAs based on MoS₂-loaded molecular beacons (MBs) and duplex-specific nuclease (DSN)-mediated signal amplification (DSNMSA). In the system, MoS₂ nanosheets not only exhibited a high affinity toward MBs but also acted as an efficient quencher for absorbed MBs. The biosensor showed superior sensitivity with a limit of detection 4 orders of magnitude lower than that of traditional hybridization methods. Moreover, the biosensor also showed high selectivity for discriminating homogenous miRNA sequences with one-base differences.

5.5. Hybrid Nanomaterials-based Biosensor

Liu et al. [84] developed a new sensitive biosensor by combining 3-D layer-by-layered nanostructures (SWCNTs-ox@nanodiamonds (NDs) with a hybridization chain reaction. The system showed an ultrasensitive detection for miRNA-21 with a dynamic range from 10 fM-1.0 nM and a detection limit of 1.95 fM.

Shuai et al. [154] developed an ultrasensitive electrochemical biosensor for microRNA based on tungsten oxide-graphene (WOₓ-Gr) composites coupled with catalyzed hairpin assembly target recycling and enzyme signal amplification. The biosensor could detect target miRNA down to 50 aM with a linear range from 0.1 fM to 100 pM, and discriminate the target miRNA from mismatched miRNA with high selectivity.

Ma et al. [141] reported a novel fluorescence-quenching platform for ultrasensitive detection of miRNA-155 based on multi-walled carbon nanotube-gold nanocomposites (MWCNT/AuNCs). In the presence of duplex-specific nuclease (DSN), DNA-RNA heteroduplexes became a substrate for the enzymatic hydrolysis of DNA strands and triggered a cyclic signal amplification. The biosensor showed high sensitivity with a detection limit down to 33.4 fM. Additionally, the strategy showed excellent selectivity for miRNA-155 detection against other miRNAs, including miRNA-21, miRNA-141, and mutated miRNA-155 strands.

Yu et al. [155] designed an ultrasensitive electrochemical biosensor for miRNA-21 detection based on CoFe₂O₄ magnetic nanoparticles (CoFe₂O₄ MNPs) and padlock exponential rolling circle amplification (P-ERCA). In the assay system, the nanoelectrocatalysis could be carried out without a substrate for signal amplification. The nanocatalyst (CoFe₂O₄ MNPs) and redox molecule (Tb) were co-immobilized onto the graphene (Gr) surface and formed Au@CoFe₂O₄/Tb-Gr probe. Through multiple polymerization and nicking reactions, plenty of P-ERCA product was produced and exponential signal amplification was circumvented; the biosensor showed a wide dynamic range of 1 fM to 2 nM and a low detection limit of 0.3 fM.

Liu et al. [121] developed an ultrasensitive electrochemiluminescence (ECL) biosensor for the detection of microRNA, based on the combination of the N-QDs immobilized on GO/Au composite and nicking enzyme Nb.BbvCl mediated signal amplification (NESA). First, the hairpin probe1-N-QDs with assistant probe and microRNA (miRNA) formed a Y junction structure which was cleaved with the addition of nicking enzyme Nb.BbvCl with release miRNA and assistant probe. The released miRNA and assistant probe triggered the next recycling process and the recycling process continued repeatedly. The resultant numerous nitrogen-doped carbon quantum dots DNA having intermediate sequences (N-QDs-DNA) further hybridized with hairpin probe2 on GO/Au composite to modify the electrode
surface, and subsequently, the ECL intensity was enhanced. The ECL intensity increased with the concentration of the target miRNA. The schematic diagrams of the mechanism are represented in Fig. (3). The biosensor exhibited high sensitivity and selectivity for miRNA-21 detection with a very wide linear range from 10 aM to 10 nM and a relatively low detection limit of 10 aM.

Lu et al. [156] constructed a novel electrochemiluminescence (ECL) biosensor for miRNA-141 detection based on a Faraday cage-type strategy via graphene oxide (GO) lapped on Fe₃O₄@SiO₂@AuNPs and hybridization chain reaction (HCR)-assisted cascade amplification. A capture probe (CP) was immobilized on Fe₃O₄@SiO₂@Au nanoparticles as a capture unit, which could catch miRNA-141, and the immobilization of the signal unit (Ru(phen)₃²⁺-HCR/GO) was allowed via nucleic acid hybridization. The proposed sensor displayed ultrasensitivity for miRNA-141 with a low detection limit of 30 aM.

Shuai et al. [153] developed an ultrasensitive electrochemical biosensor for detecting miRNAs based on hollow molybdenum disulfide (MoS₂) microcubes coupled with duplex-specific nuclease (DSN) and electrochemical-chemical-redox cycling. The biotinylated ssDNA capture probes were first immobilized on Au nanoparticles (AuNP-
s)/MoS₂ modified electrodes to combine with streptavidin-conjugated alkaline phosphatase (SA-ALP). In the presence of miRNAs, the capture probes hybridized with miRNAs, a duplex-specific nuclease that cleaves the formative duplexes. The proposed biosensor showed a good linear range from 0.1 fM to 0.1 pM and a detection limit of 0.086 fM.

Shuai et al. [157] developed an ultrasensitive sandwich-type electrochemical biosensor for miRNA detection, based on magnesium oxide (MgO) nanoflower and graphene oxide-gold nanoparticles (GO-AuNPs) hybrid coupled with electrochemical chemical-chemical (ECC) detection system. In this bioassay system, MgO nanoflowers and AuNPs were modified on the electrode to act as a sensing platform. The thiolated capture probe was then self-assembled onto AuNPs/MgO substrate via the formation of Au-S bonding. Subsequently, a biotinylated DNA signal probe was conjugated to GO–AuNPs hybrids. In the presence of miRNA-21, a sandwich complex was formed and a lot of signal indicator streptavidin-conjugated alkaline phosphatases (SA-ALP) were immobilized upon the electrode by the specific reaction between avidin and biotin. Finally, an ECC reaction occurred in the system to improve the detection signal. The proposed assay exhibited a good dynamic range from 0.1 to 100 fM and a low detection limit of 50 aM.

Chen et al. [61] developed a sandwich-type electrochemical biosensing platform based on carbon sphere-MoS₂ (CS-MoS₂) and target recycling amplification of catalyzed hairpin assembly (CHA) strategy for ultrasensitive determination of miRNA. The capture probe was first modified on Au nanoparticles/CS-MoS₂ modified glassy carbon electrode through their Au-S bonding, and the hairpin DNA1 (H1) then reacted with it and unfolded its loop domain by hybridizing with the target miRNA-21. The opened H1 was subsequently assembled with biotin-labeled hairpin DNA 2 (H2) to displace the target. The liberated miRNA-21 went back to the original position to proceed next cycling, resulting in the generation of large amounts of H1–H2 duplexes. The resultant was catalyzed by horseradish peroxidase (HRP) to produce a strong electrochemical response. The proposed assay exhibited a very wide linear relation ranging from 0.1 fM to 0.1 nM and an extremely low detection limit of 16 aM.

Zhao et al. [158] developed a gold nanoparticle-coated polystyrene microbead (PS@Au microspheres)-based DNA probe and DSN signal amplification platform. The 5’fluorochrome-labeled molecular beacons (MBs) were immobilized on PS@Au microspheres via their 3’-thiol group. Target miRNAs were captured by the PS@Au microsphere-based DNA probe through DNA/RNA hybridization. DSN subsequently cleaved the DNA to recycle the target miRNA and release fluorophores, thereby triggering the signal amplification with more free fluorophores. The RGB value due to the fluorescence quenching by nanoparticle surface energy transfer (NSET) was measured. The sensor showed a detection limit of 50 fM, almost 4 orders of magnitude lower than PS@Au microsphere-based DNA probe detection without DSN. Moreover, by the different encoding of dyes, miRNA-21 and miRNA-10b were simultaneously detected in the same sample. The characteristics of miRNA detection by a combinatorial approach are summarized in Table 4 [159, 160].

| Table 4. The characteristics of miRNA detection based on a combinatorial approach. |
|-----------------------------------------------|-----------------|---------------|--------|---------------|----------|-----------|
| **Strategy**                               | **Target**      | **Signal**    | **LOD**| **Dynamic Range**| **Refs.**|
| Nanoparticle-based biosensor                |                 |               |       |               |          |
| PEGylated-AuNPs coupled with DSN mediated target recycling | miRNA-203       | Fluorescence  | 0.2 fM | -              | [39]     |
| TSA based on coupling DNA–AuNPs with target-triggered cyclic DSN digestion | miRNA-21       | Electrochemical | 6.8 aM | 10 aM ~ 10 nM | [145]   |
| Magnetic superstructure coupled with DSN-mediated target cycling | miRNA-let-7b | Current | 4.8 fM | 10 fM ~ 10 nM | [143] |
| MNPs coupled with on-particle RCA and DSN-assisted target recycling | miRNA-let-7b | Current | 1 fM | 5 orders of magnitude | [146] |
| CuO-CuWO₄ coupled with RCA and nicking endonuclease triggered exponential amplification | miRNA-319a | PEC | 0.47 fM | 1 fM to 0.1 nM | [140] |
| QDs-based biosensor                        |                 |               |       |               |          |
| QD coupled with two-stage EXPAR            | Let-7a, let-7b, let-7c and miRNA-21 | FRET | 0.1 aM | -              | [144] |
| Nanoassembled QDs coupled with CHA         | miRNA-21       | Fluorescence quenching | 37 fM | 100 fM to 10 nM | [148] |
| QDs coupled with NESA                      | miRNA-21       | ECL           | 10 aM | 10 aM ~ 10 PM  | [121]   |
| RTP-QDs@PDDAC coupled with DSN             | miRNA-21       | PRET          | 0.16 nM | 0.25 ~ 40 nM | [149] |
| Carbon nanomaterial-based biosensor        |                 | Electrochemical | 1.2 fM | -              | [44]     |

(Table 4 contd....)
| Strategy                                                                 | Target          | Signal            | LOD          | Dynamic Range            | Refs. |
|-------------------------------------------------------------------------|-----------------|-------------------|--------------|--------------------------|-------|
| GO coupled with catalyzed signal cyclic amplification and TMSD          | miRNA-21        | FA                | 1.09 nM      | 10 ~ 330 nM              | [150] |
| GO coupled with ISDPR                                                   | -               | Fluorescence quenching | 2.1 fM       | 4 orders of magnitude    | [40]  |
| Chacogenide-based biosensor                                            |                 |                   |              |                          |       |
| WS₂ nanosheets coupled with DSNSA                                       | -               | Fluorescence quenching | 300 fM       | -                        | [142] |
| MoS₂NS@MBs coupled with DSNSA                                           | -               |                   |              | 4 orders of magnitude    | [152] |
| Nanohybrid-based biosensor                                             |                 |                   |              |                          |       |
| SWCNTs-ox@NDs coupled with HCR                                          | miRNA-21        | Electrochemical    | 1.95 fM      | 0 fM-1.0 nM              | [84]  |
| WO₃-Gr composites coupled with CHA target recycling and ESA             | -               | Electrochemical    | 50 aM        | 0.1 fM ~ 100 pM          | [154] |
| AuNPs@Cu-MOFs coupled with hairpin assembly target recycling            | miRNA-155       | Electrochemical    | 0.35 fM      | 1.0 fM ~ 10 nM           | [159] |
| MWCNT/AuNCs coupled with DSNSA                                          | miRNA-155       | Fluorescence quenching | 33.4 fM     | -                        | [141] |
| PDANSs/AuNCs coupled with DNase-I-assisted target recycling amplification | miRNA-21 and miRNA-let-7a | FRET | 4.2 & 3.6 pM | -                        | [160] |
| Au@CoFe₂O₄/Tb-Gr coupled with P-ERCA                                    | miRNA-21        | Electrochemical    | 0.3 fM       | 1 fM ~ 2 nM              | [155] |
| N-CQDs/GO@Au composite coupled with NESA                                | miRNA-21        | ECL               | 10 aM        | 10 aM ~ 10 nM            | [121] |
| GO-lapped on Fe₂O₃@SiO₂@AuNPs coupled with HCR-assisted cascade amplification | miRNA-141      | ECL               | 30 aM        | -                        | [156] |
| Hollow MoS₂ microcubes coupled with DSN and ECC redox cycling           | miRNA-21        | ECC               | 86 aM        | 0.1 fM ~ 0.1 pM          | [153] |
| MgO nanoflower and GO-AuNPs hybrids coupled with enzyme signal amplification | miRNA-21        | ECC               | 50 aM        | 0.1 ~ 100 fM             | [157] |
| CS-MoS₂, and target recycling amplification of CHA                       | miRNA-21        | Electrochemical    | 16 aM        | 0.1 fM ~ 0.1 nM          | [61]  |
| Nanohybrid-based biosensor                                             |                 |                   |              |                          |       |
| SWCNTs-ox@NDs coupled with HCR                                          | miRNA-21        | Electrochemical    | 1.95 fM      | 0 fM-1.0 nM              | [84]  |
| WO₃-Gr composites coupled with CHA target recycling and ESA             | -               | Electrochemical    | 50 aM        | 0.1 fM ~ 100 pM          | [154] |
| AuNPs@Cu-MOFs coupled with hairpin assembly target recycling            | miRNA-155       | Electrochemical    | 0.35 fM      | 1.0 fM ~ 10 nM           | [159] |
| MWCNT/AuNCs coupled with DSNSA                                          | miRNA-155       | Fluorescence quenching | 33.4 fM     | -                        | [141] |
| PDANSs/AuNCs coupled with DNase-I-assisted target recycling amplification | miRNA-21 and miRNA-let-7a | FRET | 4.2 & 3.6 pM | -                        | [160] |
| Au@CoFe₂O₄/Tb-Gr coupled with P-ERCA                                    | miRNA-21        | Electrochemical    | 0.3 fM       | 1 fM ~ 2 nM              | [155] |
| N-CQDs/GO@Au composite coupled with NESA                                | miRNA-21        | ECL               | 10 aM        | 10 aM ~ 10 nM            | [121] |
| GO-lapped on Fe₂O₃@SiO₂@AuNPs coupled with HCR-assisted cascade amplification | miRNA-141      | ECL               | 30 aM        | -                        | [156] |
| Hollow MoS₂ microcubes coupled with DSN and ECC redox cycling           | miRNA-21        | ECC               | 86 aM        | 0.1 fM ~ 0.1 pM          | [153] |
| MgO nanoflower and GO-AuNPs hybrids coupled with enzyme signal amplification | miRNA-21        | ECC               | 50 aM        | 0.1 ~ 100 fM             | [157] |

(Table 4 contd....)
Kilic et al. [42] argued that a standardized miRNA detection method should have better sensitivity than pM, with high specificity to identify a single nucleotide difference, less than 1-hour assay time, and high-throughput analysis. However, it is already reported that some biosensing systems, including TSA based on coupling DNA–AuNPs with target-triggered cyclic DSN digestion [145], WO$_3$-Gr composites coupled with CHA target recycling and ESA [154], N-CQDs/GO@Au composite coupled with NESA [121], etc, reached the detection limit of the tens of aM, as shown in Table 4.

The next-generation miRNA biosensing should have ultrasensitivity for detecting a single particle in a single cell (50 zM is almost equal to 1 miRNA particle in 30 μL of the specimen), a linear range of 5 ~ 7 orders of magnitude, and high specificity for identifying a single nucleotide difference between miRNA family members, and should be small enough to enable portable POC analysis to be conducted.

As shown in Table 4, the combinational approach-based miRNA detection method has already implemented the linear range of 3 ~ 5 orders of magnitude, ultrasensitivity of the tens of aM as a detection limit, and ultraspécificity for identifying a single nucleotide difference between miRNA family members. Zhang et al. [161] succeeded in the single-molecule analysis of miRNA-21 by designing a smart plasmonic nanobiosensor based on an individual Au@Ag core-shell nanocube (Au@Ag NC) modified with tetrahedron-structured DNA (tsDNA) (Fig. 4). In this system, a single miRNA-21 hybridization event on the nanobiosensor caused an average localized surface plasmon resonance (L-SPR) scattering spectral wavelength shift of approximately 0.4 nm. The system detected miRNA-21 with an aM level sensitivity over a large dynamic range from 1 aM to 1 nM in real-time. In the near future, a more active combinational approach (especially, a combination of physical biosensing with physical signals like surface plasmonic resonance) is expected to realize the requirements of the next generation of miRNA biosensing [162-164].

Fig. (4). The schematic diagram for single-molecule analysis of microRNA and logic operations using a smart plasmonic nanobiosensor [161]. (A higher resolution / colour version of this figure is available in the electronic copy of the article).
CONCLUSION

Since their dysregulation correlates with the development and progress of many diseases, particularly cancer, miRNAs are considered promising biomarkers. Therefore, it is essential to develop an efficient detection system for miRNA and target sequences not only in the basic research of life science but also in the diagnosis and treatment of diseases and the development of customized medicines. Accordingly, various miRNA detection methods have been developed and applied.

In this review, the advantages and disadvantages of traditional, NAA-based, and nanomaterial-based miRNA detection methods were compared and analyzed. Particularly, recent strategies were discussed with emphasis on combinational approaches to improve detection efficiency.

Traditional methods, such as Northern blotting, microarray, and RT-PCR have flaws, such as the labor-intensive nature of multiple steps, time consumption, and high cost. Most of all, they fail to meet the basic requirements of biosensing, including sensitivity, specificity, and biocompatibility. Nucleic acid amplification methods, including IEA, RCA, and LCR, and signal amplification methods based on nanomaterials, such as gold or silver nanoparticles, carbon nanomaterials, TMDCs, and quantum dots can solve these problems. However, they, in turn, have their own strengths and weaknesses. A new strategy based on various combinations of NAA-based and nanomaterial-based techniques has been observed to secure a wide linear range of 3 ~ 5 orders of magnitude and successfully lower the detection limit to tens of attomolar.

MiRNAs are small and feature, however, in only a small fraction (about 1% of total RNAs), a diverse number of copies in cells from 50,000 to several thousand, exhibiting sequence similarity within miRNA family members. Moreover, one miRNA is related to multiple diseases, and one disease is caused by multiple miRNAs.

The requirements of the next-generation miRNA biosensing are ultrasensitivity for detecting a single particle in an individual cell, ultraspecificity for identifying a single nucleotide difference between miRNA family members, and a small size for conducting portable POC analysis. To make this possible, nanotechnology is an alternative avenue to explore. When it is combined with a nucleic acid amplification technique, it is possible to produce a synergistic effect which is expected to realize the goals of the next-generation miRNA biosensing.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

This work was supported by the Seokyeong University in 2020, Korea (Grant number: 2020-0051).

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The author would like to acknowledge the Seokyeong University, Korea.

REFERENCES

[1] Ambros, V. The functions of animal microRNAs. Nature, 2004, 431(7006), 35-35. http://dx.doi.org/10.1038/nature02871 PMID: 15372042
[2] Moazed, D. Small RNAs in transcriptional gene silencing and genome defence. Nature, 2009, 457(7228), 413-420. http://dx.doi.org/10.1038/nature07756 PMID: 19158787
[3] Carthew, R.W.; Sontheimer, E.J. Origins and mechanisms of miRNAs and siRNAs. Cell, 2009, 136(4), 642-655. http://dx.doi.org/10.1016/j.cell.2009.01.035 PMID: 19293886
[4] Crookshanks, H.; Filipowicz, W. Molecular biology: The expanding world of small RNAs. Nature, 2008, 451(7177), 414-416. http://dx.doi.org/10.1038/451414a PMID: 18216846
[5] Dostie, J.; Mourelatos, Z.; Yang, M.; Sharma, A.; Dreyfuss, G. Numerous microRNPs in neuronal cells containing novel microRNAs. RNA, 2003, 9(2), 180-186. http://dx.doi.org/10.1012/rna.2141503 PMID: 12554860
[6] Wang, Y.; Keys, D.N.; Au-Young, J.K.; Chen, C. MicroRNAs in embryonic stem cells. J. Cell. Physiol., 2009, 218(2), 251-255. http://dx.doi.org/10.1002/jcp.21607 PMID: 18821562
[7] Xu, P.; Vernoyy, S.Y.; Guo, M.; Hay, B.A. The Drosophila microRNA Mir-14 suppresses cell death and is required for normal fat metabolism. Curr. Biol., 2003, 13(9), 790-795. http://dx.doi.org/10.1016/S0960-8223(03)00250-5 PMID: 12725740
[8] Bartel, D.P. MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell, 2004, 116(2), 281-297. http://dx.doi.org/10.1016/S0092-8674(04)00045-5 PMID: 14744438
[9] Chen, C.Z.; Li, L.; Lodish, H.F.; Bartel, D.P. MicroRNAs modulate hematopoietic lineage differentiation. Science, 2004, 303(5654), 83-86. http://dx.doi.org/10.1126/science.1091903 PMID: 14657504
[10] Hatfield, S.D.; Shcherbata, H.R.; Fischer, K.A.; Nakahara, K.; Carthew, R.W.; Ruohola-Baker, H. Stem cell division is regulated by the microRNA pathway. Nature, 2005, 435(7044), 974-978. http://dx.doi.org/10.1038/nature03816 PMID: 15944714
[11] Cho, W.C.S. MicroRNAs in cancer - from research to therapy. Biochim. Biophys. Acta, 2010, 1805(2), 209-217. PMID: 19931352
[12] Ruan, K.; Fang, X.; Ouyang, G. MicroRNAs: Novel regulators in the hallmarks of human cancer. Cancer Lett., 2009, 285(2), 116-126. http://dx.doi.org/10.1016/j.canlet.2009.04.031 PMID: 19464788
[13] Kocerha, J.; Kauppinen, S.; Wahlstedt, C. microRNAs in CNS disorders. Neuromolecular Med., 2009, 11(3), 162-172. http://dx.doi.org/10.1007/s12119-009-8066-1 PMID: 19536656
[14] Pandey, A.K.; Agarwal, P.; Kaur, K.; Datta, M. MicroRNAs in diabetes: Tiny players in big disease. Cell. Physiol. Biochem., 2009, 23(4-6), 221-232. http://dx.doi.org/10.1159/000218169 PMID: 19471090
[15] Chen, X.M. MicroRNA signatures in liver diseases. World J. Gastroenterol., 2009, 15(14), 1665-1672. http://dx.doi.org/10.3748/wjg.15.1665 PMID: 19360090
[16] Feng, M.J.; Shi, F.; Qiu, C.; Peng, W.K. MicroRNA-181a, -146a and -146b in spleen CD4+ T lymphocytes play proinflammatory roles in a murine model of asthma. Int. Immunopharmacol., 2012, 13(3), 347-353. http://dx.doi.org/10.1016/j.intimp.2012.05.001 PMID: 22580216
[17] Tsitsiou, E.; Lindsay, M.A. microRNAs and the immune response. Curr. Opin. Pharmacol., 2009, 9(4), 514-520.
A Combinational Approach for More Efficient miRNA Biosensing

Current Genomics, 2022, Vol. 23, No. 1

129(7), 5437-5443.
http://dx.doi.org/10.2174/1876511X129737707141306
Cardoso, A.R.; Moreira, F.T.C.; Fernandes, R.; Sales, M.G.F. Novel and simple electrochemical biosensor monitoring attainable levels of miRNAS in breast cancer. Biosens. Bioelectron., 2016, 80, 621-630.
http://dx.doi.org/10.1016/j.bios.2016.02.035 PMID: 26901495
Lee, H.; Park, J.E.; Nam, J.M. Bio-barcode gel assay for microRNA. Nat. Commun., 2014, 5(1), 3367.
http://dx.doi.org/10.1038/ncomms4367 PMID: 2469571
Labib, M.; Khan, N.; Gholabio, S.M.; Cheng, J.; Pezacki, J.P.; Bereczkovsky, M.V. Three-mode electrochemical sensing of ultralow microRNA levels. J. Am. Chem. Soc., 2013, 135(8), 3027-3038.
http://dx.doi.org/10.1021/ja303821e PMID: 23362834
Hosseini, M.; Akbari, A.; Ganjali, M.R.; Dadmehr, M.; Rezayan, A.H. A novel label-free microRNA-155 detection on the basis of fluorescent silver nanoclusters. J. Fluoresc., 2015, 25(4), 925-929.
http://dx.doi.org/10.1007/s10895-015-1754-5 PMID: 25953605
Chi, B.Z.; Liang, R.P.; Qiu, W.B.; Yuan, Y.H.; Qiu, J.D. Direct fluorescence detection of microRNA based on enzymatically engineered primer extension poly-thymine (EPEFT) reaction using copper nanoparticles as nano-dye. Biosens. Bioelectron., 2017, 87, 216-221.
http://dx.doi.org/10.1016/j.bios.2016.08.042 PMID: 27566394
Borghei, Y.S.; Hosseini, M.; Ganjali, M.R.; Hosseinkhani, S. Label-free fluorescent detection of microRNA-155 based on synthesis of hairpin DNA-templated copper nanoclusters by etching (top-down approach). Sens. Actuators B Chem., 2017, 249, 133-139.
http://dx.doi.org/10.1016/j.snb.2017.03.148
Medintz, I.L.; Uyeda, H.T.; Goldman, E.R.; Mattoussi, H. Quantum dot bioconjugates for imaging, labelling and sensing. Nat. Mater., 2005, 4(6), 455-466.
http://dx.doi.org/10.1038/nmat1390 PMID: 15928695
Huang, X.; Ren, J. “Nanomaterial-based chemiluminescence resonance energy transfer: A strategy to develop new analytical methods,” TAC-Trend. Anal. Chem., 2012, 40, 77-89.
http://dx.doi.org/10.1016/j.talanta.2015.07.059 PMID: 26388371
Deng, H.; Liu, Q.; Wang, X.; Huang, R.; Liu, H.; Lin, Q.; Zhou, X.; Xing, D. Quantum dots-labeled strip biosensor for rapid and sensitive detection of microRNA based on target-recycled nonenzymatic amplification strategy. Biosens. Bioelectron., 2017, 87, 931-940.
http://dx.doi.org/10.1016/j.bios.2016.09.043 PMID: 27664413
Lv, S.; Chen, F.; Chen, C.; Chen, X.; Gong, H.; Cai, C. 2-amino-phenylurea probe in combination with catalyzed hairpin assembly signal amplification for simple and sensitive detection of microRNA. Talanta, 2017, 174, 336-340.
http://dx.doi.org/10.1016/j.talanta.2017.06.028 PMID: 28738589
Zhang, H.; Wang, Q.; Yang, X.; Wang, K.; Li, Q.; Li, Z.; Gao, L.; Nie, W.; Zheng, Y. An isothermal electrochemical biosensor for the sensitive detection of microRNA based on a catalytic hairpin assembly and suprasandwich amplification. Analyst (Lond.), 2017, 142(2), 389-396.
http://dx.doi.org/10.1039/C6AN02390H PMID: 28009023
Wu, X.Y.; Chai, Y.Q.; Yuan, R.; Zhou, Y.; Chen, Y. Dual signal amplification strategy for enzyme-free electrochemical detection of microRNAs. Sens. Actuators B Chem., 2014, 203, 296-302.
http://dx.doi.org/10.1016/j.snb.2014.06.131
Zhang, P.; Wu, X.; Chai, Y.; Yuan, R. An electrochemiluminescent microRNA biosensor based on hybridization chain reaction coupled with hemin as the signal enhancer. Analyst (Lond.), 2014, 139(11), 2748-2753.
http://dx.doi.org/10.1039/C4AN00284A PMID: 24722579
Yan, Y.; Shen, B.; Wang, H.; Sun, X.; Cheng, W.; Zhao, H.; Ju, H.; Ding, S. A novel and versatile nanomachine for ultrasensitive and specific detection of microRNAs based on molecular beacon initiated strand displacement amplification coupled with catalytic hairpin assembly with DNAzyme formation. Analyst (Lond.), 2015, 140(16), 5469-5474.
http://dx.doi.org/10.1039/C5AN00920K PMID: 26134555
Deng, R.; Tang, L.; Tang, Q.; Wang, Y.; Lin, L.; Li, J. Toehold-initiated rolling circle amplification for visualizing individual microRNAs in situ in single cells. Angew. Chem. Int. Ed. Engl., 2014, 53(8), 2389-2393.
http://dx.doi.org/10.1002/anie.201309388 PMID: 24469913
Fan, Y.; Chen, X.; Trigg, A.D.; Tung, C.H.; Kong, J.; Gao, Z. Detection of MicroRNAs using target-guided formation of conducting polymer nanowires in nanopores. J. Am. Chem. Soc., 2007, 129(7), 5437-5443.
composites as a novel fluorescence quenching platform. Sens. Actuators B Chem., 2018, 266, 221-227.
http://dx.doi.org/10.1016/j.snb.2018.03.071

[142] Xi, Q.; Zhou, D.M.; Kan, Y.Y.; Ge, J.; Wu, Z.K.; Yu, R.Q.; Jiang, J.H. Highly sensitive and selective strategy for microRNA detection based on WS2 nanosheet mediated fluorescence quenching and duplex-specific nuclease signal amplification. Anal. Chem., 2014, 86(3), 1361-1365.
http://dx.doi.org/10.1021/ac403944c PMID: 24446758

[143] Tian, B.; Ma, J.; Qiu, Z.; Zardán Gómez de la Torre, T.; Donolato, M.; Hansen, M.F.; Svedlindh, P.; Strömberg, M. Optomagnetic detection of MicroRNA based on duplex-specific nuclease-assisted target recycling and multilayer core-satellite magnetic superstructures. ACS Nano, 2017, 11(2), 1798-1806.
http://dx.doi.org/10.1021/acsnano.6b07676 PMID: 28176611

[144] Zhang, Y.; Zhang, C.Y. Sensitive detection of microRNA with isothermal amplification and a single-quantern-dot-based nanosensor. Anal. Chem., 2012, 84(1), 224-231.
http://dx.doi.org/10.1021/ac202405g PMID: 22103863

[145] Bo, B.; Zhang, T.; Jiang, Y.; Cui, H.; Miao, P. Triple signal amplification for ultrasensitive determination of miRNA based on duplex specific nuclease and bridge DNA-Gold nanoparticles. Anal. Chem., 2018, 90(3), 2395-2400.
http://dx.doi.org/10.1021/acs.analchem.7b07401 PMID: 29127312

[146] Yuan, R.; Yu, X.; Zhang, Y.; Xu, L.; Cheng, W.; Tu, Z.; Ding, S.; Tu, Z.; Ding, S. DNAzyme-modulated double quenching for ultrasensitive microRNA detection of MicroRNA based on duplex-specific nuclease-assisted target recycling and multilayer core-satellite magnetic superstructures. ACS Nano, 2017, 11(2), 1798-1806.
http://dx.doi.org/10.1021/acsnano.6b07676 PMID: 28176611

[147] Nakayama, S.; Sintim, H.O. Isothermal amplified detection of microRNA via strand displacement reaction and metal-organic frameworks. Sens. Actuators B Chem., 2018, 257, 406-411.
http://dx.doi.org/10.1016/j.snb.2018.03.071

[148] Yuan, R.; Yu, X.; Zhang, Y.; Xu, L.; Cheng, W.; Tu, Z.; Ding, S. Target-triggered DNA nanoassembly on quantum dots and DNAzyme-modulated double quenching for ultrasensitive microRNA biosensing. Biosens. Bioelectron., 2017, 92, 342-348.
http://dx.doi.org/10.1016/j.bios.2016.11.002 PMID: 27836609

[149] Yang, J.J.; Zhang, Z.F.; Yan, G.Q. Facile detection of microRNA based on phosphorescence resonance energy transfer and duplex-specific nuclease-assisted signal amplification. Anal. Biochem., 2017, 539, 127-133.
http://dx.doi.org/10.1016/j.ab.2017.10.021 PMID: 29107578

[150] Jing, T.; Xue, X.; Li, C.H.; Chen, M.; Zhen, S.J. A new graphene oxide enhanced fluorescence anisotropy strategy for microRNA detection. Scientia Sinica Chimica, 2018, 48(1), 85-92.
http://dx.doi.org/10.1007/s11431-017-9245-1

[151] Wang, Y.H.; Huang, K.J.; Wu, X. Recent advances in transition-metal dichalcogenides based electrochemical biosensors: A review. Biosens. Bioelectron., 2017, 97, 305-316.
http://dx.doi.org/10.1016/j.bios.2016.10.011 PMID: 28621207

[152] Xiao, M.; Tan, M.; Zhu, C.; Pei, H.; Shi, J.; Li, L.; Qu, X.; Shen, X.; Li, J. MoS2 nanoprobe for microRNA quantification based on duplex-specific nucleic acid signal amplification. ACS Appl. Mater. Interfaces, 2018, 10(9), 7852-7858.
http://dx.doi.org/10.1021/acsami.7b17894 PMID: 29431420

[153] Shuai, H.L.; Huang, K.J.; Chen, Y.X.; Fang, L.X.; Jia, M.P. Au nanoparticles/hollow molybdenum disulfide micorubes based biosensor for microRNA-21 detection coupled with duplex-specific nucleic acid and enzyme signal amplification. Biosens. Bioelectron., 2017, 89(2), 989-997.
http://dx.doi.org/10.1016/j.bios.2016.10.051 PMID: 27825521

[154] Shuai, H.L.; Huang, K.J.; Xing, L.L.; Chen, Y.X. Ultrasensitive electrochemical sensing platform for microRNA based on tungsten oxide-graphene composites coupling with catalyzed hairpin assembly target recycling and enzyme signal amplification. Biosens. Bioelectron., 2016, 66, 337-345.
http://dx.doi.org/10.1016/j.bios.2016.06.057 PMID: 27392235

[155] Yu, N.; Wang, Z.; Wang, C.; Han, J.; Bu, H. Combining padlock exponential rolling circle amplification with CoFeO2 magnetic nanoparticles for microRNA detection by nanoelectrocatalysis without a substrate. Anal. Chim. Acta, 2018, 102(2), 243-301.
http://dx.doi.org/10.1016/j.aca.2017.01.069 PMID: 28231877

[156] Lu, J.; Wu, L.; Hu, Y.; Wang, S.; Guo, Z. Ultrasensitive Faraday cage-type electrochemiluminescence assay for femtomolar microRNA-141 via graphene oxide and hybridization chain reaction-assisted cascade amplification. Biosens. Bioelectron., 2018, 109, 13-19.
http://dx.doi.org/10.1016/j.bios.2018.02.062 PMID: 29529269

[157] Wang, H.; Jian, Y.; Kong, Q.; Liu, H.; Lan, F.; Liang, L.; Ge, S.; Yu, J. Ultrasensitive electrochemical paper-based biosensor for microRNA via strand displacement reaction and metal-organic frameworks. Sens. Actuators B Chem., 2018, 257, 561-569.
http://dx.doi.org/10.1016/j.snb.2017.10.188

[158] Zhao, Q.; Piao, J.; Peng, W.; Wang, Y.; Zhang, B.; Gong, X.; Chang, J. Simple and sensitive quantification of microRNAs via FTS@Au microspheres-based DNA probes and DSN-assisted signal amplification platform. ACS Appl. Mater. Interfaces, 2018, 10(4), 3324-3332.
http://dx.doi.org/10.1021/acsami.7b16733 PMID: 29300448

[159] Wang, H.; Jian, Y.; Kong, Q.; Liu, H.; Lan, F.; Liang, L.; Ge, S.; Yu, J. Ultrasensitive electrochemical paper-based biosensor for microRNA via strand displacement reaction and metal-organic frameworks. Sens. Actuators B Chem., 2018, 257, 561-569.
http://dx.doi.org/10.1016/j.snb.2017.10.188

[160] Xiao, M.; Nie, Y.; Jiang, L.; Wang, J.; Xu, G.; Wang, W.; Luo, X. Polydopamine nanosphere/gold nanocluster (Au NC)-based nano-platform for dual color simultaneous detection of multiple tumor-related microRNAs with DSNase-l-assisted target recycling amplification. Anal. Chem., 2018, 90(6), 4039-4045.
http://dx.doi.org/10.1021/acs.analchem.7b05253 PMID: 29488338

[161] Zhang, Y.; Shuai, Z.; Zhou, H.; Luo, Z.; Liu, B.; Zhang, Y.; Zhang, L.; Chen, S.; Chao, J.; Wang, L.; Fan, Q.; Fan, C.; Huang, W.; Wang, L. Single-molecule analysis of microRNA and logic operations using a smart plasmonic nanobiosensor. J. Am. Chem. Soc., 2018, 140(11), 3988-3993.
http://dx.doi.org/10.1021/jacs.7b12772 PMID: 29504757

[162] Zeng, K.; Li, H.; Peng, Y. Gold nanoparticle enhanced surface plasmon resonance imaging of microRNA-155 using a functional nucleic acid-based amplification machine. Microchim. Acta, 2017, 4(8), 1-8.
http://dx.doi.org/10.1007/s00604-017-2276-2

[163] Jebeili, A.; Oroojalian, F.; Fathi, F.; Mokhtarzadeh, A.; Guardia, M. Recent advances in surface plasmon resonance biosensors for microRNA detection. Biosens. Bioelectron., 2020, 169, 112599.
http://dx.doi.org/10.1016/j.bios.2020.112599 PMID: 32931990

[164] Singh, M.K.; Pal, S.; Prajapati, Y.K.; Saini, J.P. Highly sensitive antimonene based SPR biosensor for miRNA detection. Mater. Today Proc., 2020, 28, 1776-1780.
http://dx.doi.org/10.1016/j.matpr.2020.05.183