Biosensors and Bioelectronics 68 (2015) 83–94

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Electrochemical sensing of microRNAs: Avenues and paradigms

Mahmoud Labib, Maxim V. Berezovski *

Department of Chemistry, University of Ottawa, 10 Marie Curie, Ottawa, Canada ON K1N 6N5

ARTICLE INFO

Article history:
Received 1 October 2014
Received in revised form
24 November 2014
Accepted 9 December 2014
Available online 16 December 2014

Keywords:
Amperometric
Biosensor
Conductometric
Electrochemical
Field-effect transistor
Impedimetric
MicroRNA
Potentiometric
Voltammetric

ABSTRACT

Twenty years has passed since the first discovery of microRNA (miRNA) lin-4 in *Caenorhabditis elegans*. Over the last two decades, the study of miRNAs has attracted tremendous attention. These new stars of biomarkers are naturally occurring non-coding RNAs that regulate gene expression posttranscriptionally and have been demonstrated to be dysregulated in many diseases. Since their profiles reflect pathological conditions, miRNAs have recently been proposed as biomarkers of the onset, prognosis and risk of diseases, as well as in the classification of different types of cancer. The establishment of miRNA profiles for diseases and the detection of different types and levels of miRNAs in biological samples are therefore critical milestones in diagnostics. This provides powerful impetus and a growing demand for researchers to develop simple analytical techniques to allow for an accurate, sensitive, selective, and cost effective miRNA analysis at point-of-care settings. Among several methods proposed for miRNA detection, electrochemical nucleic acid biosensors exhibit many attractive features and could play a leading role in future miRNA detection and quantification. This review gives an overview of recent advances in the rapidly growing area of electrochemical detection of miRNAs. The fundamentals of the different strategies adopted for miRNA detection are discussed and some examples of relevant approaches are highlighted, along with future prospects and challenges.

Contents

1. Introduction

RNA is one of three major macromolecules, including DNA and proteins, that are essential for all forms in life. There are several types of RNA in the body: messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), small nuclear RNA (snRNA), and non-coding RNA (ncRNA) which is not translated into proteins. Once class of ncRNAs is microRNAs (miRNAs) (Cheng et al., 2005). miRNAs are ~22 nucleotide-long ncRNA molecules that regulate the expression of target genes at the post-transcriptional level by either translational repression or degradation of mRNAs (Ambros, 2004). It has been estimated that the human genome may encode for over 1000 miRNAs, which are thought to regulate about 60% of the mammalian genes (Friedman et al., 2009). Altered expression patterns of miRNAs have been associated with many diseases, including several types of cancer (Lawrie et al., 2008), cardiovascular disorders (Edwards et al., 2010), diabetes (Nielsen et al., 2012),

* Corresponding author.
E-mail address: Maxim.Berezovski@uottawa.ca (M.V. Berezovski).

http://dx.doi.org/10.1016/j.bios.2014.12.026
0956-5663/

rheumatic diseases (Alevizos and Illei, 2010), and neurological disorders (Miller and Wahlestedt, 2010). Since unique miRNA expression signatures have been demonstrated in many pathological conditions, it is not surprising that these molecules have been proposed as novel biomarkers in diagnostics (Keller et al., 2009). In addition, determination of miRNA profiles in clinical samples will not only signify the presence of a disease, it can also help in the prediction of its course and provision of personalized treatments (Zhang et al., 2009b). These miRNAs were detected in blood serum, plasma, urine, saliva, semen, sweat, and other body fluids. Once thought of as unstable RNA molecules, circulating miRNAs are in fact highly stable in blood inside exosomes or in a complex with an RNA binding protein, Nucleophosmin1 (Zen and Zhang, 2012). Typical levels of circulating miRNAs in serum were estimated to be in the 200 aM to 20 pM range (Tsujiura et al., 2010). Detection of miRNAs is challenging because of their short and highly homologous sequences, low concentrations and wide dynamic range, large variation in base composition (G/C or A/T rich), and its secondary structure. For instance, miRNA profiles for pancreatic cancer have been reported by four independent research groups with discrepancies between the results (Bloomston et al., 2007; Lee et al., 2007; Roldo et al., 2006; Zhang et al., 2009b). Therefore, discovering miRNAs that could be consistently used as biomarkers for a specific diseases along with the development of accurate and inexpensive miRNA diagnostics are some of the current challenges for using miRNA signatures in clinical settings. In addition, data reproducibility is also a challenge, since the results obtained using the same profiling technique are not always consistent (Sato et al., 2009). Nevertheless, detection of miRNAs in diagnostics is solely based on the variation of their expression profiles in patients and healthy subjects.

To date, several analytical approaches have been developed for miRNA detection, including Northern blot analysis (Valoczi et al., 2004), real-time PCR methods (Chen et al., 2005), microarrays (Thomson et al., 2004), *in situ* hybridization (de Planell-Saguer et al., 2010), bioluminescence-based methods (Cissell et al., 2008), fluorescence correlation spectroscopy (FCS) (Neely et al., 2006), surface-enhanced Raman spectroscopy (SERS) (Driskell et al., 2008), surface Plasmon resonance imaging (SPRI) (Fang et al., 2006), and high-throughput sequencing techniques (Schulte et al., 2010). Electrochemical biosensors hold a great promise to serve as suitable devices for point-of-care diagnostics (Labib et al., 2011a, 2011b, 2011c, 2013c, 2012a, 2012b, 2012c). They can allow for a simple, rapid, sensitive, and inexpensive multiplexed analysis of nucleic acids. This review summarizes and evaluates recent developments of electrochemical biosensors for miRNA analysis.

2. Electrochemical biosensors for microRNA analysis

In this section we will focus on the different types of electrochemical biosensors developed for miRNA analysis, including amperometric, voltammetric, potentiometric, conductometric, impedimetric, and field-effect transistor-based biosensors.

2.1. Amperometric and voltammetric biosensors

Amperometry allows the detection of an analyte by measuring the current at a constant applied potential and relating the measured current to the analyte concentration. Whereas, voltammetry allows measuring the current as the potential is ramped at a given rate. Both techniques have been extensively used for miRNA analysis even simultaneously at several occasions and hence they were included in one subsection. Due to the small size of miRNAs, direct labeling of the target miRNAs coupled with electrocatalytic amplification offers an amenable approach to enhance the sensitivity of detection compared to other conventional techniques. Transduction often relies on an electrochemically active reporter species, whose properties can be used to infer changes in interfacial properties caused by miRNA hybridization. Commonly used reporters include small molecules and enzyme–substrate pairs. Gao and Yang reported an amperometric assay for measurement of miRNAs (Gao and Yang, 2006). In this assay, an electrocatalytic system was formed by hybridizing a periodate-treated target miRNA with an oligonucleotide capture probe immobilized onto an indium tin oxide (ITO) electrode followed by chemical modification of the captured miRNA with isoniazid-capped OsO_2 nanoparticles *via* a condensation reaction. An amplified signal was obtained as a result of the electrocatalytic oxidation of hydrazine at −0.1 V, which allowed for miRNA analysis with a detection limit of 80 fM. In 2007, Gao and Yu covalently modified the target miRNA with an isoniazid-substituted osmium complex, $Os(4,4'\text{-dimethyl-}2,2'\text{-bipyridine})_2(IN)Cl^+$, *via* a condensation reaction (Gao and Yu, 2007b). The tagged miRNA from a total RNA sample was captured by complementary oligonucleotide capture probe immobilized onto the gold surface. An amplified signal was gained due to the electrocatalytic oxidation of ascorbic acid and allowed for miRNA detection at levels down to 800 fM. In addition, the same group attached an electrocatalytic moiety, $Ru(1,10\text{-phenanthroline-}5,6\text{-dione})_2Cl_2$, to the target miRNA in a total RNA sample *via* coordinative bonds with the purine bases in the miRNA molecule (Gao and Yu, 2007a). Subsequently, the labeled target miRNAs were hybridized with immobilized complementary oligonucleotides immobilized onto the surface of an ITO electrode. The electrocatalytic oxidation of hydrazine allowed for an ultrasensitive interrogation of the miRNA level with a detection limit of 0.2 pM. In 2010, Peng and Gao developed an amperometric miRNA biosensor based on a self-assembled monolayer (SAM) of peptide nucleic acid (PNA) capture probes immobilized onto a gold electrode (Peng et al., 2010). The target miRNA molecules were modified with RuO_2 nanoparticles which served as a catalyst for polymerization of aniline. Upon hybridization with PNA capture probes, the formed hybrid acted as a template for the deposition of polyanailine. It is noteworthy that PNA was utilized as a capture probe to alleviate the electrostatic interaction with the cationic aniline molecules in the buffer and subsequently reduce the background current. This amplification-by-polymerization technique allowed for a sensitive analysis of miRNA with a detection limit of 2 fM.

In 2012, Yin et al. reported a highly sensitive label free electrochemical miRNA biosensor based on dendritic gold nanostructures and graphene nanosheets modified glassy carbon electrode (Yin et al., 2012). In this work, a thiol-modified locked nucleic acid (LNA) hairpin molecular beacon (MB) was utilized as a capture probe. After hybridization with the target miRNA, the formed duplex was incubated with a short detection probe complementary to the distal terminus of the capture probe and modified with biotin coated gold nanoparticles. Subsequently, streptavidin-modified horseradish peroxidase was brought to the electrode surface through the specific interaction with the biotin tag of the gold nanoparticles to catalyze the chemical oxidation of hydroquinone to benzoquinone in presence of H_2O_2, as shown in Fig. 1. The developed biosensor exhibited excellent sensitivity and low detection limit of 0.06 pM. In 2013, Bettazzi et al. developed an amperometric biosensor for miRNA detection based on paramagnetic beads and enzyme amplification (Bettazzi et al., 2013). The proposed assay is based on biotinylated DNA capture probes immobilized on streptavidin-coated paramagnetic beads. Total RNA was extracted from a cell sample, enriched for small RNAs, biotinylated, and then hybridized with the immobilized capture probes. This was followed by incubation with streptavidin-labeled alkaline phosphatase and exposure to the enzyme–substrate,

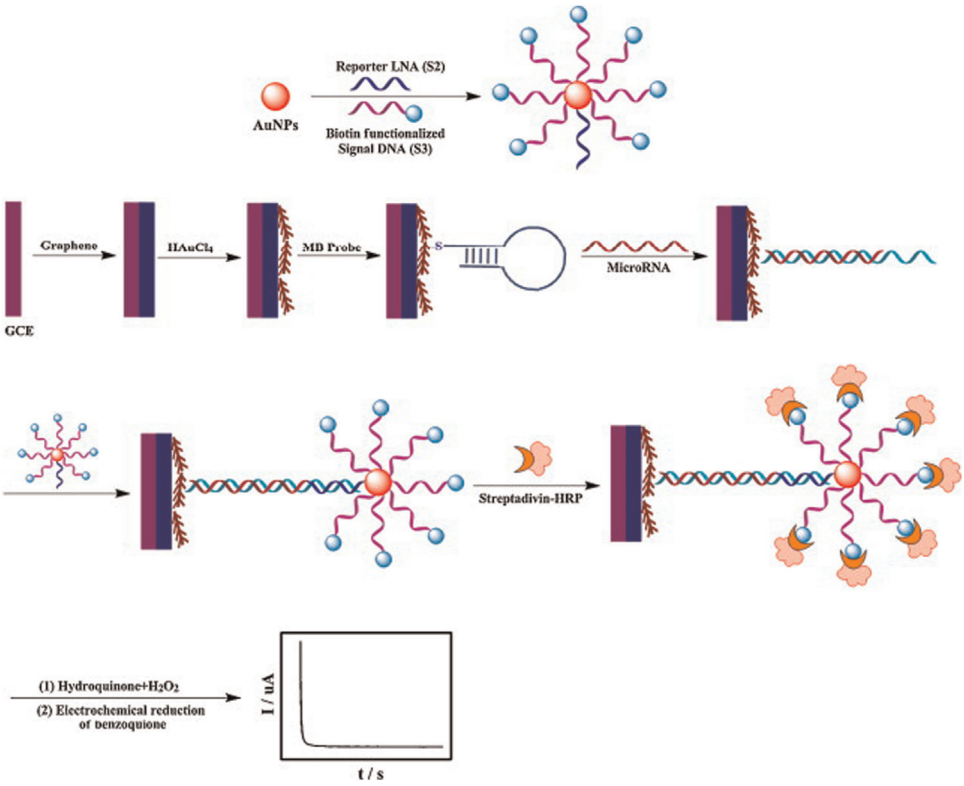

Fig. 1. Electrochemical amperometric analysis of miRNA based on graphene nanosheets, locked nucleic acid (LNA) integrated molecular beacon, gold nanoparticles, biotin multifunctional bio barcodes and enzymatic assay. From Yin et al. (2012), with permission.

α-naphthyl phosphate. The product of the enzymatic reaction, α-naphthol, was electrochemically measured which allowed for a sensitive detection of miRNA levels down to 7 pM.

A different strategy for amperometric miRNA detection has eliminated the need for ligation and PCR amplification. On this note, a miRNA biosensor was described based on a stem-loop oligonucleotide consisting of a miRNA complementary segment at one end and a capturing segment for a glucose oxidase-labeled PNA detection probe at the other end. Upon hybridization with its target miRNA, the stem loop is unlocked exposing the detection probe capturing segment. A subsequent hybridization with the detection probe brings the activated glucose oxidase onto the electrode surface. The activated glucose oxidase demonstrated high catalytic activity towards electrooxidation of glucose in the measurement buffer. Based on this scheme, the number of glucose oxidase molecules correlates to the number of hybridized miRNA molecules, allowing for a simple and straightforward quantification of miRNA with detection limits of 4 fM (Gao, 2012) and 10 fM (Gao and Peng, 2011). In 2013, Cai et al. reported a label-free electrochemical biosensor based on functional allosteric molecular beacons (Cai et al., 2013). In the presence of the target miRNA, horseradish peroxidase was captured by the formed aptamer to catalyze the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) by H_2O_2. Measurement of the reduction current allowed for the quantification of extremely low miRNA concentrations down to 13.6 aM. Pöhlmann and Sprinzl reported a rapid and sensitive gap hybridization assay for detection of miRNAs based on four components DNA/RNA hybridization and electrochemical detection using esterase 2-oligodeoxynucleotide conjugates (Pohlmann and Sprinzl, 2010). The gab hybridization assay is based on the complementary binding of miRNA to a gap built of capture and detection oligonucleotides which produce an electrochemical signal when the reporter enzyme is brought to the vicinity of the electrode. In the absence of miRNA, the gap between capture and detection probes is not filled, and the missing base stacking energy destabilizes the hybridization complex. The gab hybridization assay allowed for an ultrasensitive interrogation of the miRNA level with a detection limit of 2 aM.

In 2013, Wu and his colleagues described an amperometric miRNA biosensor based on a conductive self-assembled multilayer of nafion, thionine and palladium nanoparticles (Wu et al., 2013). In this work, a bare glassy carbon electrode was first coated with nafion and subsequently thionine was adsorbed by the cation-exchanger, nafion. Afterward, the palladium nanoparticles were attached to the amino groups of the thionine molecules and used to immobilize the capture oligonucleotides. Upon hybridization with the target miRNA, an excellent electrocatalytic activity toward H_2O_2 reduction was obtained, allowing a linear quantification of miRNA with a detection limit of 1.87 pM. In 2013, Wang et al. published a significant report concerning an amperometric biosensor based on a home-made electrically magnetic-controllable gold electrode (Wang et al., 2013). The use of this electrode combines the merits of heated and magnetic electrodes since the temperature of the electrode surface and direction of magnetic field can be easily regulated. Briefly, two probes were designed according to the "junction-probe" strategy proposed by the coauthors. The first probe is a capture probe functionalized with a biotin group at one end and was immobilized on the surface of streptavidin-coated magnetic beads. The second probe is a signal probe modified with biotin tag at both ends. In the presence of target miRNA, both probes can hybridize to form a ternary "Y" junction structure on the surface of the magnetic beads. The hybrid-attached magnetic beads can capture streptavidin-horseradish peroxidase by the biotinylated signal probe. After the capture of streptavidin-horseradish peroxidase, the resulting complex catalyzes the oxidation of TMB by H_2O_2. Then, the tagged magnetic beads were adsorbed on the surface of the electrically magnetic-controllable working electrode by adding a voltage on the electric

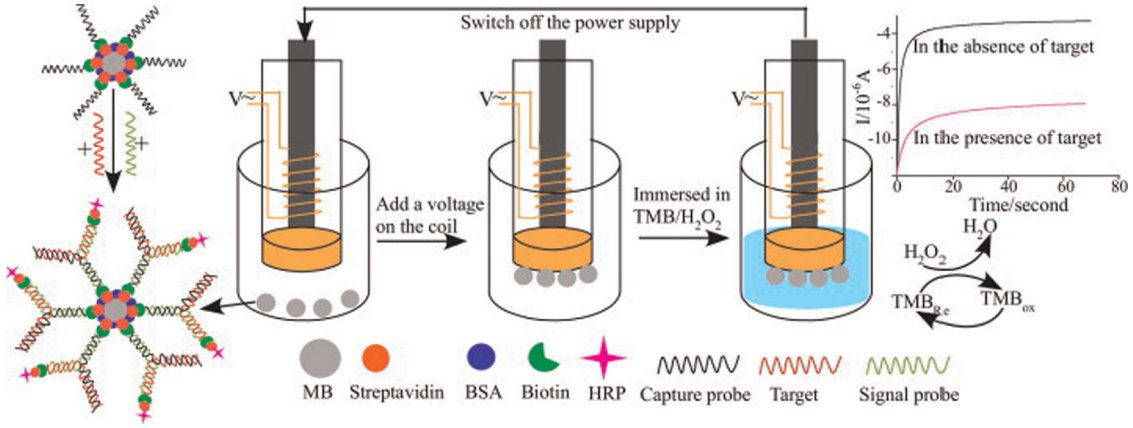

Fig. 2. A schematic representation of the magnetic-controllable electrochemical miRNA assay based on a home-made electrically magnetic-controllable gold electrode. From Wang et al. (2013), with permission.

coil, and the working electrode was then used for electrochemical measurement. Upon dipping the working electrode into the TMB–H_2O_2 solution, the enzyme catalyzes the H_2O_2-mediated oxidation of TMB, giving rise to an increasing current intensity, as shown in Fig. 2. Incorporation of the "junction probe" strategy with magnetic bead-based enzymatic catalysis allowed for an exceptional ultralow detection limit of 0.2 aM.

In 2009, Lusi and his colleagues reported a miRNA biosensor based on the guanine oxidation after hybridization between the inosine-modified capture probes and the target miRNA (Lusi et al., 2009). The developed biosensor allowed a linear quantification of miRNA with a detection limit of 0.1 pM. In 2009, Yang and his coworkers described a multiplexed chip for miRNA detection using an electrode pattern generated by photolithography (Yang et al., 2009). This chip was made by depositing a pattern of gold on the surface of a silicon wafer to provide a multiplexed set of external contacts. Subsequently, a layer of silicon oxide was deposited on the gold layer to passivate the metal. A SAM of thiol-modified PNA capture probes was formed on the chip surface. The approach relied on the primary electron acceptor $[Ru(NH_3)_6]^{3+}$, which is attracted electrostatically to the electrode surface at levels that are correlated with the amount of bound probe molecules. The inclusion of $[Fe(CN)_6]^{3-}$ during the electrochemical measurement serves to regenerate the Ru^{III} and allow multiple reductions per metal center. Subsequently, the Fe^{III} is repelled electrostatically from the electrode and undergoes chemical reduction by Ru^{II} (Lapierre et al., 2003). Upon hybridization with the target miRNA, an electrocatalytic signal was generated allowing for a sensitive quantification of miRNA with a detection limit of 10 fM.

In 2012, Wang and his colleagues explored the possibility of analyzing miRNAs through a competitive hybridization approach (Wang et al., 2012). In this work, a biotin-tagged miRNA whose sequence is identical to that of the target miRNA was introduced into the tested sample and allowed to displace the target miRNA from its hybrid with an oligonucleotide capture probe immobilized onto the electrode surface. Subsequently, the newly formed biotin-tagged hybrid was quantified using ferrocene-labeled gold nanoparticles/streptavidin conjugates. The ferrocene oxidation current was inversely proportional to the miRNA concentration and allowed for a detection limit of 10 fM. In 2012, Kilic et al. described a miRNA voltammetric assay based on the immobilization of the oligonucleotide capture probes onto a pencil graphite electrode (PGE) using a mixture of *N*-(dimethylamino)propyl-*N*-ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxysulfosuccinimide (NHS) as a coupling agent (Kilic et al., 2012). Upon hybridization with a biotin-tagged target miRNA, a streptavidin-labeled alkaline phosphatase enzyme was brought to the sensor surface *via* the interaction between the avidin and biotin molecules. Subsequently, the electrode surface was exposed to the electro-inactive enzyme–substrate, α-naphthyl phosphate, as shown in Fig. 3. The product of the enzymatic reaction, α-naphthol, was electrochemically oxidized at +23 V *vs.* Ag/AgCl which allowed for a sensitive analysis of miRNA with a detection limit of 0.67 μg mL^{-1}. In 2012, Dong and his coworkers reported a voltammetric biosensor based on oligonucleotide encapsulated silver nanoclusters (Ag-NCs) (Dong et al., 2012). In this work, a SAM of a molecular beacon (MB) probe was formed onto a gold electrode surface. A functional oligonucleotide probe was formed and it comprised a recognition sequence for hybridization and a template sequence for *in situ* formation of Ag-NCs. In the presence of the target miRNA, the MB probe hybridized with the target miRNA and oligonucleotide encapsulated Ag-NCs were brought to the surface to produce a detection signal, in response to H_2O_2 reduction. The developed assay allowed the detection of as low as 67 fM of miRNA. In addition, this work represents the first application of the electrocatalytic activity of Ag-NCs in bioanalysis.

In 2013, Labib and his coworkers developed a novel three-mode electrochemical biosensor for miRNA detection that brought together the advantages of electrochemical genosensors and the unique binding properties of p19 protein (from Carnation Italian Ringspot Virus, CIRV) to small double-stranded RNAs (Labib et al., 2013b). In this work, the developed biosensor was based on the self-assembly of a thiol-modified RNA capture probe onto gold nanoparticles-modified screen-printed carbon electrode (GNPs-SPGE). The three-way analysis can detect one or multiple miRNAs by a hybridization-based modality (H-SENS), a protein-based modality (P-SENS), and a universal displacement-based modality (D-SENS). An electrocatalytic reporter of $[Ru(NH_3)_6]^{3+}$/$[Fe(CN)_6]^{3-}$ was utilized for measurements to amplify the current signal. In the H-SENS, the hybridization of the target miRNA to its complementary immobilized probe caused an increase in the current signal due to the increase in the number of Ru^{III} cations electrostatically attracted to the anionic phosphate groups of the RNA molecules. However, addition of the p19 protein dimer to the formed hybrid caused a large decrease in current density, thus amplifying the signal. A universal displacement-based sensor (D-SENS) was formed on the basis of the self-assembled RNA capture probe bound to the saturation concentration of a miRNA, whereas the p19 was attached to the formed hybrid. Subsequently, a mixture of a target miRNA and a non-thiolated RNA probe was incubated with the p19-modified sensor. In the presence of the target miRNA, a newly formed hybrid at relatively higher concentration can force the p19 to dissociate from the surface attached hybrid and to bind to the added hybrid causing an increase

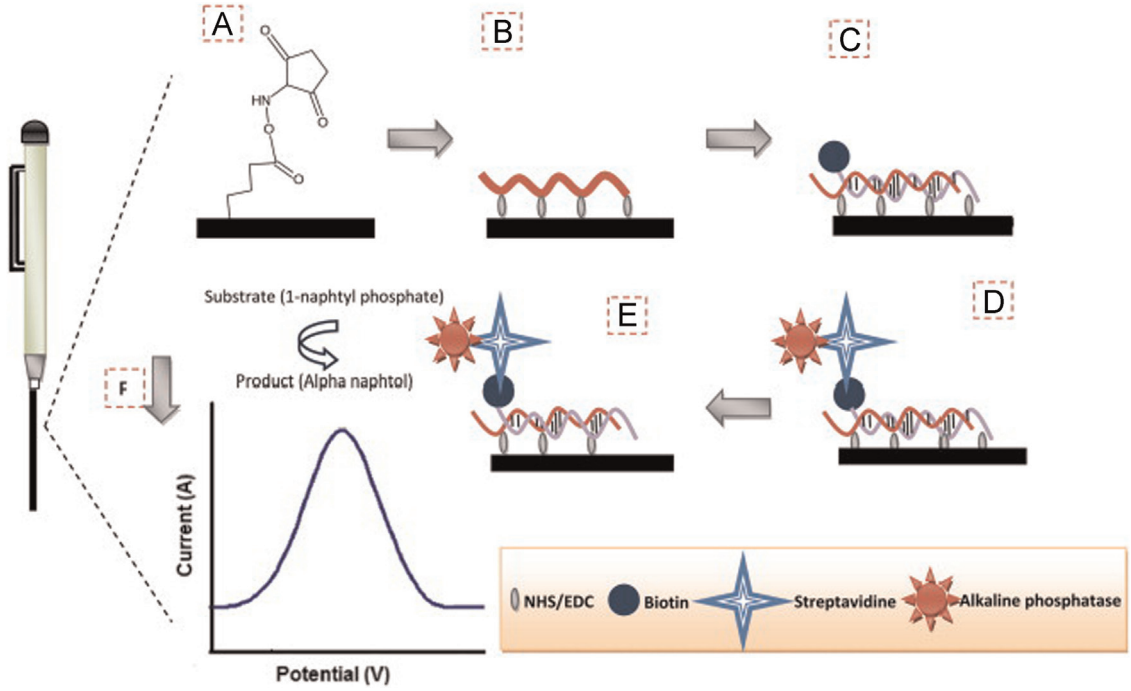

Fig. 3. Schematic diagram of the steps involved in the fabrication of a miRNA biosensor, including (A) surface activation with the pencil graphite electrode (PGE) with EDC/NHS, (B) probe immobilization onto the PGE surface, (C) hybridization of the probe with biotinylated target miRNA, (D) capture of the streptavidin-labeled alkaline phosphatase enzyme, and (E) enzyme–substrate interaction and production of α-naphthol. From Kilic et al. (2012), with permission.

in the current density, as shown in Fig. 4. Therefore, this displacement-based sensor can be used for detection of any type of miRNAs and eliminates the need for thiol-modified capture probes. The developed biosensor combining the three analytical modalities allowed for a broad dynamic range of miRNA concentrations with a detection limit of 5 aM.

The three mode analysis technique was recently adopted by Tran et al. who developed a miRNA biosensor based on

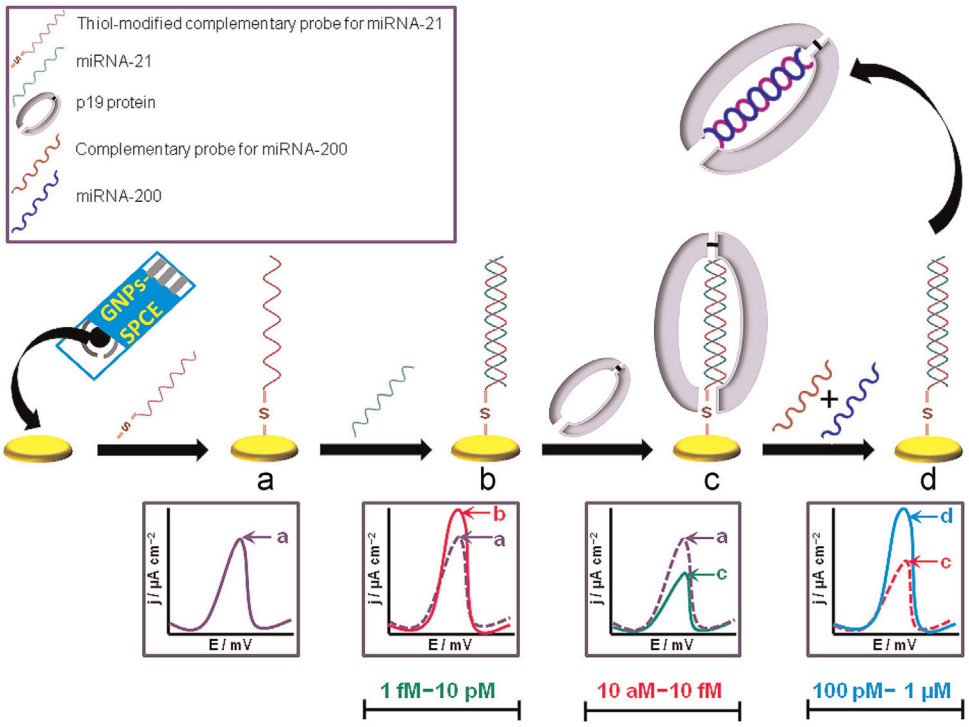

Fig. 4. Schematic representation of the three-mode electrochemical sensor for detection of miRNAs. (a) A thiol-modified complementary probe for miR-21 was self-assembled onto a gold nanoparticles-modified screen-printed carbon electrode (GNPs-SPCE). (b) A hybridization-based sensor (H-SENS) where the binding of the target miR-21 caused an increase in the current intensity, measured by square wave voltammetry (SWV). (c) A p19 protein-based sensor (P-SENS) where the binding of the p19 protein caused a large decrease in current density and thus improved the detection range. (d) A displacement-based sensor (D-SENS) where the hybridization product of miR-200 and its complementary probe forced the p19 protein to dissociate from the previous immobilized hybrid, causing a shift-back in the signal. From Labib et al. (2013b), with permission.

oligonucleotide capture probes immobilized onto a conducting polymer/reduced graphene oxide-modified electrode (Tran et al., 2013a). Upon hybridization with the target miRNA, an increase in the current was observed. On the other hand, incubation of the immobilized hybrid with an RNA/DNA specific antibody caused a large decrease in current. Subsequently, the antibody/hybrid complex was dissociated by incubation with a new miRNA/DNA hybrid causing a shift-back in the signal. The developed biosensor allowed a sensitive analysis of miRNAs with a detection limit of 8 fM. Also, the same group reported a different label-free miRNA biosensor based on an interpenetrated network of carbon nanotubes and an electroactive polymer (Tran et al., 2013b). The nanostructured polymer film exhibited well-defined redox peaks due to the two quinone redox couples. Upon hybridization with the target miRNA, an increase in the current signal was observed due to the more compact nature of the formed hybrid compared to single-stranded capture probes which significantly improved the electron transfer properties of the underlying electrode surface. The developed miRNA biosensor allowed for a detection limit of 8 fM. Kilic and his coworkers also utilized the unique binding ability of p19 to RNA duplexes to develop a voltammetric miRNA assay (Kilic et al., 2013). In this work, an RNA capture probe was immobilized onto the surface of a PGE. After hybridization with the target miRNA, the formed hybrid was incubated with p19 protein. Subsequently, the oxidation signal of the tryptophan residues of p19 was measured and allowed a sensitive analysis of the miRNA level with a detection limit of 1.6 pmole in 10 μL sample volume.

A novel strategy for miRNA analysis was recently developed by Labib and his colleagues who reported a DNA four-way junction based electrochemical sensor (4J SENS) for miRNA analysis (Labib et al., 2013a). The developed biosensor is multi-component and consists of a universal interfacial probe (UIP) and two DNA adaptor strands, namely, strand C and a biotin-labeled strand H, which cooperatively hybridize with both UIP and the target miRNA. Each adaptor strand has a fragment complementary to the UIP (UIP-binding arm) and a fragment complementary to the analyzed RNA (miRNA-binding arm). In the absence of the target miRNA, the adaptor strands do not interact with the surface-bound UIP, because the UIP stem-loop structure is more thermodynamically stable than its hybrid with the adaptor strands. In the presence of the target miRNA, the two adaptor strands hybridize to UIP and miRNA, thus resulting in the formation of a quadripartite complex with a four-way junction structure. This complex can capture a large streptavidin molecule to produce a significant increase in the interfacial resistance hence improve the sensitivity, as shown in Fig. 5. The developed biosensor allowed for an ultrasensitive miRNA analysis with a detection limit of 2 aM. In 2013, Hong and his coworkers proposed a miRNA biosensor based on DNA concatamers which can serve as carriers for signal amplification (Hong et al., 2013). In this work, the coauthors immobilized two auxiliary probes onto the surface of a screen-printed gold electrode to form one-dimensional DNA concatamers. Hybridization of the surface concatamers with the target miRNA, in presence of $[Ru(NH_3)_6]^{3+}$, allowed for a sensitive analysis of miRNA with a detection limit of 100 aM.

In 2014, Bartosik and his colleagues developed a miRNA assay by immobilizing a biotin-labeled oligonucleotide capture probe onto the surface of streptavidin-coated magnetic beads (Bartosik et al., 2014). Afterward, the target miRNA was modified with an electroactive complex of osmium (VI) and 2,2′-bipyridine, which specifically binds to the 3′ end ribose of the miRNA strand. Subsequently, hybridization of the labeled target miRNA with the capture probe was measured using a hanging mercury drop electrode with a detection limit of 10 nM. In 2014, Zhou and his coworkers developed a miRNA biosensor based on mimic enzyme catalysis and signal amplification (Zhou et al., 2014). In this work, gold nanoparticles were electrochemically deposited onto the surface of a glassy carbon electrode. Afterward, a thiol-modified stem-loop DNA capture probe was immobilized onto the surface of the gold nanoparticles. Next, the stem-loop structure of the capture probe was unfolded after hybridization with the target

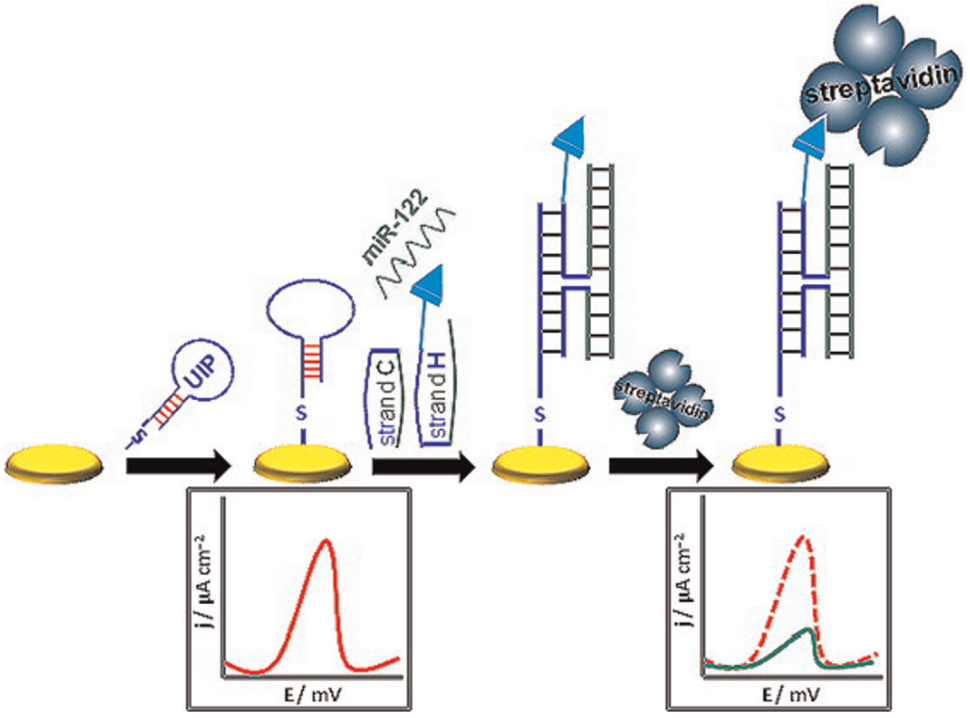

Fig. 5. Schematic illustration of the four-way junction-based biosensor for detection of miR-122. A thiol-modified universal interfacial probe (UIP was self-assembled onto the electrode surface). Afterward, the sensor was incubated with a mixture of strand C, biotin-labeled strand H, and miR-122. Incubation of the sensor with streptavidin causes a decrease in the current density, measured by square wave voltammetry (SWV). From Labib et al. (2013a), with permission.

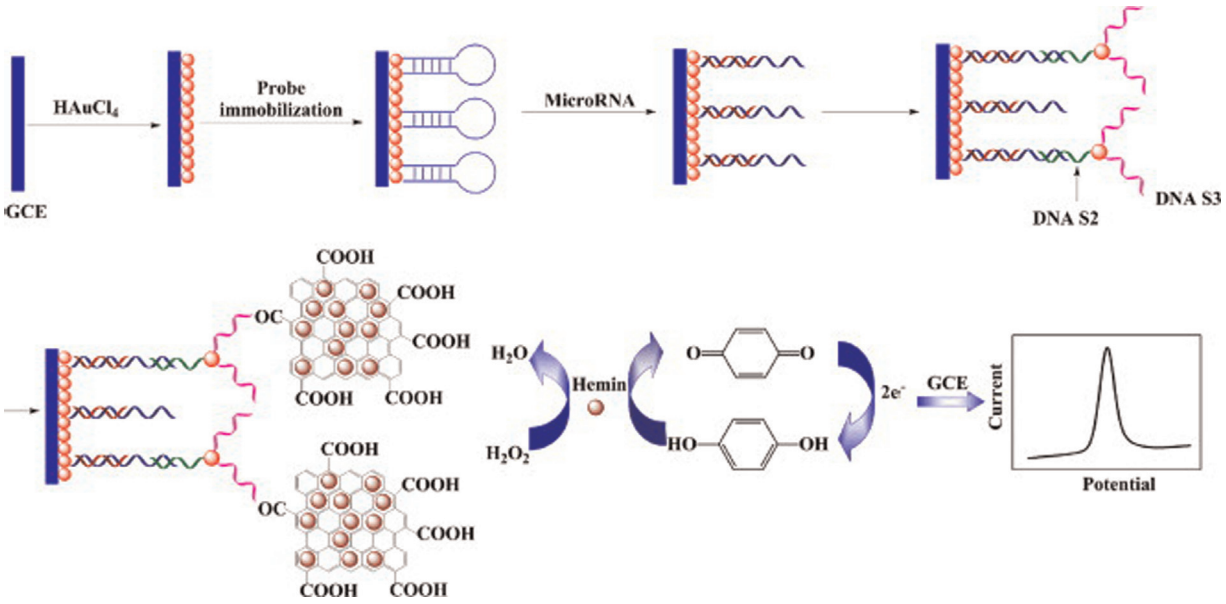

Fig. 6. Schematic representation of the miRNA assay using mimic enzyme catalysis based electrochemical biosensor. From Zhou et al. (2014), with permission.

miRNA. Then, the unhybridized terminal segment of the capture probe was hybridized with a DNA strand 2 (DNA S2) on bio barcode functionalized gold nanoparticles which led to the successful immobilization of the amino-functionalized DNA S3. After activation with EDC and NHS, a carboxylic graphene-hemin complex was assembled onto the electrode surface *via* coupling of the DNA S3 to the carboxylic graphene. The carboxylic graphene–hemin complex catalyzed the oxidation of hydroquinone into benzoquinone in the presence of H_2O_2, as shown in Fig. 6. Subsequently the product was electrochemically reduced which allowed for a sensitive interrogation of the miRNA level with a detection limit of 0.17 pM. Recently, Liu et al. developed a voltammetric biosensor in which the target miRNA was hybridized with a pre-immobilized DNA capture probe onto the surface of a gold electrode (Liu et al.,

Table 1
Analytical performance of amperometric microRNA detection platforms.

S/N	Sensing platform	Target miRNA	Detection limit	Reference
1	Amperometry using isoniazid-capped OsO_2 nanoparticles	miR-106, miR-139, and let-7b	80 fM	Gao and Yang (2006)
2	Amperometry using $Os(dmpy)_2(IN)Cl^+$ electrocatalytic moiety	miR-106, miR-139, and let-7b	800 fM	Gao and Yu (2007b)
3	Amperometry using $Ru(PD)_2Cl_2$ electrocatalytic moiety	miR-92, miR-320, and let-7b	0.2 pM	Gao and Yu (2007a)
4	Amperometry based on RuO_2 nanoparticles-catalyzed polymerization of aniline	Let-7c	2 fM	Peng et al. (2010)
5	Chronoamperometry using capture LNA molecular beacons	miR-21	0.06 pM	Yin et al. (2012)
6	Chronoamperometry based on paramagnetic beads and enzyme amplification	miR-222	7 pm	Bettazzi et al. (2013)
7	Amperometry using glucose oxidase detection probe	miR-720, miR-1248, and let-7c	4 fM	Gao (2012)
8	Amperometry using glucose oxidase detection probe	Let-7a, let-7b, and let-7c	10 fM	Gao and Peng (2011)
9	Amperometry using allosteric molecular beacons	Let-7a	13.6 aM	Cai et al. (2013)
10	Amperometry based on gap hybridization assay	miR-16	2 aM	Pohlmann and Sprinzl (2010)
11	Amperometry using Pd nanoparticles as enhancer and linker	miR-155	1.87 pM	Wu et al. (2013)
12	Chronoamperometry using magnetic-controllable electrodes	miR-200a	0.2 aM	Wang et al. (2013)
13	Differential pulse voltammetry based on guanine oxidation of target miRNA	miR-122	0.1 pM	Lusi et al. (2009)
14	Differential pulse voltammetry based on nanostructured microarrays	miR-21	10 aM	Yang et al. (2009)
15	Cyclic voltammetry using a competitive hybridization approach	miR-182	10 fM	Wang et al. (2012)
16	Differential pulse voltammetry based on enzyme amplification	miR-21	0.67 $\mu g\ mL^{-1}$	Kilic et al. (2012)
17	Differential pulse voltammetry using probe encapuslated Ag nanoclusters	miR-16	67 fM	Dong et al. (2012)
18	Square wave voltammetry using p19 protein in a three-mode assay format	miR-21, miR-32, miR-122, miR-141, miR-200	5 aM	Labib et al. (2013b)
19	Square wave voltammetry using RNA/DNA specific antibody in a three-mode assay format	miR-29b-1 and miR-141	8 fM	Tran et al. (2013a)
20	Square wave voltammetry using an interpenetrated network of carbon nanotubes and an electroactive polymer	miR-141	8 fM	Tran et al. (2013b)
21	Differential pulse voltammetry based on the oxidation of tryprophan residues of p19 protein	miR-21	1.6 pmole	Kilic et al. (2013)
22	Square wave voltammetry using a DNA four-way junction assay format	miR-122	2 aM	Labib et al. (2013a)
23	Differential pulse voltammetry using DNA concatamers	miR-21	100 aM	Hong et al. (2013)
24	Differential pulse voltammetry using an electroactive complex of osmium (VI) and 2,2′-bipyridine	miR-261 and miR-522	10 nM	Bartosik et al. (2014)
25	Differential pulse voltammetry based on mimic enzyme catalysis and signal amplification	miR-159a	0.17 pM	Zhou et al. (2014)
26	Cyclic voltammetry and amperometry based on enzyme amplification	miR-21	3 fM	Liu et al. (2014)

2014). Afterward, the cis-diol of the ribose sugar at the miRNA terminus was modified with 3-aminophenylboronic acid/biotin-tagged gold nanoparticles. Subsequently, streptavidin-labeled alkaline phosphatase (ALP) was coupled to the formed hybrid *via* interaction with biotin. After incubation with the enzyme–substrate, 4-aminophenylphosphate (p-APP), ALP converted p-APP to p-aminophenol (p-AP). The formed p-AP was then reduced by tris (2-carboxyethyl)phosphine (TCEP) after its electrooxidation on the electrode surface. As a result, an increase in the anodic current was measured and allowed for the detection of miRNA levels down to 3 fM. Table 1 summarizes the analytical performance of various amperometric and voltammetric biosensors currently employed for miRNA analysis.

2.2. Potentiometric biosensors

Potentiometry, or measuring the potential of a solution between two electrodes to determine the concentration of one or more analytes, was first adopted by Rothberg et al. for nucleic acid sequencing in 2011 (Rothberg et al., 2011). In 2012, Goda et al. developed a potentiometric biosensor for detecting encapsulated miRNAs in exosomes using a microelectrode-based assay after a reverse transcription polymerase chain reaction (RT-PCR) (Goda et al., 2012). In this work, PCR amplicons which retained the original miRNA sequences were captured *via* hybridization with oligonucleotide probes immobilized onto the surface of a microarray sensor, as shown in Fig. 7. The developed biosensor relied on the changes in surface potential as a result of changes in molar ratios in a mixed SAM. Using a mixed SAM significantly improved the signal-to-noise ratio compared to other conventional label-free methods which were prone to high noise ratios due to the non-specific adsorption of interfering molecular species on the sensor surface. The developed assay allowed for the detection of miRNA levels down to 20 pM. However, a major drawback of this method is the need for a tedious reverse-transcription scheme prior to electrochemical sensing.

2.3. Conductometric biosensors

Conductometry, or measuring the electrolytic conductivity to assess the progress of a chemical reaction, was successfully employed for miRNA detection. In 2007, Fan et al. reported a nanogapped microelectrode-based sensor array for ultrasensitive analysis of miRNAs (Fan et al., 2007). In this work, PNA capture probes were immobilized in the nanogaps of a pair of interdigitated microelectrodes. Compared to DNA, PNA is neutral and does not have an anionic phosphate backbone. The repulsion between PNA and RNA is therefore less than between DNA and RNA, resulting in an enhanced hybridization efficiency. Upon hybridization with the target miRNA, conducting polyaniline nanowires, were deposited onto the hybridized miRNA by virtue of the electrostatic interaction between the cationic aniline molecules and anionic phosphates groups in the miRNA molecules using an enzymatically catalyzed method, as shown in Fig. 8. It was observed that the conductance of the deposited nanowires correlates with the amount of miRNA, which allowed a linear quantification of miRNA with a detection limit of 5 fM.

2.4. Impedimetric biosensors

Electrochemical impedance spectroscopy (EIS), or measuring the effective resistance of an electric circuit or component to alternating current signal of different frequencies arising from the combined effects of ohmic resistance and reactance, has been utilized for miRNA analysis. In 2011, Peng and Gao reported a label-free impedimetric miRNA biosensor that employs RuO_2 nanoparticles-catalyzed polymerization of 3,3′-dimethoxybenzidine (DB) and miRNA-templated deposition of an insulating film of poly (3,3′-dimethoxybenzidine) (PDB) (Peng and Gao, 2011). In this work, a mixed SAM of the oligonucleotide capture probes and 4-mercaptoanailine was formed on the gold electrode surface. Upon hybridization with the RuO_2-tagged target miRNA, the formed hybrid was incubated with a mixture of DB/H_2O_2. The amount of deposited PDB and its insulating effect were directly proportional to the miRNA concentration. EIS measurements allowed for a linear interrogation of the miRNA level with a detection limit of 3 fM. In a related study, SAM of charge-neutral morpholino capture probes were formed on the surface of an ITO-coated glass slide (Gao et al., 2013b). Upon hybridization with the target miRNA, the neutral surface of the biosensor becomes anionic by virtue of the negatively charged phosphate groups of miRNA strands. Afterward, deposition of the insulating polymer film, PDB, was carried out by the horseradish peroxidase-catalyzed polymerization of DB in the presence of H_2O_2. The amount of deposited PDB and its insulating effect correlated directly with the level of the target miRNA. Under optimized condition, EIS was employed for the miRNA expression profiling with a detection limit of 2 fM.

In 2013, Shen and his coworkers described an impedimetric miRNA biosensor based on a SAM of oligonucleotide capture probes on a gold electrode (Shen et al., 2013). After hybridization with the target miRNA, the unhybridized capture strands were digested with nuclease. Subsequently, a second hybridization was carried out using an oligonucleotide-tailed DANzyme. Upon

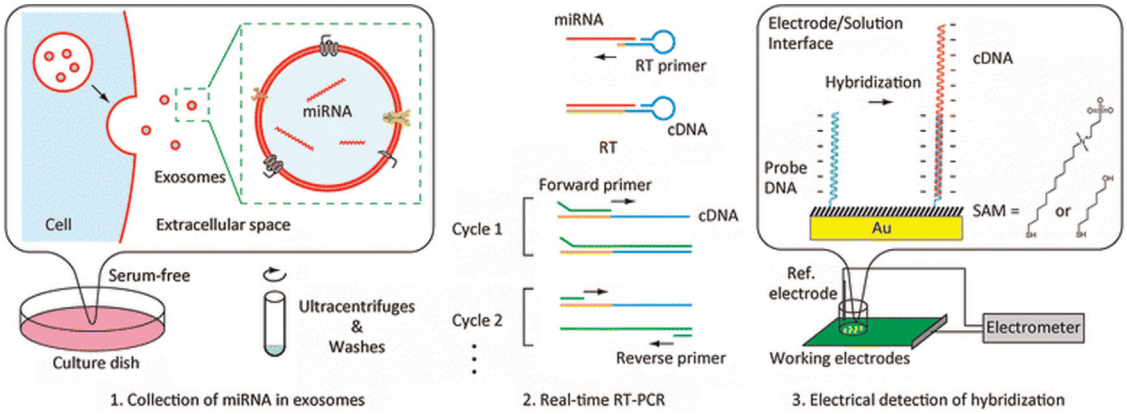

Fig. 7. Schematic illustration of a potentiometric miRNA biosensor using microelectrodes array after RT-PCR. (1) Exosomes were collected from serum-free medium by ultracentrifugation, (2) miRNA was transformed into cDNA by the stem-loop RT primer, followed by PCR, and (3) an electrometer was used for direct electrical detection of the charges in target cDNAs following hybridization. From Goda et al. (2012), with permission.

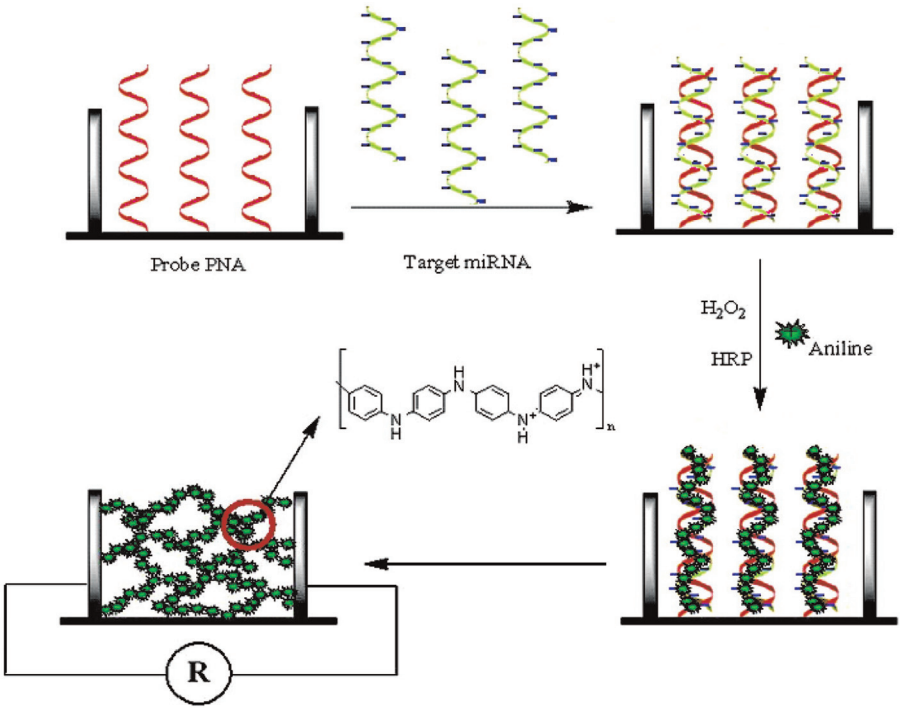

Fig. 8. Schematic illustration of the conductometric miRNA assay using target-guided formation of conducting polymer nanowires in nanogaps. From Fan et al. (2007), with permission.

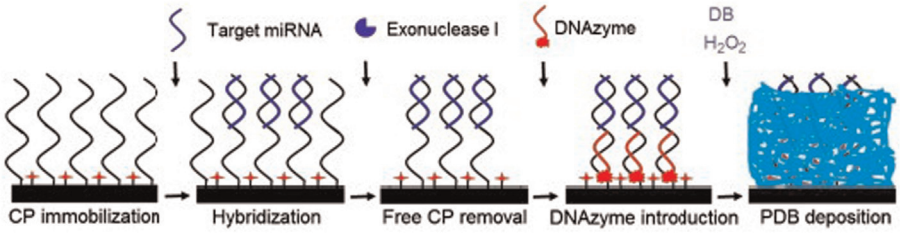

Fig. 9. Schematic representation of impedimetric miRNA biosensor based on the immobilization of a capture probe (CP) onto the surface of gold electrode. After hybridization with the target miRNA, the unhybridized capture strands were digested with nuclease. Subsequently, a second hybridization was carried out using an oligonucleotide-tailed DANzyme. Upon incubation with a mixture of 3,3′-dimethoxybenzidine (DB)/H_2O_2, the DNAzyme triggered the polymerization of DB and the hybrid served as a template to guide the deposition of poly(3,3′-dimethoxybenzidine) (PDB). From Shen et al. (2013), with permission.

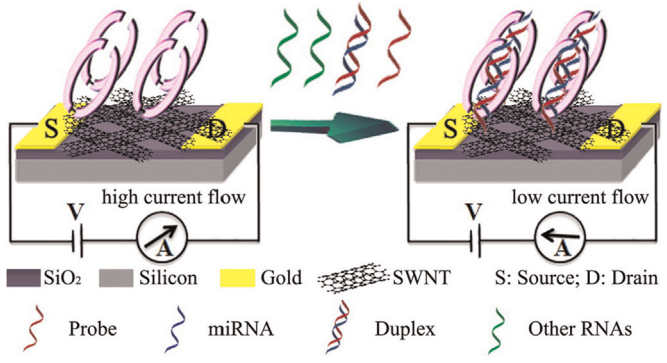

Fig. 10. Schematic illustration of the miRNA detection principle using the p19 protein functionalized carbon nanotubes field-effect transistor. From Ramnani et al. (2013), with permission.

incubation with a mixture of DB/H_2O_2, the DNAzyme triggered the polymerization of DB and the hybrid served as a template to guide the deposition of PDB, as shown in Fig. 9. The formed PDB caused a change in the interfacial resistance proportional to the miRNA concentration with a detection limit of 2 fM. An interesting approach was adopted by Ren and his colleagues who reported an impedimetric biosensor based on a SAM of thiol-modified DNA as a capture probe immobilized onto the surface of a gold electrode (Ren et al., 2013). After hybridization with the target miRNA, the formed hybrid was cleaved off using a duplex-specific nuclease releasing the target strand back to the sample solution for further hybridization, thus forming an isothermal signal amplification cycle. The distinct difference in impedance between the control and the nuclease treated biosensor allowed for a sensitive analysis of miRNA with a detection limit of 1 fM.

2.5. *Field-effect transistor-based biosensors*

Field-effect transistor-based biosensor (Bio-FET) is a type of transistors that changes its source–drain "channel" conductivity depending on the electric field of the environment. The electric resistance at the channel is inversely proportional to its carrier density which can be measured from the change in the source–drain voltage–current. Bio-FETs provide an attractive platform for analysis of biological samples due to their ability to directly convert the interaction with the target molecules into an electronic signal. Hence, Bio-FETs were employed for detection of a variety of analytes, including ions, proteins, nucleic acids, viruses, and cells.

Table 2
Analytical performance of potentiometric, conductometric, impedimetric, and field-effect transistor microRNA detection platforms.

S/N	Sensing platform	Target miRNA	Detection limit	Reference
1	Potentiometry using microelectrode array	miR-143 and miR-146a	20 pM	Goda et al. (2012)
2	Conductometry using nanogapped microelectrode based sensor array	let-7b	5 fM	Fan et al. (2007)
3	Electrochemical impedance spectroscopy based on RuO_2-catalyzed deposition of poly(3,3′-dimethoxybenzidine) (PDB)	miR-720, miR-1248, and let-7c	3 fM	Peng and Gao (2011)
4	Electrochemical impedance spectroscopy based on horseradish peroxidase catalyzed deposition of PDB	let-7c	2 fM	Gao et al. (2013b)
5	Electrochemical impedance spectroscopy based on DNAzyme-catalyzed and miRNA-guided deposition of PDB	let-7c	2 fM	Shen et al. (2013)
6	Electrochemical impedance spectroscopy based on isothermal signal amplification	Let-7b	1 fM	Ren et al. (2013)
7	Field-effect transistor based on silicon nanowires (SiNWs)	Let-7b	1 fM	Zhang et al. (2009a)
8	Field-effect transistor based on SiNWs	miR-21	1 fM	Gao et al. (2013a)
9	Field-effect transistor based on CIRV p19 protein	miR-122a	1 aM	Ramnani et al. (2013)
10	Field-effect transistor based on complementary metal oxide semiconductor (CMOS)-compatible SiNWs	miR-21 and miR-205	1 zeptomole	Lu et al. (2014)

One-dimensional silicon nanowires (SiNWs) configured with FETs offered a promising sensing platform for miRNAs due to their high sensitivity, selectivity, and real-time response. In 2009, Zhang and his colleagues reported a label-free hybridization assay for miRNA detection using SiNWs)-based field-effect transistors (Zhang, 2011; Zhang et al., 2009a). In this work, PNA, as a capture probe, was immobilized onto the surface of the SiNWs. Upon hybridization with the target miRNA, a significant change in resistance was registered and allowed for a sensitive analysis of miRNA with a detection limit of 1 fM. In 2013, Gao and his colleagues reported a SiNW-FET nanosensor for miRNA analysis. The developed sensor allowed for a sensitive analysis of miRNA with a detection limit of 1 fM (Gao et al., 2013a). In 2013, Ramnani and his coworkers utilized carbon nanotubes field-effect transistor functionalized with the CIRV p19 protein to develop a highly sensitive miRNA biosensor (Ramnani et al., 2013). After hybridization between the target miRNA and the capture probe, the formed hybrid was quantified by measuring the change in the interfacial resistance resulting from its binding to the surface p19, as shown in Fig. 10. The developed biosensor allowed for a sensitive quantification of miRNA with a detection limit of 1 aM. In 2014, Lu and his coworkers developed a complementary metal oxide semiconductor (CMOS)-compatible SiNW-FET biosensor using anisotropic wet etching technology to facilitate miRNA analysis at low cost and ultrahigh sensitivity. The developed assay allowed for the detection of miRNA levels down to 1 zeptomole (ca. 600 copies) in one minute (Lu et al., 2014). Table 2 summarizes the analytical performance of potentiometric, conductometric, impedimetric, and field-effect transistor-based biosensors currently employed for miRNA analysis.

3. Conclusions and future perspectives

Detection of circulating miRNAs in body fluids has become a glimmer of hope for the early diagnosis of cancer and assessment of disease progression. Although the field of circulating miRNA analysis is still in its infancy, it has a great potential since miRNAs are relatively stable molecules and can be easily accessible in a noninvasive manner. Typical levels of circulating miRNAs in serum range between 200 aM and 20 pM. Electrochemical sensing platforms offer many attractive analytical features and could play a leading role in future miRNA diagnostics. Many of the electrochemical sensors described in this review allow rapid, selective and highly sensitive analysis of miRNAs without involving preamplification steps. In addition, these techniques have addressed the required high sensitivity through various strategies, including supporting highly favourable hybridization conditions using PNA or LNA capture probes, signal amplification through enzyme–substrate turnover, and comprising nanostructured sensors and/or electrocatalytic steps. However, there are still several issues that must be addressed before they are implemented in POC diagnostics. First, the ongoing research of electrochemical miRNA analysis using nano-sized transducers drives the need for developing methods to overcome mass transfer limitations at nanoscales. Novel methods for actuating fluid motion at nano-scales and techniques for integrating them into sensing platforms will be an area for future developments. Second, the trend toward nanomaterials and miniaturized devices highlights the urge to evaluate the biocompatibility and environmental friendliness of these nanomaterials. In addition, research needs to be devoted to simplify their fabrication techniques. Unfortunately, preparation and manipulation of nanomaterials in a reproducible and economic fashion is still premature. Third, sensor biofouling by the background biologics still presents a significant challenge for the development of POC devices. Hence, techniques that employ chemical, electrical, and mechanical passivation approaches to reduce the degree of nonspecific binding are of great interest for clinical analysis of miRNAs. Fourth, little attention has been paid to the long-term storage stability of these biosensors, which is of utmost importance for large-scale production of POC devices on the commercial level. In this sense, optimization of surface chemistry and interfacial design using mild functionalization approaches would lead to the development of highly stable genosensors. Fifth, integration of electrochemical genosensors into automated systems that are fast enough to relay results quickly is essential to accomplish a widespread use of the POC device. In summary, as miRNA research enters its third decade, there are significant challenges to meet before electrochemical POC miRNA diagnostics become a reality. The efforts devoted by the scientific community in this research arena is unprecedented, allowing a great optimism that these diagnostics might become a reality in the near future.

Acknowledgments

Authors thank the Mitacs Elevate Fellowship Program (grant # IT02722) (M.L.) and the Natural Sciences and Engineering Research Council of Canada (NSERC) (grant #RGPIN/385739-2010) (M.V.B.) for funding this work.

References

Alevizos, I., Illei, G.G., 2010. MicroRNAs as biomarkers in rheumatic diseases. Nat. Rev. Rheumatol. 6 (7), 391–398.

Ambros, V., 2004. The functions of animal microRNAs. Nature 431 (7006), 350–355.

Bartosik, M., Hrstka, R., Palecek, E., Vojtesek, B., 2014. Magnetic bead-based hybridization assay for electrochemical detection of microRNA. Anal. Chim. Acta 813 (0), 35–40.

Bettazzi, F., Hamid-Asl, E., Esposito, C., Quintavalle, C., Formisano, N., Laschi, S., Catuogno, S., Iaboni, M., Marrazza, G., Mascini, M., Cerchia, L., De Franciscis, V., Condorelli, G., Palchetti, I., 2013. Electrochemical detection of miRNA-222 by use of a magnetic bead-based bioassay. Anal. Bioanal. Chem. 405 (2–3), 1025–1034.

Bloomston, M., Frankel, W.L., Petrocca, F., Volinia, S., Alder, H., Hagan, J.P., Liu, C.G., Bhatt, D., Taccioli, C., Croce, C.M., 2007. MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. JAMA 297 (17), 1901–1908.

Cai, Z., Song, Y., Wu, Y., Zhu, Z., James Yang, C., Chen, X., 2013. An electrochemical sensor based on label-free functional allosteric molecular beacons for detection target DNA/miRNA. Biosens. Bioelectron. 41 (0), 783–788.

Chen, C., Ridzon, D.A., Broomer, A.J., Zhou, Z., Lee, D.H., Nguyen, J.T., Barbisin, M., Xu, N.L., Mahuvakar, V.R., Andersen, M.R., Lao, K.Q., Livak, K.J., Guegler, K.J., 2005. Real-time quantification of microRNAs by stem-loop RT-PCR. Nucl. Acids Res. 33 (20), e179.

Cheng, J., Kapranov, P., Drenkow, J., Dike, S., Brubaker, S., Patel, S., Long, J., Stern, D., Tammana, H., Helt, G., Sementchenko, V., Piccolboni, A., Bekiranov, S., Bailey, D. K., Ganesh, M., Ghosh, S., Bell, I., Gerhard, D.S., Gingeras, T.R., 2005. Transcriptional maps of 10 human chromosomes at 5-nucleotide resolution. Science 308 (5725), 1149–1154.

Cissell, K.A., Rahimi, Y., Shrestha, S., Hunt, E.A., Deo, S.K., 2008. Bioluminescence-based detection of microRNA, miR21 in breast cancer cells. Anal. Chem. 80 (7), 2319–2325.

de Planell-Saguer, M., Rodicio, M.C., Mourelatos, Z., 2010. Rapid in situ codetection of noncoding RNAs and proteins in cells and formalin-fixed paraffin-embedded tissue sections without protease treatment. Nat. Protoc. 5 (6), 1061–1073.

Dong, H., Jin, S., Ju, H., Hao, K., Xu, L.-P., Lu, H., Zhang, X., 2012. Trace and Label-Free MicroRNA Detection Using Oligonucleotide Encapsulated Silver Nanoclusters as Probes. Analytical Chemistry 84 (20), 8670–8674.

Driskell, J.D., Seto, A.G., Jones, L.P., Jokela, S., Dluhy, R.A., Zhao, Y.P., Tripp, R.A., 2008. Rapid microRNA (miRNA) detection and classification via surface-enhanced Raman spectroscopy (SERS). Biosens Bioelectron 24 (4), 923–928.

Edwards, J.K., Pasqualini, R., Arap, W., Calin, G.A., 2010. MicroRNAs and ultraconserved genes as diagnostic markers and therapeutic targets in cancer and cardiovascular diseases. J. Cardiovasc. Transl. Res. 3 (3), 271–279.

Fan, Y., Chen, X., Trigg, A.D., Tung, C.H., Kong, J., Gao, Z., 2007. Detection of MicroRNAs using target-guided formation of conducting polymer nanowires in nanogaps. J. Am. Chem. Soc. 129 (17), 5437–5443.

Fang, S., Lee, H.J., Wark, A.W., Corn, R.M., 2006. Attomole microarray detection of microRNAs by nanoparticle-amplified SPR imaging measurements of surface polyadenylation reactions. J. Am. Chem. Soc. 128 (43), 14044–14046.

Friedman, R.C., Farh, K.K., Burge, C.B., Bartel, D.P., 2009. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. 19 (1), 92–105.

Gao, A., Lu, N., Dai, P., Li, T., Wang, Y., 2013a. Label-free and ultrasensitive detection of microrna biomarkers in lung cancer cells based on silicon nanowire FET biosensors. In: Proceedings of the 17th International Conference on Solid-State Sensors, Actuators and Microsystems (Transducers & Eurosensors XXVII), pp. 2439–2442.

Gao, Z., 2012. A highly sensitive electrochemical assay for microRNA expression profiling. Analyst 137 (7), 1674–1679.

Gao, Z., Deng, H., Shen, W., Ren, Y., 2013b. A label-free biosensor for electrochemical detection of femtomolar microRNAs. Anal. Chem. 85 (3), 1624–1630.

Gao, Z., Peng, Y., 2011. A highly sensitive and specific biosensor for ligation- and PCR-free detection of microRNAs. Biosens. Bioelectron. 26 (9), 3768–3773.

Gao, Z., Yang, Z., 2006. Detection of microRNAs using electrocatalytic nanoparticle tags. Anal. Chem. 78 (5), 1470–1477.

Gao, Z., Yu, Y.H., 2007a. Direct labeling microRNA with an electrocatalytic moiety and its application in ultrasensitive microRNA assays. Biosens. Bioelectron. 22 (6), 933–940.

Gao,, z, Yu, Y.H., 2007b. A microRNA biosensor based on direct chemical ligation and electrochemically amplified detection. Sens. Actuators B 121 (2), 552–559.

Goda, T., Masuno, K., Nishida, J., Kosaka, N., Ochiya, T., Matsumoto, A., Miyahara, Y., 2012. A label-free electrical detection of exosomal microRNAs using microelectrode array. Chem. Commun. 48 (98), 11942–11944.

Hong, C.-Y., Chen, X., Liu, T., Li, J., Yang, H.-H., Chen, J.-H., Chen, G.-N., 2013. Ultrasensitive electrochemical detection of cancer-associated circulating microRNA in serum samples based on DNA concatamers. Biosens. Bioelectron. 50 (0), 132–136.

Keller, A., Leidinger, P., Lange, J., Borries, A., Schroers, H., Scheffler, M., Lenhof, H.P., Ruprecht, K., Meese, E., 2009. Multiple sclerosis: microRNA expression profiles accurately differentiate patients with relapsing-remitting disease from healthy controls. PLoS One 4 (10), e7440.

Kilic, T., Nur Topkaya, S., Ozsoz, M., 2013. A new insight into electrochemical microRNA detection: a molecular caliper, p19 protein. Biosens. Bioelectron. 48 (0), 165–171.

Kilic, T., Topkaya, S.N., Ozkan Ariksoysal, D., Ozsoz, M., Ballar, P., Erac, Y., Gozen, O., 2012. Electrochemical based detection of microRNA, mir21 in breast cancer cells. Biosens. Bioelectron. 38 (1), 195–201.

Labib, M., Ghobadloo, S.M., Khan, N., Kolpashchikov, D.M., Berezovski, M.V., 2013a. Four-way junction formation promoting ultrasensitive electrochemical detection of microRNA. Anal. Chem. 85 (20), 9422–9427.

Labib, M., Khan, N., Ghobadloo, S.M., Cheng, J., Pezacki, J.P., Berezovski, M.V., 2013b. Three-mode electrochemical sensing of ultralow microRNA levels. J. Am. Chem. Soc. 135 (8), 3027–3038.

Labib, M., Martić, S., Shipman, P.O., Kraatz, H.B., 2011a. Electrochemical analysis of HIV-1 reverse transcriptase serum level: exploiting protein binding to a functionalized nanostructured surface. Talanta 85 (1), 770–778.

Labib, M., Shipman, P.O., Martić, S., Kraatz, H.-B., 2011b. A bioorganometallic approach for rapid electrochemical analysis of human immunodeficiency virus type-1 reverse transcriptase in serum. Electrochim. Acta 56 (14), 5122–5128.

Labib, M., Shipman, P.O., Martić, S., Kraatz, H.B., 2011c. Towards an early diagnosis of HIV infection: an electrochemical approach for detection of HIV-1 reverse transcriptase enzyme. Analyst 136 (4), 708–715.

Labib, M., Zamay, A.S., Berezovski, M.V., 2013c. Multifunctional electrochemical aptasensor for aptamer clones screening, virus quantitation in blood and viability assessment. Analyst 138 (6), 1865–1875.

Labib, M., Zamay, A.S., Kolovskaya, O.S., Reshetneva, I.T., Zamay, G.S., Kibbee, R.J., Sattar, S.A., Zamay, T.N., Berezovski, M.V., 2012a. Aptamer-based impedimetric sensor for bacterial typing. Anal. Chem. 84 (19), 8114–8117.

Labib, M., Zamay, A.S., Kolovskaya, O.S., Reshetneva, I.T., Zamay, G.S., Kibbee, R.J., Sattar, S.A., Zamay, T.N., Berezovski, M.V., 2012b. Aptamer-based viability impedimetric sensor for bacteria. Anal. Chem. 84 (21), 8966–8969.

Labib, M., Zamay, A.S., Muharemagic, D., Chechik, A.V., Bell, J.C., Berezovski, M.V., 2012c. Aptamer-based viability impedimetric sensor for viruses. Anal. Chem. 84 (4), 1813–1816.

Lapierre, M.A., O'Keefe, M., Taft, B.J., Kelley, S.O., 2003. Electrocatalytic detection of pathogenic DNA sequences and antibiotic resistance markers. Anal. Chem. 75 (22), 6327–6333.

Lawrie, C.H., Gal, S., Dunlop, H.M., Pushkaran, B., Liggins, A.P., Pulford, K., Banham, A.H., Pezzella, F., Boultwood, J., Wainscoat, J.S., Hatton, C.S., Harris, A.L., 2008. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br. J. Haematol. 141 (5), 672–675.

Lee, E.J., Gusev, Y., Jiang, J., Nuovo, G.J., Lerner, M.R., Frankel, W.L., Morgan, D.L., Postier, R.G., Brackett, D.J., Schmittgen, T.D., 2007. Expression profiling identifies microRNA signature in pancreatic cancer. Int. J. Cancer 120 (5), 1046–1054.

Liu, L., Xia, N., Liu, H., Kang, X., Liu, X., Xue, C., He, X., 2014. Highly sensitive and label-free electrochemical detection of microRNAs based on triple signal amplification of multifunctional gold nanoparticles, enzymes and redox-cycling reaction. Biosens. Bioelectron. 53 (0), 399–405.

Lu, N., Gao, A., Dai, P., Song, S., Fan, C., Wang, Y., Li, T., 2014. CMOS-compatible silicon nanowire field-effect transistors for ultrasensitive and label-free microRNAs sensing. Small 10 (10), 2022–2028.

Lusi, E.A., Passamano, M., Guarascio, P., Scarpa, A., Schiavo, L., 2009. Innovative electrochemical approach for an early detection of microRNAs. Anal. Chem. 81 (7), 2819–2822.

Miller, B.H., Wahlestedt, C., 2010. MicroRNA dysregulation in psychiatric disease. Brain Res. 1338, 89–99.

Neely, L.A., Patel, S., Garver, J., Gallo, M., Hackett, M., McLaughlin, S., Nadel, M., Harris, J., Gullans, S., Rooke, J., 2006. A single-molecule method for the quantitation of microRNA gene expression. Nat. Methods 3 (1), 41–46.

Nielsen, L.B., Wang, C., Sorensen, K., Bang-Berthelsen, C.H., Hansen, L., Andersen, M. L., Hougaard, P., Juul, A., Zhang, C.Y., Pociot, F., Mortensen, H.B., 2012. Circulating levels of microRNA from children with newly diagnosed type 1 diabetes and healthy controls: evidence that miR-25 associates to residual beta-cell function and glycaemic control during disease progression. Exp. Diabetes Res. 2012, 896362.

Peng, Y., Gao, Z., 2011. Amplified detection of microRNA based on ruthenium oxide nanoparticle-initiated deposition of an insulating film. Anal. Chem. 83 (3), 820–827.

Peng, Y., Yi, G., Gao, Z., 2010. A highly sensitive microRNA biosensor based on ruthenium oxide nanoparticle-initiated polymerization of aniline. Chem. Commun. 46 (48), 9131–9133.

Pohlmann, C., Sprinzl, M., 2010. Electrochemical detection of microRNAs via gap hybridization assay. Anal. Chem. 82 (11), 4434–4440.

Ramnani, P., Gao, Y., Ozsoz, M., Mulchandani, A., 2013. Electronic detection of microRNA at attomolar level with high specificity. Anal. Chem. 85 (17), 8061–8064.

Ren, Y., Deng, H., Shen, W., Gao, Z., 2013. A highly sensitive and selective electrochemical biosensor for direct detection of microRNAs in serum. Anal. Chem. 85 (9), 4784–4789.

Roldo, C., Missiaglia, E., Hagan, J.P., Falconi, M., Capelli, P., Bersani, S., Calin, G.A., Volinia, S., Liu, C.G., Scarpa, A., Croce, C.M., 2006. MicroRNA expression abnormalities in pancreatic endocrine and acinar tumors are associated with distinctive pathologic features and clinical behavior. J. Clin. Oncol. 24 (29), 4677–4684.

Rothberg, J.M., Hinz, W., Rearick, T.M., Schultz, J., Mileski, W., Davey, M., Leamon, J. H., Johnson, K., Milgrew, M.J., Edwards, M., Hoon, J., Simons, J.F., Marran, D., Myers, J.W., Davidson, J.F., Branting, A., Nobile, J.R., Puc, B.P., Light, D., Clark, T.A., Huber, M., Branciforte, J.T., Stoner, I.B., Cawley, S.E., Lyons, M., Fu, Y., Homer, N., Sedova, M., Miao, X., Reed, B., Sabina, J., Feierstein, E., Schorn, M., Alanjary, M., Dimalanta, E., Dressman, D., Kasinskas, R., Sokolsky, T., Fidanza, J.A., Namsaraev, E., McKernan, K.J., Williams, A., Roth, G.T., Bustillo, J., 2011. An integrated

semiconductor device enabling non-optical genome sequencing. Nature 475 (7356), 348–352.
Sato, F., Tsuchiya, S., Terasawa, K., Tsujimoto, G., 2009. Intra-platform repeatability and inter-platform comparability of microRNA microarray technology. PLoS One 4 (5), e5540.
Schulte, J.H., Marschall, T., Martin, M., Rosenstiel, P., Mestdagh, P., Schlierf, S., Thor, T., Vandesompele, J., Eggert, A., Schreiber, S., Rahmann, S., Schramm, A., 2010. Deep sequencing reveals differential expression of microRNAs in favorable versus unfavorable neuroblastoma. Nucl. Acids Res. 38 (17), 5919–5928.
Shen, W., Deng, H., Ren, Y., Gao, Z., 2013. A label-free microRNA biosensor based on DNAzyme-catalyzed and microRNA-guided formation of a thin insulating polymer film. Biosens. Bioelectron. 44 (0), 171–176.
Thomson, J.M., Parker, J., Perou, C.M., Hammond, S.M., 2004. A custom microarray platform for analysis of microRNA gene expression. Nat. Methods 1 (1), 47–53.
Tran, H.V., Piro, B., Reisberg, S., Duc, H.T., Pham, M.C., 2013a. Antibodies directed to RNA/DNA hybrids: an electrochemical immunosensor for microRNAs detection using graphene-composite electrodes. Anal. Chem. 85 (17), 8469–8474.
Tran, H.V., Piro, B., Reisberg, S., Tran, L.D., Duc, H.T., Pham, M.C., 2013b. Label-free and reagentless electrochemical detection of microRNAs using a conducting polymer nanostructured by carbon nanotubes: application to prostate cancer biomarker miR-141. Biosens. Bioelectron. 49 (0), 164–169.
Tsujiura, M., Ichikawa, D., Komatsu, S., Shiozaki, A., Takeshita, H., Kosuga, T., Konishi, H., Morimura, R., Deguchi, K., Fujiwara, H., Okamoto, K., Otsuji, E., 2010. Circulating microRNAs in plasma of patients with gastric cancers. Br. J. Cancer 102 (7), 1174–1179.
Valoczi, A., Hornyik, C., Varga, N., Burgyan, J., Kauppinen, S., Havelda, Z., 2004. Sensitive and specific detection of microRNAs by northern blot analysis using LNA-modified oligonucleotide probes. Nucl. Acids Res. 32 (22), e175.
Wang, J., Yi, X., Tang, H., Han, H., Wu, M., Zhou, F., 2012. Direct quantification of microRNA at low picomolar level in sera of glioma patients using a competitive hybridization followed by amplified voltammetric detection. Anal. Chem. 84 (15), 6400–6406.
Wang, Z., Zhang, J., Guo, Y., Wu, X., Yang, W., Xu, L., Chen, J., Fu, F., 2013. A novel electrically magnetic-controllable electrochemical biosensor for the ultra sensitive and specific detection of attomolar level oral cancer-related microRNA. Biosens. Bioelectron. 45 (0), 108–113.
Wu, X., Chai, Y., Yuan, R., Su, H., Han, J., 2013. A novel label-free electrochemical microRNA biosensor using Pd nanoparticles as enhancer and linker. Analyst 138 (4), 1060–1066.
Yang, H., Hui, A., Pampalakis, G., Soleymani, L., Liu, F.F., Sargent, E.H., Kelley, S.O., 2009. Direct, electronic microRNA detection for the rapid determination of differential expression profiles. Angew. Chem. Int. Ed. Engl. 48 (45), 8461–8464.
Yin, H., Zhou, Y., Zhang, H., Meng, X., Ai, S., 2012. Electrochemical determination of microRNA-21 based on graphene, LNA integrated molecular beacon, AuNPs and biotin multifunctional bio bar codes and enzymatic assay system. Biosens. Bioelectron. 33 (1), 247–253.
Zen, K., Zhang, C.Y., 2012. Circulating microRNAs: a novel class of biomarkers to diagnose and monitor human cancers. Med. Res. Rev. 32, 326–348, published online EpubMar, 10.1002/med.20215.
Zhang, G-J, 2011. Silicon Nanowire Biosensor for Ultrasensitive and Label-Free Direct Detection of miRNAs. MicroRNA and Cancer, Methods in Molecular Biology, ed Wu W (Humana Press) 676, 111–121.
Zhang, G.-J., Chua, J.H., Chee, R.-E., Agarwal, A., Wong, S.M., 2009a. Label-free direct detection of MiRNAs with silicon nanowire biosensors. Biosens. Bioelectron. 24 (8), 2504–2508.
Zhang, Y., Li, M., Wang, H., Fisher, W.E., Lin, P.H., Yao, Q., Chen, C., 2009b. Profiling of 95 microRNAs in pancreatic cancer cell lines and surgical specimens by real-time PCR analysis. World J. Surg. 33 (4), 698–709.
Zhou, Y., Wang, M., Xu, Z., Ni, C., Yin, H., Ai, S., 2014. Investigation of the effect of phytohormone on the expression of microRNA-159a in *Arabidopsis thaliana* seedlings based on mimic enzyme catalysis systematic electrochemical biosensor. Biosens. Bioelectron. 54 (0), 244–250.