

REVIEW ARTICLE

Electrochemical miRNA Biosensors: The Benefits of Nanotechnology

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ARTICLE INFO

Article History:

Received 08 November 2016

Accepted 29 December 2016

Published 31 December 2016

Keywords:

Nanotechnology

Biomedical diagnosis

miRNA

Electrochemical Nanobiosensor

ABSTRACT

The importance of nanotechnology in medical technologies, especially biomedical diagnostics, is indubitable. By taking advantages of nanomaterials, many medical diagnostics methods have been developed so far, including electrochemical nanobiosensors. They have been used for quantification of different clinical biomarkers for detecting, screening, or follow up a disease. microRNAs (miRNAs) are one of the most recent and reliable biomarkers used for biomedical diagnosis of various diseases including different cancer types. In addition, there are many electrochemical nanobiosensors explained in publications, patents, and/or a commercial device which have been fabricated for detection or quantification of valuable miRNAs. The aim of this article is to review the concept of medical diagnostics, biosensors, electrochemical biosensors and to emphasize the role of nanotechnology in nanobiosensor development and performance for application in microRNAs detection for biomedical diagnosis. We have also summarized recent ideas and advancements in the field of electrochemical nanobiosensors for miRNA detection, and the important breakthroughs are also explained.

How to cite this article

Azimzadeh M, Rahaie M, Nasirizadeh N, Daneshpour M, Naderi-Manesh H, Electrochemical miRNA Biosensors: The Benefits of Nanotechnology. *Nanomed Res J*, 2017; 2(1):36-48. DOI: 10.22034/nmrj.2017.23336

INTRODUCTION

Nanotechnology has revolutionized our life through its different advantages and applications [1]. Different nanomaterials from gold [2] to carbon [3], have been used so far in biomedical applications such as diagnostics [4, 5]. The term nanobiosensor, which is the application of nanotechnology in biosensors, has developed for medical applications in various scientific papers and patents, especially electrochemical nanobiosensors [6, 7]. The electrochemical nanobiosensors are pulling together the advantages of electrochemical methods, nanotechnology and selectivity of the

biological molecules [8, 9]. For that reason, they are one of the most important ways of medical diagnostics today [10, 11].

With the increasing of human knowledge about the mechanisms of development, spread, and transmission of various diseases, novel strategies are provided for prevention, diagnosis, and treatment. Although these processes have been upgraded faster and more efficient in recent years due to the advancement in science and technology, however the increasing number of cases with lethal diseases such as cancers reveals the importance and emergency of developing early detection systems that can provide rapid, precise, and low-priced

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diagnosis results [12, 13].

One of the most common used approaches in biomedical detection of diseases is determining the level of valuable specific biomarkers in different tissues. Biomarkers are biological molecules which can indicate particular medical or clinical conditions including the infections, emergence of a disease, mal/well-functioned biological process, etc. [14]. Due to the intelligent nature of biomolecules, biomarkers offer higher sensitivity, selectivity, specificity, and accuracy compared to the chemical indicators. The biomarkers can be successfully used for screening, early detection, and prognosis of different diseases and malignancies. Various types of biomarkers (proteins, nucleic acids, metabolites, etc.) have been reported that in some cases are currently the common targets of commercial diagnosis kits [6, 15].

The biomarkers can be divided into two groups based on their application: diagnostic biomarkers and screening biomarkers. The evaluation of diagnosis biomarkers is frequently done by analysis of enzymes activity, exploring antigen-antibody interaction, or detecting a specific nucleic acid sequence that can be achieved via biotechnological or biochemical techniques including PCR, ELISA, FISH, IHC, etc. [10, 16-18]. An ideal diagnostic biomarker should provide high sensitivity, remarkable specificity, and agreeable accuracy in differentiating between diseases. These biomarkers should additionally have the potential for investigating the drug efficacy and estimating the result of chosen treatment, particularly in cancers [16, 18]. On the other hand, a perfect screening biomarker should be extremely specific, reduce false positive errors, be able to show the different stages of the disease, be determined easily and without the need for complex medical procedures, and also be affordable. Biomolecules that are released into the body liquids (e.g. blood, saliva, and urine) are of the beneficial targets for early screening acute and chronic illnesses, infectious diseases, genetic disorders, and cancers [19-21].

microRNAs are of the newest and most reliable biomarkers reported for the early detection, diagnosis, metastasis, prognosis, and assessment of treatment [22, 23]. In following section, these valuable tiny biomolecules are briefly discussed. The aim of this article is to review the concept of medical diagnostics, biosensors, electrochemical

biosensors and to emphasize the role of nanotechnology in nanobiosensor development and performance for application in microRNA (miRNA), as a new and reliable biomarker, detection. We have also summarized recent ideas and advancements in the field of electrochemical nanobiosensors for miRNA detection, and the important breakthroughs are explained.

microRNAs (miRNAs) AS VALUABLE BIOMARKERS FOR MEDICAL DIAGNOSIS

miRNAs are a class of small, non-coding endogenous RNAs with about 22 nucleotides long that can negatively control their target gene expression at post-transcription levels. Since their discovery in 1993, the essential role of miRNAs has been frequently reported in regulating different genes of humans [24, 25]. The Figure 1 schematically shows the steps of generation and maturation of a miRNA and also, its mode of function in gene regulation.

Due to the involvement of miRNAs in regulating critical genes of vital biological pathways and mechanisms including cell cycle and apoptosis, considerable differences in their expression patterns in healthy and cancerous cells/tissues has been found so far [22, 24]. In addition, a large group of miRNAs categorized as “circulating miRNA” are released into the blood and their expression level is specifically related with the disease stage, beginning of tumorigenesis, cancer development, or metastasis [11, 26]. Circulating miRNAs are considerably stable and can be easily evaluate through blood sampling and following molecular analysis. Having all these advantageous characteristics makes these highly-conserved molecules as ideal biomarkers for being used in medical diagnosis [11, 26].

There are numerous methods designed and suggested for miRNA quantification and some of them including real-time qPCR, northern blotting, microarray, and Deep sequencing of transcriptome (RNAseq) are more preferable due to their specific advantages [11, 28]. Northern blotting is the most ancient method introduced in 1977 and consists of size-based separation of RNA samples and their final hybridization with a complementary probe. The application of this technique is limited because of its low sensitivity, time-consuming procedure, and the use of dangerous radioactive labels [29,

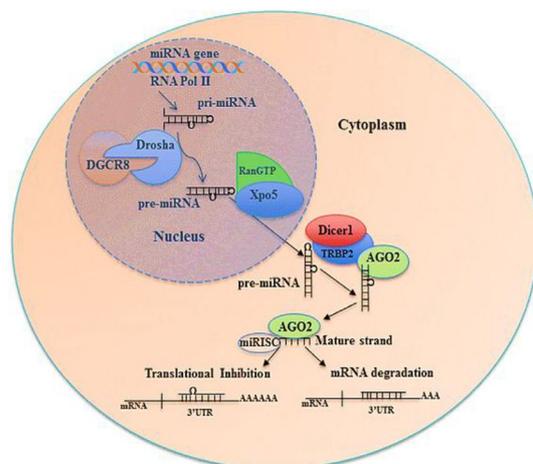


Fig. 1. The way undergone by a miRNA from generation to maturation and function. Reprinted with the permission from ref. [27]

30]. Microarray-based methods were of the interest for years due to their potential for simultaneous examinations of huge number of samples and gaining massive data. Despite of easy manual, microarray-based analysis has been known as a qualitative technique that cannot provide essential sensitivity in miRNA quantification [31, 32]. Recently, deep sequencing of transcriptome has been also used for investigating miRNA in biological samples. Deep sequencing of transcriptome benefits greatly from high-throughput technology for parallel analyses and the ability to explore expression of all annotated miRNAs [33, 34]. However, this technique has shown promising application for discovery of novel miRNAs instead of being used as a quantification handy tool for diagnosis or screening purposes. Nowadays, real-time qPCR is the most common used method for miRNA evaluation. It is a sensitive method which provides multi-analysis even in small labs [1]. In recent years, various strategies such as using the stem-loop primers have been introduced for optimizing this method toward miRNA evaluation [35]. However, the small size of miRNAs still limits this technique in discriminating between miRNA precursors and mature miRNA. Furthermore, real-time qPCR is generally considered as an advanced expert-dependent technique that needs expensive reagents and equipment [7]. Based on all the advantages and shortcomings of conventional methods, the search for better strategies that can lead to rapid inexpensive miRNA determination is

still essentially warranted.

Development of miRNA biosensors has become one of the most attractive field of research in last decade. This interesting approach has presented many biosensing systems that in spite of significant variation, have some key features in common [4]. In the next parts, we will discuss the electrochemical miRNA biosensors and see how they have risen the hope for precise screening and early diagnosis of lethal diseases due to remarkable advantages over the commonly-used methods.

ELECTROCHEMICAL BIOSENSORS FOR miRNA QUANTIFICATION

As IUPAC defined, a biosensor is a sensor which uses biological/biochemical interaction for detection of the analyte. Biosensors generally consist of two major parts: biorecognition element also called “bioreceptor” is the one which interacts with the analyte and is made of biomolecules (protein, nucleic acids, PNA, etc.), cell receptors, enzymes, organelles, tissue, or even a whole cell [5]. This part is assembled with a physicochemical transducer that is responsible for converting the signal resulting from bioreceptor-analyte interaction into measurable signal. The nature of transducer significantly influences the sensitivity of the biosensor and usually is the basis of biosensor classification [4, 36] (Figure 2).

Among the various types of biosensors, the electrochemical ones have been found more applications in biomedical fields due to their impressive sensitivity, high compatibility with advanced technologies, portability, and affordability [3]. Particularly, the association of remarkable features of electrochemical methods with high-selective nature of nucleic acids has led to fabricate successful biosensors for detecting all types of nucleic acids [4, 6]. As far as the detection of specific nucleic acid sequences is really valuable in the diagnosis of bacterial/viral infections and contaminations, these electrochemical biosensing devices can be effectively helpful in biomedical, pharmaceutical, and environmental applications. In the case of miRNAs, a well-designed electrochemical analysis can promisingly make the early detection of lethal diseases possible, lead to apply more efficient treatment, and enhance the patients' quality of life [7, 9, 38, 39].

Electrochemical biosensors can be categorized into

five main groups based on the type of transducer and measurement method: Potentiometry [40], Amperometry [41], Voltammetry [8], Conductometry [42], Impedimetry [43], and Ion charge or field effect [44]. The conductometry- and impedimetry-based biosensors are commonly known as label-free electrochemical biosensors and can determine the analyte according to the behavior of electroactive species on the electrode surface [45]. However, as shown in Figure 3, the voltammetric modes are the most frequently used electrochemical techniques preferred for qualitative and quantitative analyses. In electrochemical miRNA biosensors, cyclic voltammetry (CV), differential pulse voltammetry (DPV), and square wave voltammetry (SWV) are the most attractive techniques due to higher sensitivity [7, 46, 47]. In almost all of the miRNA biosensors, the detection procedure has been done based on the specific hybridization of miRNA target with complementary probe. The probe is generally labeled with the materials which are able to generate an electrochemical signal [48]. In another strategy, miRNA biosensors have been fabricated

using electroactive labels such as ferroceneboronic acid [49], methylene blue [50], or oracet blue [9] which showed higher tendency to intercalate into the double stranded nucleic acids compared to single strands. Hence, there are three main steps in fabricating an electrochemical miRNA biosensor: 1) immobilization of complementary probe on the electrode surface; 2) hybridization of miRNA target with the probe on the surface of electrode; and 3) electrochemical analysis of the modified electrode [5, 7].

Although the design of biosensor structure including the probe immobilization methodology, the biosensing platform, and the modification of electrode surface play the crucial role in fabricating an electrochemical biosensor and is usually presented as the identifying features of a device. However, the target is also considered as an important part that should be valuable to be screened or measured. Figure 4 shows the frequent miRNAs chosen as the target of reported miRNA biosensors. As it can be seen, miR-21 is the most common miRNA among the others (32%) that can be explained due to established contribution of this

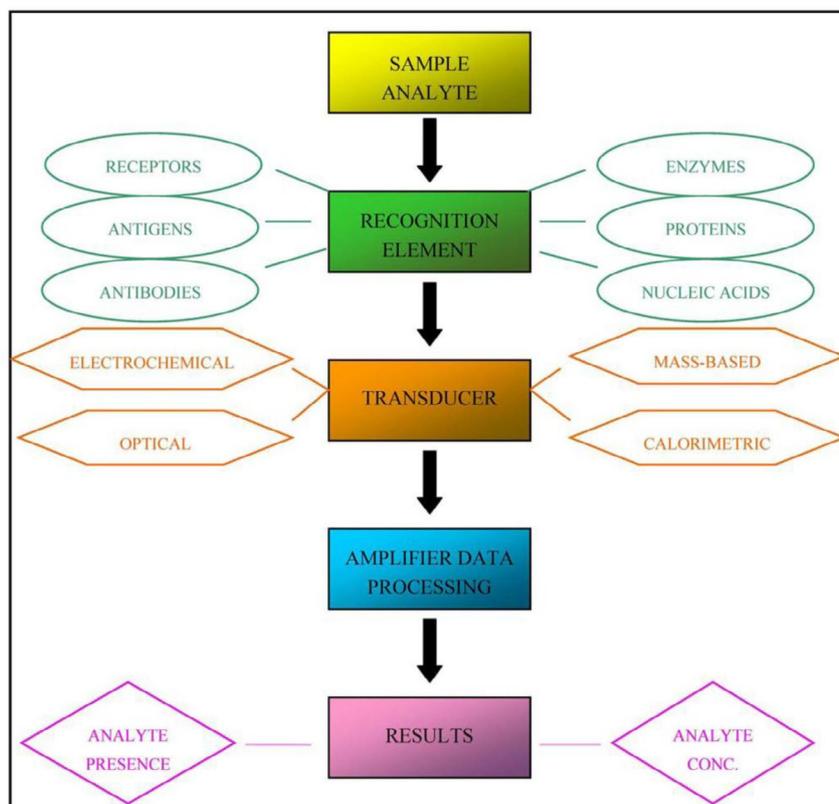


Fig. 2. Different types of bioreceptors and transducers used in biosensor fabrication. Reprinted with the permission from ref. [37].

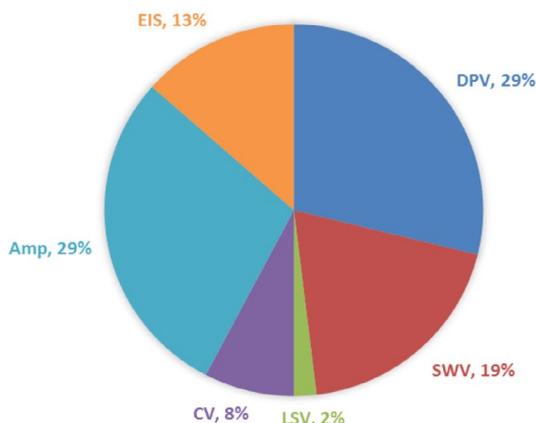


Fig. 3. The most common electrochemical methods used for biosensing. Data collected from Web of Science database.

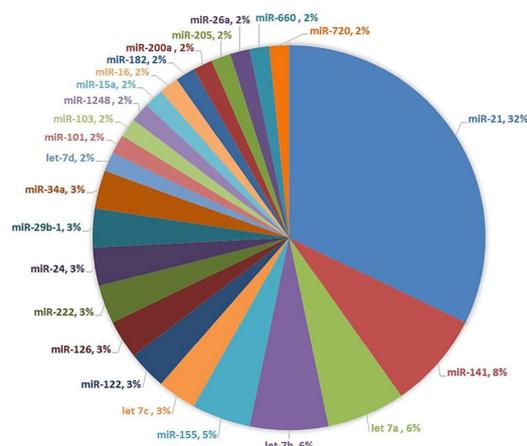


Fig. 4. The frequency of miRNAs reported as the target in reported miRNA-nanobiosensors. Data collected from Web of Science database.

miRNA in numerous types of malignancies [51]. Moreover, the electrochemical miRNA biosensors have been presented as the more sensitive devices with promising medical application, but, enhancing the sensitivity, increasing the selectivity, expanding the linear range, lowering the detection limit, decreasing the device size, and facilitating the portability have been always the desirable criteria to meet [52, 53]. Therefore, various approaches have been presented in order to reach an ideal miRNA biosensor. Using different enzymes involved in oxidoreduction reactions has been reported in lots of electrochemical miRNA biosensors while the enzymes are responsible for generating the final electrochemical signal [54, 55]. In these biosensors, the substrate of the enzyme is added to the reaction zone as the label of the probe. It worth noting that such enzyme-based biosensors need strict optimized condition in order to keep the enzyme stable and active during biosensor fabrication and electrochemical measurement [56]. Another idea is employing conductive polymers for electrode modification. These polymers and their derivatives can considerably expedite the electron transfer on the electrode that lead to improvement of electrochemical behavior of electrode and amplification of detection signal [57]. Furthermore, during the recent decade, the engagement of nanomaterial in biosensor technology has opened interesting new doors for miniaturizing the electrochemical biosensors while improving their analytical capabilities [53]. The great impact of nanotechnology in electrochemically miRNA

biosensors construction will be discussed in the next section.

ELECTROCHEMICAL NANOBIOSENSORS FOR miRNA QUANTIFICATION

In recent years, the advancements of science and technology at the nanoscale have entered extensively into the biosensor technology, especially electrochemical ones [53]. Nanotechnology is defined as the potentials for producing, fabricating, and manipulating materials at molecular/atomic levels in order to control their properties and applications [58]. Since nanomaterials (with a size range of 1-100 nm) generally show novel and different optical, electrical, electrochemical, and magnetic properties compared to bulk forms, using them in design and construction of biosensing systems not only reduces the device size, but also adds extra useful characteristics to biosensors leading to effective improvements in accuracy and reproducibility of bioanalysis result [58, 59]. Remarkable surface to volume ratio and a size range similar to the biomolecules are responsible for efficient non-destructive reactions between nanomaterials and biomarkers [7, 53, 60]. A wide range of nanoparticles, nanorods, nanowires, nanoclusters, nanocomposites, etc. have been used in electrochemical biosensor fabrication for miRNA determination (Figure 5). These nanomaterials have mainly participated in electrochemical signal amplification, bioseparation, and biomolecule immobilization platform [52, 53]. Most of the recent advances in the field of

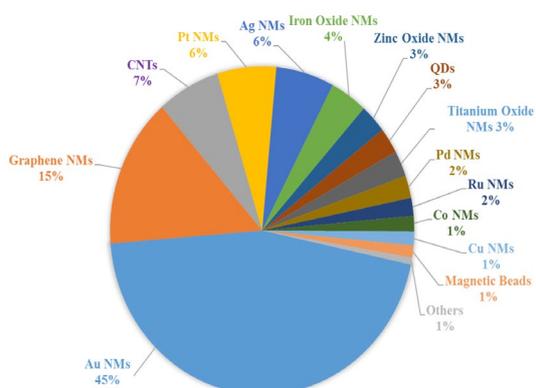


Figure 5. The most commonly used nanomaterials in biosensor fabrication and their frequencies. Data collected from Web of Science database.

electrochemical nanobiosensors for miRNA quantification have been summarized in the Table 1. The Table does contain specifications of nanobiosensors such as detected miRNA, Electrode and Electrochemical method, Mechanism, applied nanomaterials, Dynamic range and Limit of Detection (LOD).

One of the first studies on electrochemical miRNA nanobiosensors was published in 2006 where the authors reported the use of electrocatalytic OsO₂ nanoparticles for signal amplification [76]. The detection procedure was begun with miRNA peroxidation for generating dialdehydes at the 3' end. After the hybridization of oxidized miRNA with previously-immobilized probe on the electrode surface, the isoniazid-modified OsO₂ nanoparticles were added. The final determination was done via oxidation of hydrazine. The detection limit of this biosensor was evaluated to be 80 fM [76]. In another study by Jolly et al., a highly sensitive dual mode electrochemical platform has been developed for miRNA detection [87]. As the Figure 6A demonstrates, the gold electrode was first modified with a thiolated peptide nucleic acids probe sequence and 6-mercapto-1-hexanol (MCH). After hybridization with the target miR-145, positively-charged gold nanoparticles have been added and by using thiolated ferrocene, the square wave voltammograms have been recorded. A wide dynamic range from 1 fM to 100 nM along with a limit of detection of 0.37 fM have been reported for this biosensing platform [87]. In another study by Dong et al., a miRNA biosensor was fabricated using oligonucleotide encapsulated

silver nanoclusters as electrochemical probe. This nanoprobe played a dual role in specific detection of miRNA sequence and effective electrochemical signal amplification. The silver nanoclusters mimicked enzymatic behavior for reducing H₂O₂ and generating the final detection signal. This miRNA nanobiosensor showed a linear range of 100 fM-10 nM and reached a detection limit of 67 fM [88].

Magnetic nanomaterials have been generally employed for bio-separation during the washing steps in electrochemical biosensing [89]. For this purpose, these nanoparticles should be coated with some kinds of polymers or noble metals prior to functionalizing by biomolecules [53, 90]. These additional layers significantly enhance the biocompatibility of nanoparticle and provide a welcoming surface for immobilization of biomolecules. Moreover, such coating step prevents or at least considerably decreases the aggregation and oxidation of magnetic nanoparticles [90]. In a study by Wang et al., a magnetic-controllable electrochemical biosensor was developed for evaluation of miRNAs involved in oral cancers [91]. In this work, the capture probes were immobilized on the magnetic beads surface through streptavidin-biotin reaction. After the addition of target miRNA and signal probe, the resulted hybrid formed a ternary "Y" junction structures on the surface of magnetic beads. By employing a voltage on the electric coil, the magnetic nanostructures anchored to the surface of working gold electrode and the enzyme horseradish peroxidase (HRP) produced the final electrochemical signal due to interact with TMB/H₂O₂ as its substrate. This miRNA nanobiosensor reached a linear range between 1 aM and 10 fM and also a detection limit of 0.22 aM [91]. Recently, Campuzano et al. fabricated a miRNA magnetosensor in order to quantification of miR-21 in raw samples. The complementary anti-miR-21 was biotin-labeled and acted as the capture probe which specifically hybridized with the target sequence. The hybrid was then captured by p19-modified magnetic beads and exposed to streptavidin labeled-HRP. The nanocomplex was magnetically disposed on the screen printed carbon electrodes and the enzymatic reaction upon the addition of H₂O₂ and hydroquinone generated a measurable amperometric signal. A linear range of 0.14-10 nM and a detection limit of 0.04 nM have

Table 1. Summary of recent electrochemical nanobiosensor components and performances.

No	miRNA	Electrode/Electrode method	Mechanism	Nano	Dynamic range	LOD	Ref
1	miR-21 miR-122	SPCE/SWV	Hybridization/ p19 Binding, displacement	AuNP	10.0 aM-1.0 μ M	5.0 aM	[61]
2	miR-122	SPCE/SWV	Four-Way Junction/MBE CP/three probes/Str/Bi	AuNP	10.0 aM- 1.0 fM	2.0 aM	[62]
3	miR-21	AuE/Amp	ALP/p-aminophenol/3-aminophenylboronic acid	AuNP	10.0 fM- 5.0 pM	3.0 fM	[63]
4	miR-21	GCE/SWV	CP/probes/star trigon/endonuclease/Pol	AuNP	100.0 aM- 1.0 nM	30.0 aM	[64]
5	let-7a	AuE/DPV	Str-ALP/DNA polymerase	AuNP	100.0 fM- 1.0 nM	99.2 fM	[55]
6	miR-182	AuE/CV	ferrocene (Fc)/Str/Bi	AuNP	10.0 fM- 2.0 pM	10.0 fM	[65]
7	miR-21	AuE/Amp	LNA hairpin/Bi- DNA-bio-barcode/Str-HRP	AuNP	0.01 pM-700.0 pM	6.0 aM	[66]
8	miR-21	AuE/EIS	hemin-G-quadruplex/ hairpin CP /AP DNAzyme /hairpin	AuNP	5.0 pM-5000.0 pM	3.96 pM	[43]
9	miR-26a	AuE/EIS	CP/probes/hemin/H ₂ O ₂	AuNP	30.0 aM- 10.0 fM	15.0 aM	[67]
10	miR-21	AuE/DPV	3D DNA stem-loop probe/ferrocene	AuNP, DNA NS	100.0 pM- 1.0 mM	10.0 pM	[68]
11	miR-141	GCE/SWV	GO/conducting polymer/CP/AB	GO	1.0 fM-1.0 nM	5.0 fM	[69]
12	miR-34a	PGE/EIS	GO/CP	GO	20.5 pM- 10.0 pM	2.5 pM	[70]
13	miR-141 miR-29b	SPGE/SWV	ELISA-like amplification/AB/HRP	GO/MWCNTs	10.0 fM- 1.0 nM	10.0 fM	[71]
14	miR-21	GCE/SWV	GO/AuNP/3 CP/Ru(NH ₃) ₆ ³⁺	GO/ Titanium	1.0 aM- 10.0 pM	0.76 aM	[72]
15	miR-126	GCE/DPV	PNA CP/AB/PAMAM dendrimer/HRP/H ₂ O ₂	Graphene/ Au-Ag NC	1.0 fM- 10.0 nM	0.79 fM	[73]
16	miR-21	GCE/Amp	LNA MBE/Biotin-barcode/ Str-HRP/H ₂ O ₂	Graphene/ AuNP	0.1 pM- 70.0 pM	0.06 pM	[41]
17	miR-21	AuE/Amp	Bi-LNA MBE/Str-HRP/ H ₂ O ₂	Graphene/ AuNP	1.0 pM-5000.0 pM	0.4 pM	[74]
18	miR-155	GCE/ DPV	hairpin CP/hybridization chain reaction/MB	GO/AuNP	10.0 fM- 1.0 nM	3.3 fM	[75]
19	miR-155	AuE/Amp	CP/probe/TMB/HRP	Graphene QD	1.0 fM – 100.0 pM	0.14 fM	[76]
20	let-7a	ITO/Amp	CP/hydrazine	Osmium dioxide NP	0.3 pM -200.0 pM	80.0 fM	[77]
21	let-7a	AuE/LSV	3 probes/phi29 DNA polymerase	AgNP	0.1 fM -10.0 nM	50.0 aM	[78]
22	miR-101	AuE/Amp	CP/ H ₂ O ₂	Cu NP	30.0 fM- 250.0 fM	8.2 fM	[79]
23	let-7c	AuE/SWV	PNA CP/aniline/ H ₂ O ₂	Ruthenium oxide NP	5.0 fM – 2.0 pM	2.0 fM	[80]
24	miR-155	GCE/ CV	Nafion/thionine/ H ₂ O ₂	Pd NP	5.6 pM -56.0 μ M	1.8 pM	[46]
25	miR-155	GCE/ SWV	MB/ligase chain reaction/T4 ligase	PbS/CdS quantum dots	50.0 fM -30.0 pM	12.0 fM	[81]
26	miR-24	GCE/DPV	CP/Oxidation of Guanine	MWCNT	1.0 pM -1.0 nM	1.0 nM	[82]
27	miR-141	GCE/SWV	CP/electroactive polymer	MWCNT	1.0 fM- 10.0 nM	8.0 fM	[47]
28	miR-24	GCE/DPV	CP/PAMAM dendrimer /MB	MWCNT	1.0 fM -10.0 pM	0.10 fM	[83]
29	miR-34a	SPCE/DPV	CP/carbon NF	Carbon NF	1.0 fM- 10.0 pM	0.5 fM	[84]
30	miR-141	AuE/Amp	DNA/MBE CP/HRP/ H ₂ O ₂	DNA TNS	-	1.0 fM	[85]
31	miR-21	AuE/Amp	DNA NS/CP/HRP/ H ₂ O ₂	DNA TNS	10.0 fM- 10.0 nM	10.0 fM	[86]
32	let-7d	AuE/Amp	DNA NS/CP/HRP/H ₂ O ₂	DNA TNS	10.0 fM- 10.0 nM	10.0 aM	[87]

Table instructions: NP: nanoparticle; NS: nanostructure; SPCE: Screen-printed carbon electrodes; AuE: Gold Electrode; GCE: Glassy Carbon Electrode; PGE: pencil graphite electrode; SPGE: Screen-printed Gold electrodes; ITO: indium tin oxide; SWV: square wave voltammetry; Amp: amperometry; DPV: differential pulse voltammetry; CV: cyclic voltammetry; EIS: Electrochemical Impedance Spectroscopy; LSV: Linear sweep voltammetry; MBE: molecular beacon; CP: capture probe; Str; Bi; ALP: alkaline phosphatase; LNA: locked nucleic acid; AP: aptamer; HRP: horseradish peroxidase; GO: graphene oxide; AB: antibody; MBE: molecular beacon; MB: methylene blue; TMB: 3,3',5,5'-tetramethylbenzidine; Pl: polymer; QD: quantum dots; DNA TNS: DNA Tetrahedral Nanostructure

been reported for this miRNA nanobiosensor [92]. A hybrid structure composed of a magnetic core, an intermediate polymer layer, and an outer layer of gold nanoparticles has been used for miRNA-nanogenosensor construction by Daneshpour et al. [93]. This nanocomposite was functionalized by a biotin-labeled probe and exposed to miR-106a (the target). The target complex was then added to previously probe-modified SPCE followed by electrochemical analysis (Figure 6B). Using such simple platform and double-probe strategy, this nanogenosensor achieved a detection limit of 0.3 fM and a linear range of 1000-0.001 pM [93].

The use of nanomaterials on the surface of electrodes or electrochemical platforms in biosensor development is a very attractive strategy that mainly leads to increase the surface area, chemical stability, and numbers of binding events [53, 89]. In addition, electrode modification by metal nanostructures or conductive carbon-based nanomaterials such as carbon nanotubes (CNTs) and graphene offers unique advantages including enhancing the electron-transport ability, improving the surface conductivity, and increasing the biomolecule load capacity especially in electrochemical label-free biosensing [7, 48, 50, 53]. Congur et al. have recently introduced a miRNA-nanobiosensor based on the graphene oxide (GO) modified pencil graphite electrodes (PGEs). In this work, the capture probe firstly hybridized with miR-34a (target) and the hybrid complex was added to the previously prepared PGE. The determination of miRNA was carried out via impedimetric analysis and the detection limit of 1.9 mg/mL was assessed in the linear concentration range from 0 to 10 mg/mL [70]. Rafiee-pour and his coworkers have used multiwall CNTs (MWCNTs)-modified glassy carbon electrode (GCE) for fabricating a label-free miRNA biosensor based on employing methylene blue as a hybridization indicator [50]. After the immobilization of complementary probe on the modified GCE, the miR-21 was added as the analyte. Finally, the miR-21 concentration was determined according to the difference between the oxidation peak current of methylene blue on DNA or duplex DNA/miRNA. This miRNA biosensor reached a detection limit of 84.3 fM in linear range between 0.1 and 500.0 pM [50].

Furthermore, there are miRNA nanobiosensors

that have successfully used combined approaches for electrochemically determination of their targets. For this purpose, Azimzadeh et al simultaneously employed graphene oxide and gold nanorods for modifying GCEs (Figure 6C). This nanobiosensor showed good sensitivity and selectivity in quantification of miR-155 and obtained a low detection limit of 0.6 fM [7]. Although the final electrochemical signal was generated from the intercalating label Oracet Blue (OB), however, the impact of using nanomaterials in improving the sensitivity and stability of biosensing system can be definitely understood by comparing this work with their former study [9].

Current Challenges Of Electrochemical miRNA (Nano)Biosensor Toward Clinical Application

Recent studies demonstrate the remarkable role of nanotechnology in improvement of various aspects of electrochemical miRNA biosensing. The use of different nanomaterials in electrochemical platforms offers considerable analytical features which seem really attractive in sensitive miRNA diagnosis [48, 56, 89]. However, there are still some major challenges in the way of constructing biosensing systems for point-of-care (POC) application. One of the main issues to overcome is simplifying the portability of these devices. Although nowadays the ongoing researches at nanoscale design of electrochemical transducers and integrating all the essential parts as a functional unit are rapidly moving forward, however, the clinical application of these creatures is still in infancy phases [94]. In other hand, the use of nanomaterials has raised the points related to nano-safety, biocompatibility, and environmental friendliness [95, 96]. Furthermore, the large-scale preparation of optimized nanomaterials for analytical applications is not sufficiently economic and cannot desirably reduced the price of screening/diagnosis procedure [96]. Finally, the stable sensitive and specific response is also considered another criterion that most of presented (nano)biosensors fails to meet, while long-term storage stability is one of the most fundamental features of a POC device [53].

Despite of all the concerns, the progressive advancement of electrochemical nanoplatfoms and the devoted efforts made in research and development of miRNA diagnosis are totally

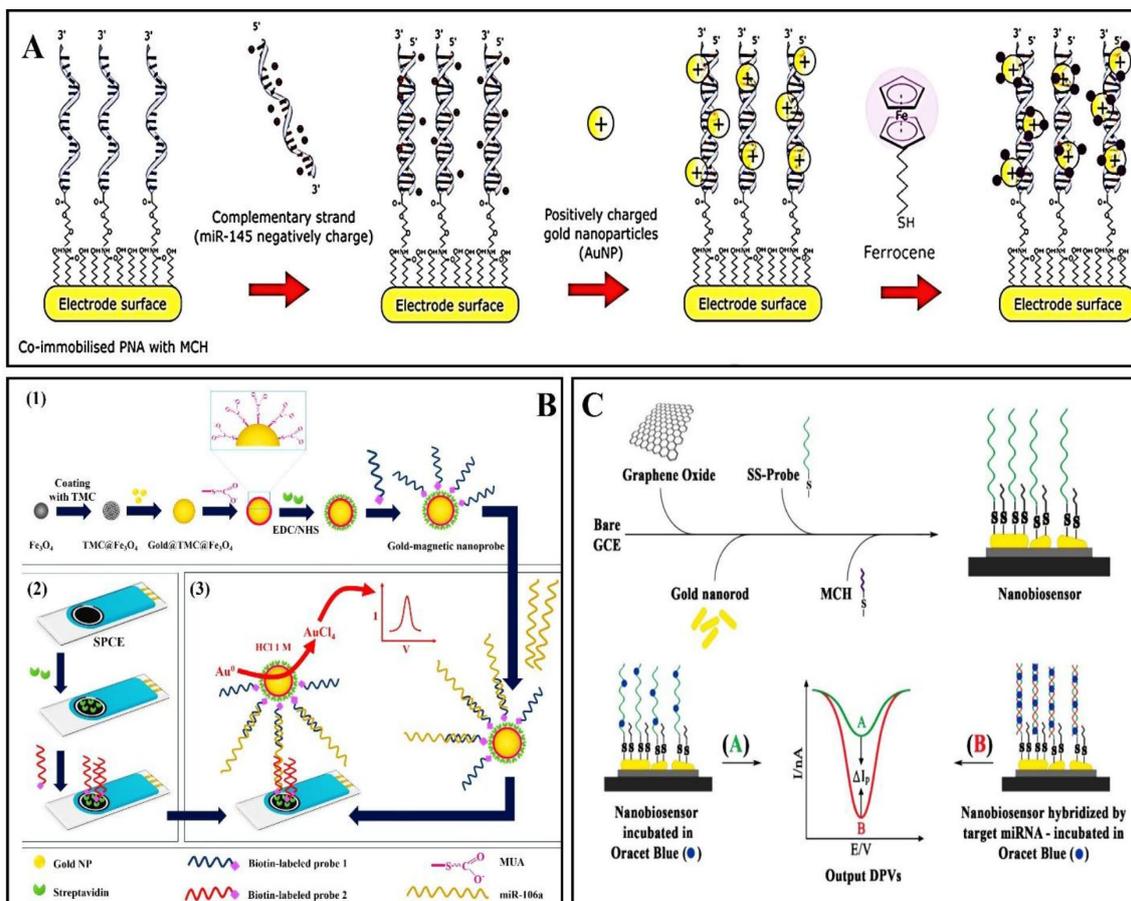


Fig 6. Examples of using nanomaterials in miRNA biosensor construction. (A) Schematic of the detection strategy using positively-charged gold nanoparticles via probe-modified gold electrode and biosensing platform. Reprinted with the permission from ref. [89] (B) Schematic illustration of the detection mechanism for miR-106a quantification by the nanogenosensor. (1) Preparation the nanoprobe; (2) modification of the electrode; and (3) hybridization steps (TMC = N-trimethylchitosan). Reprinted with the permission from ref. [95] (C) Schematic demonstration of a nanobiosensor fabricated using graphene oxide and gold nanorods for electrode modification and Oracet blue as the electrochemical label. Reprinted with the permission from ref. [7] .

promising. Therefore, obtaining a fast unexpensive miniaturized device for POC testing trace amounts of clinically-important miRNAs might become a reality in the near future.

CONCLUSIONS

In this paper, we discussed the important role of nanotechnology in medical diagnostic; in particular electrochemical nanobiosensing of miRNAs. With this review, we tried to explain the importance of miRNA biomarkers and electrochemical nanobiosensors to quantify them for medical diagnostics purposes. We summarized recent advances in electrochemical nanobiosensors of miRNA quantification for medical diagnostics application. We also talked over the challenges

in the way toward clinical applications of the electrochemical nanobiosensors for miRNA. It seems the electrochemical nanobiosensors and microRNA biomarkers are efficient and practical approaches for diseases diagnosis in clinics in future.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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