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Label-free voltammetric detection of MicroRNAs at multi-channel screen printed array of electrodes comparison to graphite sensors

Arzum Erdem*, Gulsah Congur

Ege University, Faculty of Pharmacy, Analytical Chemistry Department, Bornova, 35100 Izmir, Turkey

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ABSTRACT

The multi-channel screen-printed array of electrodes (MUX-SPE16) was used in our study for the first time for electrochemical monitoring of nucleic acid hybridization related to different miRNA sequences (miRNA-16, miRNA-15a and miRNA-660, i.e, the biomarkers for Alzheimer disease). The MUX-SPE16 was also used for the first time herein for the label-free electrochemical detection of nucleic acid hybridization combined magnetic beads (MB) assay in comparison to the disposable pencil graphite electrode (PGE).

Under the principle of the magnetic beads assay, the biotinylated inosine substituted DNA probe was firstly immobilized onto streptavidin coated MB, and then, the hybridization process between probe and its complementary miRNA sequence was performed at MB surface. The voltammetric transduction was performed using differential pulse voltammetry (DPV) technique in combination with the single-use graphite sensor technologies; PGE and MUX-SPE16 for miRNA detection by measuring the guanine oxidation signal without using any external indicator. The features of single-use sensor technologies, PGE and MUX-SPE16, were discussed concerning to their reproducibility, detection limit, and selectivity compared to the results in the earlier studies presenting the electrochemical miRNA detection related to different miRNA sequences.

1. Introduction

MicroRNAs (miRNAs) consist of a new class of endogenous 18–25-nucleotide-long RNAs. The majority of diseases (cancer, heart failure, vascular disease, diabetes etc.) has been related to the regulation of miRNAs due to their influence on the fundamental cellular process including cell proliferation, apoptosis, differentiation, and migration [1–6]. In the past 3 decades, many studies were performed in order to describe the structural and functional properties of miRNAs [2,7–11]. According to the literatures, it was found that miRNAs could be used as biomarker for diagnosis of cancer [4,9,12–14], cardiovascular disease [15–17] and Alzheimer disease [5,18–20].

The conventional miRNA detection techniques, e.g, reverse transcription polymerase chain reaction (RT-PCR) and Northern blotting are expensive, complicated, time consuming and hardly employed for on-site measurement. In contrast to these techniques, the electrochemical detection techniques have more advantages such as, simplicity, time saving analysis by offering spesific recognition process and requiring small amount of sample. There have been different studies based on electrochemical detection for miRNA analysis reported in literature [21–24]. A label-free electrochemical genosensor based on detection of guanine oxidation signal after hybridization between miRNA-122 spesific inosine substituted cDNA probe and its complementary target was developed by Lusi et al. [21]. The results obtained using different electrochemical techniques combined with passive adsorption and electrochemical deposition were compared in order to obtain more efficient miRNA determination.

Pöhlmann and Sprinzl [22] fabricated an enzymatic gap hybridization assay using gold electrode to detect miRNA10a, miRNA-16, and miRNA-21 isolated from human adenoma cell cancer line (MCF7). Expression levels of these miRNAs in MCF-7 cells were determined and unspesific binding or cross hybridization were tested.

In the study of Gao and Yang [23], an amperometric detection of miRNA in combination with the electrocatalytic isoniazid-capped OsO_2 nanoparticles and chemical ligation procedure was presented by using indium tin oxide (ITO) electrode. Total RNA was extracted from human HeLa cells and miRNA-let-7b was detected sensitively using this assay, DL was found 80 pmol/L. Similarly, this research group tagged miRNA-let-7b with $Ru(PD)_2Cl_2$ ($PD=1$, 10-phenanthroline-5,6-dione) in total RNA extract and obtained amperometric and voltammetric responses of hydrazine in the presence of hybridization between complementary sequences by using ITO electrode [24].

* Corresponding author. Tel.: +90 232 311 5131; fax: +90 232 388 5258.
E-mail address: arzum.erdem@ege.edu.tr (A. Erdem).

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Under the scope of designing novel sensor platform for miRNA detection, we present in this study a novel magnetic beads assay combined with electrochemical single-use technology for the detection of Alzheimer disease (AD). miRNA-15a, miRNA-16 and miRNA-660 were selected as the biomarkers for diagnosis of AD. The alterations of miRNA-15a, miRNA-16 and miRNA-660 in AD brain are demonstrated in literature [7,25,26].

AD is a chronic neurodegenerative disease of brain, and consequently, a reliable diagnosis of AD at early stages has a key importance for the efficient treatment of AD. Numerous studies have been reported including AD diagnosis by using different miRNAs as biomarkers [6,7,27–32].

Hebert et al. [7] studied on how AD pathogenesis occur and affect protein aggregation using both statistical and experimental studies by analyzing the expression levels of amyloid precursor protein (APP), miRNA-20a, miRNA-17-5p and miRNA-106 that were evaluated correlation in expression levels between these expressed miRNAs and APP via quantitative real time PCR (qRT-PCR) and Northern blotting techniques. Similarly, isolated miRNAs from cerebrospinal fluid [27] and cerebral cortex [28] were detected using qRT-PCR and the results were discussed in terms of disregulation of miRNAs [27,28]. In another study [29], isolated miRNAs from cerebral cortex were analyzed by using locked nucleic acid (LNA) microarray and Northern blot techniques for the purpose of showing differences on disregulation of miRNAs at different part of brain.

There have not yet been any reports in the literatures about the label-free electrochemical monitoring of miRNA using multi-channel screen printed array (MUX-SPE16) designed as disposable screen-printed array of electrodes containing one reference and auxiliary electrode in combination with 16 working electrodes. Although there are a few numbers of multi-channel screen printed array of electrodes presented in the literature [13,14,33], the MUX-SPE16 has been used for the first time in our study for voltammetric detection of miRNAs based on the label-free nucleic acid hybridization scheme.

Under the principle of the magnetic beads assay, the biotinylated inosine substituted DNA probe was firstly immobilized onto streptavidin coated MB, and then, the hybridization process between probe and its complementary miRNA sequence was performed at MB surface. The voltammetric transduction was performed in order to monitor label-free miRNA detection by measuring the guanine oxidation signal using differential pulse voltammetry (DPV) technique in combination with MUX-SPE16 system comparison to PGE.

2. Experimental

2.1. Apparatus

The oxidation signal of guanine was measured by AUTOLAB PGSTAT electrochemical analysis system and GPES 4.9 software package (Eco Chemie, The Netherlands) using DPV technique. MUXSCNR16-multichannel system was used to perform DPV measurements with MUX-SPE16s. The raw data was also treated using the Savitzky and Golay filter (level 2) of the GPES software, followed by the moving average baseline correction with a “peak width” of 0.03. The three-electrode system consisted of the PGE, an Ag/AgCl/KCl reference electrode and a platinum wire as the auxiliary electrode.

The probe immobilization onto the surface of magnetic beads and the hybridization process were performed using the magnetic separator, MCB 1200 (Sigris, USA).

2.2. Chemicals

The biotin capped DNA probes and complementary RNA oligonucleotides (ODN), were purchased (as lyophilized powder) Ella Biotech (Germany).The base sequences of ODNs are listed below (I: inosine, U: uracil):

***miRNA-15a sequences*—DNA Probe:** 5′-biotin-CAC AAA CCA TTA TIT ICT ICT A-3′; **Target RNA-1:** 5′–UAG CAG CAC AUA AUG GUU UGU G-3′; **Target RNA-2:** 5′–UAG CAG CAC AUA AUG GUU UGU GGA UUU UGA-biotin-3′.

***miRNA-16 sequences*—DNA Probe:** 5′-biotin-CIC CAA TAT TTA CIT ICT ICT A-3′; **Target RNA:** 5′–UAG CAG CUC GUA AAU AUU GGC G-3′.

***miRNA-660 sequences*—DNA Probe:** 5′-biotin-CAA CTC CIA TAT ICA ATI IIT A-3′; **Target RNA:** 5′–UAC CCA UUG CAU AUC GGA GGU G-3′

All ODN stock solutions (500 μg/mL) were prepared with Tris–EDTA buffer solution (10 mM Tris–HCl, 1 mM EDTA, pH: 8.00; TE) and kept frozen. More dilute solutions of ODNs were prepared with either TTL buffer (100 mM Tris–HCl, pH 8.0, and 0.1% Tween20 and 1 M LiCl) or 50 mM phosphate buffer solution (PBS, pH 7.40) according to the hybridization protocol. Other chemicals were in analytical reagent grade and they were supplied from Sigma and Merck.

Streptavidin coated magnetic beads (magnetic particles) in 0.94 mm diameter size were purchased from Estapor, Merck (France).

2.3. Electrode preparation

2.3.1. Pencil graphite electrodes (PGEs)

A Tombo® pencil was used as a holder for the graphite lead. Electrical contact with the lead was obtained by soldering a metallic wire to the metallic part. The pencil was held vertically with 14 mm of the lead extruded outside (10 mm of which was immersed in the solution). PGEs were pretreated by applying +1.40 V for 30 s in 0.5 M acetate buffer containing 20 mM NaCl (ABS, pH 4.80).

2.3.2. Screen-printed array of electrodes (MUX-SPE16s)

The MUXSCNR-16 compatible MUX-SPE16 was designed according to our request, and characterized by Dropsens Company (Spain) containing one main electrochemical cell with 16 working carbon electrodes combined with one reference electrode and one auxiliary electrode. Each working electrode at MUX-SPE16 was pretreated one by one by applying+0.90 V for 60 s in ABS (pH 4.80).

2.3.3. Preparation of probe-coated magnetic particles and miRNA hybridization at the surface of magnetic beads

The preparation of ODN-coated magnetic beads (magnetic particles, MBs) was performed by following the reported procedure [34,35]. 3 μL of streptavidin-coated MBs was transferred into a 1.5-mL centrifuge tube. The MBs were then washed with 90 μL TTL buffer (250 mM Tris–HCl, pH 8.0, 0.1% Tween20 and 1 M LiCl) and resuspended in 30 μL TTL buffer containing desired concentration of biotinylated probe and incubated for 15 min. at room temperature with gentle mixing. The immobilized probe was then separated and washed once with 90 μL TT buffer (250 mM Tris–HCl, pH 8.0, and 0.1% Tween20), resuspended in 20 μL PBS containing desired concentration of complementary (target of probe) RNA, or non-complementary RNA sequences. The hybridization reaction was carried out at room temperature. After the hybridized MB conjugates were then washed with 90 μl PBS, the sample was resuspended in 25 μL of 0.05 M NaOH solution for alkaline treatment and incubated for 5 min in order to release the captured hybrid products from the surface of MBs. Then, 25 μL of resulted sample was transferred into the vial containing 85 μL ABS, and thus, 110 μL sample was finally mixed for 1 min to obtain a homogenous sample.

For miRNA detection by using PGEs, the pretreated electrodes were dipped into these vials containing 110 μL sample, and kept for passive adsorption step for 15 min. The electrodes were then

rinsed with ABS for 5 s. The PGEs were connected to the electrochemical cell containing three electrode system in ABS buffer to perform the DPV measurements.

For miRNA detection by using MUX-SPE16s, 3 μL of sample was dropped consecutively onto the surface of each working electrode at MUX-SPE16s, and then it was kept for passive adsorption step during 15 min. Then, the MUX-SPE16 s was washed three times using ABS. The MUX-SPE16 were connected with multichannel system integrated to electrochemical analyzer. 850 μL ABS was then dropped onto the full surface of MUX-SPE16s to perform DPV measurements consecutively.

The schematic representation of the label-free voltammetric detection of miRNA-16 at MUX-SPE16 was presented in Scheme 1.

2.3.4. *Voltammetric transduction*

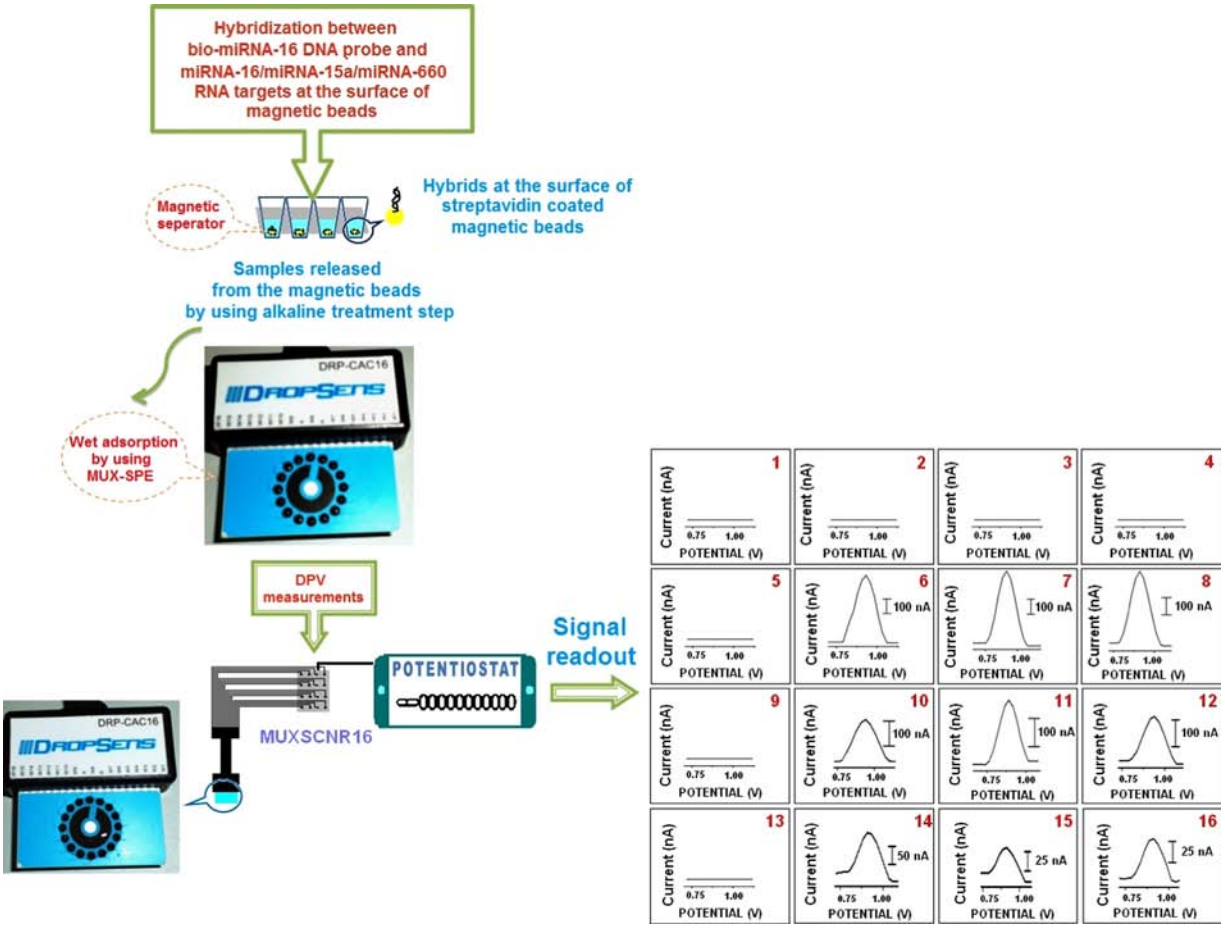
The guanine oxidation signal was measured by using DPV in ABS by scanning from +0.2 V to +1.45 V at the pulse amplitude as 50 mV with the scan rate as 50 mV/s.

3. Results and discussion

Firstly, the effect of DNA probe concentration upon the response was explored in different concentrations of probe varying from 10 to 100 μg/mL. The guanine oxidation signal was measured at +1.00 V using PGE in the presence of full hybridization between miRNA-16 specific DNA probe and its RNA target as 60 μg/mL in 10 min hybridization time (shown in Fig. S1). An increase was obtained at guanine signal while increasing probe concentration till 80 μg/mL and then it levelled off. In the case of full hybridization between 80 μg/mL DNA probe and 60 μg/mL miRNA-16 target, the average guanine signal was measured as 3.85 ± 0.11 μA with relative standart deviation (RSD%) 3.00% ($n=3$) by using single-use graphite sensor, PGE. For our further experiments, the optimum DNA probe concentration was chosen as 80 μg/mL.

Next, the hybridization was processed in different hybridization time varying from 2.5 min to 40 min, and consequently, the guanine signal was measured voltammetrically (not shown). 10 min was selected as optimum hybridization time for our further study due to a higher response with a better reproducibility.

The voltammetric miRNA detection using PGE was also performed in different target RNA concentrations in the range of 5–100 μg/mL. The changes at the magnitude of guanine signal were evaluated in the presence of hybridization between 80 μg/mL miRNA-16 spesific DNA probe and its complementary RNA target. According to full hybridization response, the guanine signal gradually increased till 80 μg/mL RNA concentration, and then decreased (shown in Fig. S2 and Fig. 1A). Since the highest sensor response with a better reproducibility (RSD% ($n=3$)=1.5%) was recorded at 80 μg/mL target RNA; the selectivity study was further



Scheme 1. Schematic representation the selectivity study of the label-free voltammetric detection of miRNA-16 at MUX-SPE16. The voltammograms representing the signals observed at the electrodes of (1), (2), (3), and (4) with control experiments performed in ABS (pH 4.8); the signals observed at the electrodes of (5), (9), and (13) in the absence of hybridization using inosine substituted the miRNA-16 DNA probe alone; the guanine signals observed at the electrodes of (6), (7), and (8) in the presence of hybridization between miRNA-16 DNA probe and its complementary RNA target; the guanine signals observed at the electrodes of (10), (11), and (12) in the presence of hybridization between miRNA-16 DNA probe and miRNA-15a RNA target-1; the guanine signals observed at the electrodes of (14), (15), and (16) guanine signals in the presence of hybridization between miRNA-16 DNA probe and miRNA-660 RNA target.

performed at this concentration level of target RNA sequences by using PGE.

The detection limit (DL) was calculated as 230 pmole in 110 μL samples according to method described by Miller and Miller [36].

The effect of alkaline medium to miRNA was also checked by using miRNA-15a sequences, and the results are presented in Fig. S3. The hybridization of 80 μg/mL miRNA-15a DNA probe with 80 μg/mL miRNA-15a RNA target-2 was performed. In addition, a biotinylated DNA probe/biotinylated miRNA-15a RNA target-2 was immobilized at the surface of MB for control. Then, each sample was treated with 0.05 M NaOH for releasing the DNA probe, or RNA target-2, or DNA–RNA hybrid from MB surface. According to the three repetitive measurements, the guanine signals were measured as 2.9 ± 0.013 μA (RSD%=0.4%, shown in Fig. S3-b) for the biotinylated miRNA-15a RNA target-2, and 3.03 ± 0.058 μA (RSD%=1.9%, shown in Fig. S3-c) for the hybridization case occured between the biotinylated miRNA-15a DNA probe and its complementary RNA target-2, whereas no guanine signal was observed (Fig. S3a) in the presence of only biotinylated miRNA-15a DNA probe since it contains inosine bases. Thus, it could be concluded that no negative effect of alkaline medium to RNA sequences upon the sensor responses was observed before or after hybridization process, as there is no significant difference between the signals (Figs. S3b and c).

In order to explore the effect of using the alkaline medium, or heating procedure for releasing the hybrid strands from MB surface upon the sensor response, the samples were also heated at 95 °C for 5 min in 25 μL of ultrapure water. Then, these samples were transferred into the vials containing 85 μL ABS following the same procedure in alkaline treatment.

After the samples were prepared by (i) alkaline treatment, or (ii) heating at 95 °C for the releasing of nucleic acids from MB surface, the guanine signals were measured. The results were shown in Fig. S4. More reproducible guanine signal obtained by using alkaline treatment (RSD%=4.2%, $n=3$) compared to the one obtained by heating procedure (RSD%=28.2%, $n=3$).

The selectivity in hybridization of each DNA probes with its complementary RNA sequences (miRNA-16, miRNA-15a target-1 and miRNA-660) was also investigated by PGE, and the results were shown in Fig. 2.

A high guanine signal was observed when a full hybridization occurred between each DNA probe and its complementary RNA target (Fig. 2A,B,C-II). There was no guanine signal measured after hybridization of each DNA probe with noncomplementary RNA sequences. These results presented that the magnetic beads assay have yielded a good selectivity for miRNA detection by the efficient magnetic separation since biotinylated DNA probe coated magnetic particles could selectively catch its complementary target RNA without any cross binding even if there were unwanted miRNAs sequences in analyte.

The magnetic beads assay combined with electrochemical single-use graphite sensor technology was examined in the present work to detect sensitively and selectively different miRNA sequences (miRNA-16, miRNA-15a and miRNA-660), that were known as the biomarkers for Alzheimer disease. The PGE presented herein some important advantages; disposable, easy to use, time-saving and cost effectiveness compared to other electrodes (e.g, hanging mercury drop electrode, carbon electrodes, and metal electrodes).

In parallel to these studies presented above, we performed miRNAs detection by using multi-channel screen-printed array of electrodes, MUX-SPE16 and consequently, these results (shown in Fig. 3) were compared to the ones obtained by using PGEs. Despite the fact that PGE has numerous advantages, it requires more sample volume per measurement at each electrode (i.e, 110 μL) in comparison to the one of MUX-SPE16 (i.e, 3 μL).

The effect of the changes at the concentration of target miRNA-16 upon the hybridization response measured using MUX-SPE16 was firstly investigated based on guanine signal in the same concentration range of target miRNA-16 optimized using PGE in 10 min hybridization time (Fig. 3). The guanine signal gradually increased up to 60 μg/mL, then it decreased (shown in Fig. S5). Since the well defined and more reproducible guanine signals (493.6 ± 44.7 nA, RSD% 9.1, $n=3$) were recorded at full hybridization case in the presence of 80 μg/mL of miRNA-16 target concentration, it was chosen as the target RNA concentration for the further selectivity studies.

The DL at MUX-SPE16 was also calculated by the method described by Miller and Miller [36] and was found as 4.3 pmole in 3 μL samples, which was approximately 53 times lower than the one estimated by using PGE.

Pöhlmann and Sprinzl [22] fabricated an electrochemical gap hybridization assay in order to detect miRNA-16 based on *p*-aminophenol signal by using gold electrode. Compared to this study [22], the electrochemical monitoring of specific miRNA-16 hybridization herein by using multi-channel screen-printed array of electrodes (MUX-SPE16) resulted only in an hour without using any extra chemical modification of the surface of single-use carbon-based electrode. In another study [37], an electrochemical assay based on enzymatic reaction was performed for miRNA detection with the DL as 6 pmole in 40 μL sample. This indirect electrochemical detection for miRNA in cell lysate was time-consuming

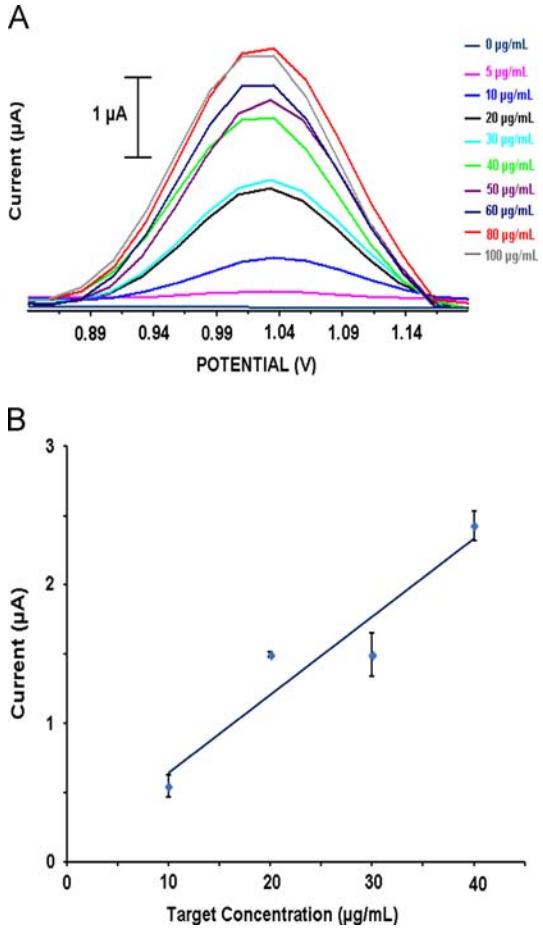


Fig. 1. The voltammograms (A), and linear graph (B) representing the guanine oxidation signal in the presence of hybridization between 80 μg/mL miRNA-16 spesific DNA probe and its RNA target in different concentrations varying from 5 μg/mL to 100 μg/mL by using PGE.

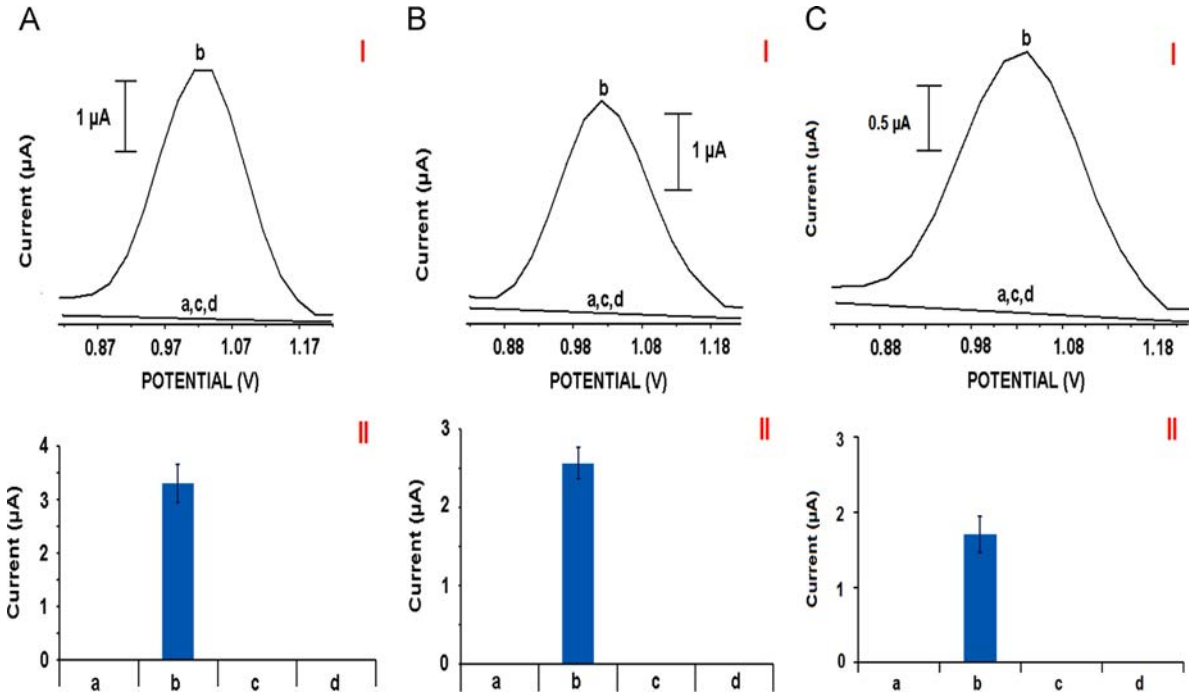


Fig. 2. Voltammograms (I) and histograms (II) representing the guanine signals in the presence of 80 μg/mL miRNA-16, miRNA-660 and miRNA-15a spesific inosine substituted DNA probe and 80 μg/mL its complementary RNA target/NC RNA sequences: (A) miRNA-16 spesific DNA probe (a), in the presence of hybridization between miRNA-16 DNA probe and miRNA-16 target (b) or, miRNA-15a target-1 (c) or, miRNA-660 target (d); (B) miRNA-15a specific DNA probe (a), in the presence of hybridization between miRNA-15a DNA probe and miRNA-15a target-1 (b) or, miRNA-16 target (c) or, miRNA-660 target (d); (C) miRNA-660 specific DNA probe **(a),** in the presence of hybridization between 80 μg/mL miRNA-660 spesific DNA probe, and miRNA-660 target (b), or, miRNA-16 target (c), or, miRNA-15a target-1 (d) by using PGE.

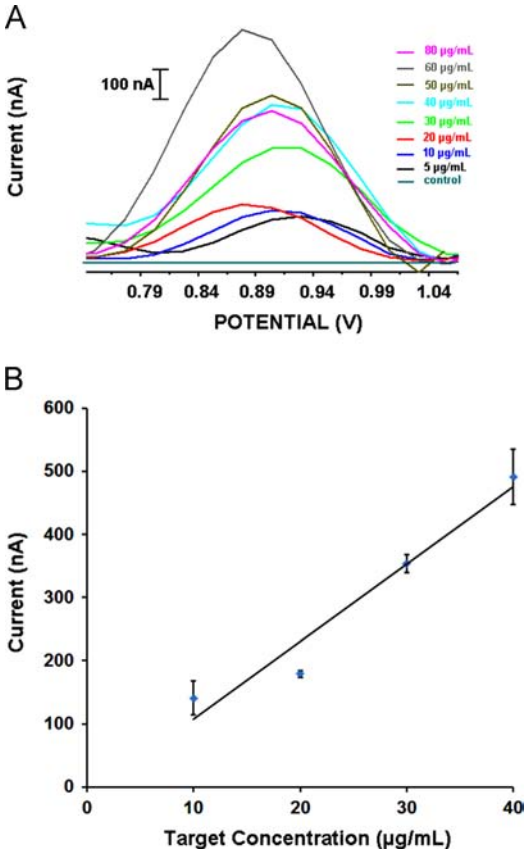


Fig. 3. Voltammograms (A) and linear graph (B) representing the guanine oxidation signal in the presence of hybridization between 80 μg/mL miRNA-16 spesific DNA probe and its RNA target in different concentrations varying from 5 μg/mL to 80 μg/mL by using MUX-SPE16.

by requiring the expensive chemicals (e.g, streptavidin alkaline phosphatase, α-naphthyl phosphate etc.), labor-intensive and less practical in contrast to our label-free detection protocol using MUX-SPEs.

The MUX-SPE16 system tested for voltammetric detection of the label-free miRNA have presented many advantages herein, easy-to-use, and cost effectiveness in total for more sensitive and selective detection protocol for miRNA by not requiring any extra chemicals like enzyme, or any types of nanomaterials (e.g, nanoparticles) in comparison to the other electrochemical assays for detection of miRNAs [22–24,37–40].

The selectivity of assay in hybridization by using MUX-SPE16 array system was also checked in the case of hybridization between miRNA-16 DNA probe and its complementary RNA sequence, or non complementary (NC) RNA sequences; miRNA-15a target-1 and miRNA-660, and the sixteen measurements were consequtively performed. When full-match hybridization occurred between miRNA-16 spesific DNA probe and the target RNA sequence (Fig. 4A-I and A-II-b) the higher guanine signal was measured compared to the signals in the presence of hybridization between miRNA-16 spesific DNA probe and NC-RNA sequences; miRNA-15a and miRNA-660 (shown in Fig. 4A I and A-II-c,d). Similiarly, a guanine signal was recorded in the case of full hybridization between miRNA-15a spesific DNA probe, or, miRNA-660 spesific DNA probe with its own RNA target sequence (Fig. 4B I and B-II-b and Fig. 4C-I and C-II-b, respectively). There was no guanine signal recorded in the case of hybridization miRNA15a DNA probe and its NC RNA sequences; miRNA-16, or miRNA 660 (Fig. 4B-I and B-II-c,d). The similar results were obtained in the presence of hybridization between miRNA-660 DNA probe and its NC RNA sequences; miRNA 15-a, or miRNA-660 (Fig. 4C-I and C-II-c,d). It was concluded that the results of selectivity studies at MUX-SPEs were complemented with the ones obtained by using PGEs. Based on three repetitive measurements, the guanine signal was

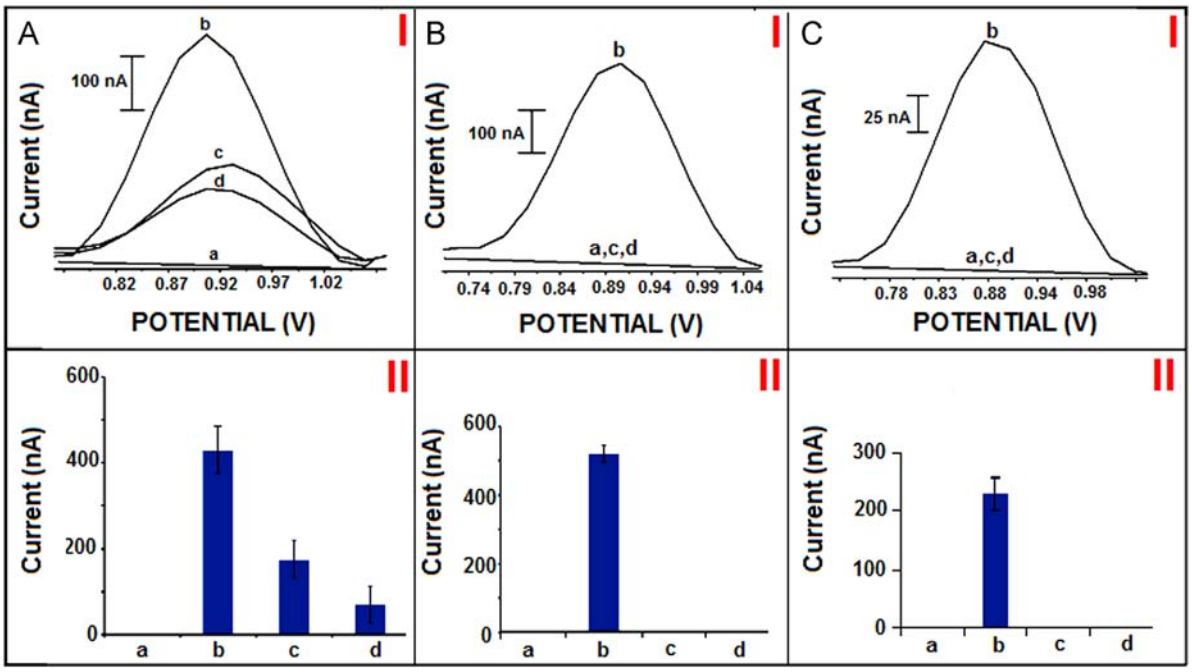


Fig. 4. Voltammograms (I) and histograms (II) representing the guanine signals in the presence of 80 μg/mL miRNA-16, miRNA-660 and miRNA-15a spesific inosine substituted DNA probe and 80 μg/mL its complementary RNA target/NC RNA sequences: (A) miRNA-16 spesific DNA probe (a), in the presence of hybridization between miRNA-16 DNA probe and miRNA-16 target (b), or miRNA-15a target-1 (c), or miRNA-660 target (d); (B) miRNA-15a specific DNA probe (a), in the presence of hybridization between miRNA-15a DNA probe and miRNA-15a target-1 (b), miRNA-16 target (c), or miRNA-660 target (d); (C) miRNA-660 specific DNA probe (a) in the presence of hybridization between miRNA-660 spesific DNA probe, and miRNA-660 target (b), or miRNA-16 target (c), or miRNA-15a target-1 (d) by using MUX-SPE16.

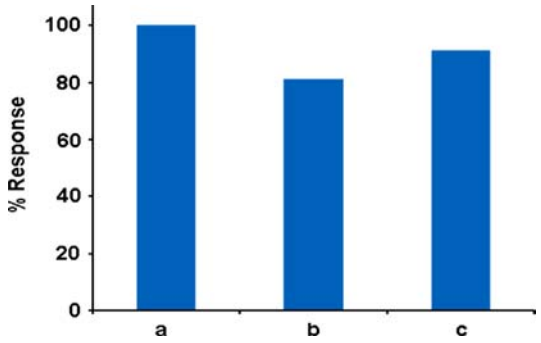


Fig. 5. % response calculated in the presence of hybridization of 80 μg/mL miRNA-16 specific DNA probe with (a) 80 μg/mL miRNA-16 target, (b) its target in the mixture of miRNA-16: miRNA-15a target-1 (1:1), and (c) its target in the mixture of miRNA-16: miRNA-660 (1:1) by using MUX-SPE16.

measured as 448.3 ± 21.8 nA (RSD%, 4.8, $n=3$) for miRNA-15a hybridization, and 228.5 ± 27.6 nA (RSD%, 12.0, $n=3$) for miRNA-660 hybridization.

The further selectivitiy test was performed at multi-channel screen printed arrays in the case of full hybridization occurred between miRNA-16 DNA probe and its target in a mixture sample containing unwanted miRNA sequences. The results showed that the electrochemical response displayed a higher specificity for electrochemical detection of miRNA-16, even if the complementary miRNA sequence of probe was in the medium in the presence of other miRNA sequences (shown in Fig. 5).

Compared to the response at full hybridization case (probe+target; P+T) (shown in Fig. 5), the response % based on the changes at current value was calculated from following equation, and found as 81.1% (Fig. 5b) for mixture sample (P+T+NC) containing miRNA-16:miRNA-15a (1:1) RNA sequences, and 91.4% (Fig. 5c) for mixture sample containing miRNA-16: miRNA-660 (1:1) RNA sequences.

$$\text{Response}\% = \left[\frac{I_{(P+T)} - I_{(P+T+NC)}}{I_{(P+T)}}\right]$$

4. Conclusion

There have not been any reports yet in the literatures about electrochemical label-free detection of miRNA at the multi-channel screen printed array (MUX-SPE16) designed as disposable screen-printed array of electrodes containing one reference and auxiliary electrode in combination with 16 working electrodes. To the best of our knowledge, the MUX-SPE16 was used as the first time herein for voltammetric detection of nucleic acid hybridization including miRNA hybridization related to different miRNA sequences (i.e, as the biomarkers for Alzheimer disease).

The label-free electrochemical detection of miRNA at MUX-SPE16 system presented many advantages; such as, more sensitive, easy-to-use, portable, cost-effective and time-saving contrary to other chip technologies developed for miRNA monitoring [22–24,37–40].

As a conclusion, the magnetic beads assay combined with PGE, or MUX-SPE16 system was developed in our study, and applied for detection of miRNA hybridization related to Alzheimer disease. The MUX-SPE16 system which was containing one reference and auxiliary electrode in combination with 16 working electrodes presented some important advantages. Only 3 μL sample per each measurement at MUX-SPE16 was required for the consecutive 16 analysis, which were shortly performed only in 15 min. Additionally, 53 times lower detection limit (4.3 pmole in 3 μL sample) for miRNA was obtained by using MUX-SPE16 system compared to the DL of PGE (230 pmole in 110 μL sample). Concerning the evaluation of results on miRNA detection with respect to DL, the possible applications on miRNA detection may be extended not only for

Alzheimer disease [25,41,42] but also for different types of cancers; such as gastric cancer [43], colon cancer [44], non-small cell lung cancer [45] and breast cancer [29] using this magnetic beads based assay in combination with multi-channel screen-printed array of electrodes. In addition, the multi-channel screen-printed array of electrodes (MUX-SPE16) has presented in our study a realistic potential for chip technology towards on its further applications not only for detection of nucleic acids, but also for gene and protein monitoring.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.09.041.

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