Paper-based electrode assemble for impedimetric detection of miRNA

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Abstract

In the present work, a paper-based electrode assemble was developed and implemented to detect target microRNA 155 (miRNA 155) via electrochemical impedance spectroscopy (EIS) measurements. In this concept, gold nanoparticles (AuNPs) modified paper based electrode assemble system (AuNP-PE) was designed, and characterized by scanning electron microscopy (SEM), cyclic voltammetry (CV) and EIS measurements. The impedimetric detection of miRNA 155 was performed by measuring the fractional change at the charge transfer resistance (R_{ct}). The detection limits were found as 33.8 nM in PBS and 93.4 nM in fetal bovine serum (FBS) medium, respectively. The selectivity of the proposed assay was tested against to non-complementary (NC) and mismatch (MM) miRNA sequences in the presence of mixture sample containing miRNA:NC (1:1) and miRNA:MM (1:1) in PBS (pH 7.40) or FBS. The analytical performance and the selectivity of impedimetric biosensor were also tested in FBS.

Keywords: paper based electrode; microRNA; gold nanoparticles; electrochemical impedance spectroscopy.

1. Introduction

microRNAs have significant roles in the regulation of differentiation, angiogenesis, proliferation, immune cell function, wound healing and apoptosis [1-4]. Especially the level of expression of Circulating miRNAs is tissue specific and have potential for use as biomarkers [2,3]. The overexpression of miRNA-155, which is known as a significant circulating miRNA, can be used as a biomarker for diagnosis and progression of cancers [5,6]. microRNAs detection in biological systems is still challenging [7-9] and upto date, several methods are reported s for miRNA detection such as RT-PCR [10-12], microarray technique [13,14], northern blotting [15,16], and in situ hybridization [17-19]. In spite of the advantages, these methods require expensive instruments and reagents, labelled miRNAs, high sample volumes and relatively pure miRNA samples [20]. Therefore, it is extremely important that miRNA detection methods should be simple, fast and label-free [21]. At this juncture, electrochemical techniques have received notable attention due to their easy controllability, good stability, reproducibility, sensitiveness and cost-effectiveness [22].

Currently, various nanostructure-based sensing platforms have been employed in constructing DNA sensors owing to nanomaterial's unique advantages (e.g., large surface areas, unique properties such as mechanical, electronic and catalytic) [23]. Among the nanomaterials, gold nanoparticles (AuNPs) have become interesting option for biomedical use due to their several kinds of effective properties [24,25]. In contrast to the graphene and iron oxides, the high affinity of the gold-sulfur bonding and other unique properties, such as high conductivity and large surface [26-28]. AuNPs, with the diameter of 1-100 nm, have high surface-to-volume ratio and high surface energy to provide a stable immobilization of a large amount of biomolecules retaining their bioactivity. Additionally, AuNPs are able to allow fast and direct electron transfer between electroactive species and electrode materials [29]. A label-free and simple electrochemical microRNA biosensor was developed by Tian et al. (2018). Toluidine blue (TB) and AuNPs superlattice were used as a redox indicator and support material, respectively. The polypyrrole coated AuNPs was self-assembled n order to obtain the maximum current. Under optimum conditions, the hybridization of single strand RNA probe with the miRNA target sequence were monitored using CV and differential pulse voltammetry (DPV) by measuring the peak current of TB [29]. In another work, DNA hybridization was investigated using AuNPs and DNA immobilized GCE. Thiol linked probe was immobilized onto GCE and the hybridization of probe and target DNA was examined using DPV technique by measuring

the Methylene blue signal [30]. A simple filtration based assay in order to develop paper based biosensor was performed by Tian et al. [31] for simultaneous detection of miRNA 144 and miRNA 21. MOF conjugated bio-probe, methylene blue (MB) and ferrocene (Fc) were used for contribution of sensor sensitivity [31].

Polymers and paper are commonly used flexile substrates for the development of bioanalytical devices [8,9,32-35]. Particularly, paper is flexible, widely available, cost-effective, hydrophilic, easy to demolish and biocompatible. Therefore, it is a convenient substrate for biosensors. Additionally, the adsorption of biomolecules or nanoparticles can be performed easily due to the porosity of the paper surface [32-35]. Paper based microfluidic electrochemical DNA biosensor was developed for the detection of Epidermal growth factor receptor (EGFR) mutations in saliva samples by Tian et al. (2017). ssDNA was adsorbed onto the surface of polypyrrole membran modified Au electrode, and the electrochemical signals were obtained. The horseradish peroxidase (HRP) recognized the methylene blue labeled DNA and showed unique electrocatalytic behavior to H_2O_2 [36]. In another study, paper-based analytical devices (µPADs) in combination with screen printed electrodes were developed by Lu et al. (2012). Thionine (TH) bound to double strand DNA (TH/D1) and complementary ssDNA (S3) immobilized on nanoporous gold (NPG) and formed S3-TH/D1-NPG conjugates. The designed µPADs nucleic acid sensor showed good performance in human serum assay [37].

To best of our knowledge, AuNPs modified paper electrode assemble system (AuNP-PE) was developed and applied for the first time herein for impedimetric detection of miRNA 155. After thiol linked DNA probe immobilization on AuNP-PE, its complementary target miRNA was recognized by capturing probe at the electrode surface during hybridization process. The selectivity of paper based biosensor specific to target miRNA (i.e, miRNA 155) was tested against to non-complementary (NC) and mismatch (MM) miRNA sequences, in the presence of mixture samples containing miRNA:NC (1:1) and miRNA:MM (1:1). In addition, the analytical performance and the selectivity of impedimetric biosensor were also tested in fetal bovine serum (FBS) medium.

2. Experimental

2.1.Instruments

Electrochemical measurements were performed with AUTOLAB-302 PGSTAT with NOVA (version 1.1.2 Eco Chemie, The Netherlands) software package (Eco Chemie, The Netherlands) in a Faraday cage.

2.2.Chemicals

Carbon paste was purchased from Daejoo Electronic Materials (Shanghai, South Korea). Silver/Silver chloride (Ag/AgCl) paste was purchased from Gwent Group (Torfaen, UK). The miRNA-155 specific DNA probe, miRNA-155 target and the other oligonucleotides (see supporting information for more information) were purchased from TIB Molbiol (Germany). All other chemicals were purchased from Sigma and Merck.

2.3.Fabrication of paper based electrodes

The most proper construction was provided using NC membrane as a structure. The printing of the hydrophobic barriers was occurred onto the NC membrane surface by using a wax printer (XEROX ColorQube™ 8570 (Norwalk, USA) after designing a pattern which includes a microfluidic channel and a working area. To provide capillary flow, channels were designed with a diameter of 2 mm and a length of 1.5 cm. In the present work, electrodes are placed in the working area and this area was designed as about 20 mm^2 with 270 angles to provide the maximum spread speed of liquid. The fabrication process of a paper electrode is shown in Fig. S1.

2.4.Design of the electrochemical detector of paper based electrode

To build the electrodes onto the patterned NC membrane, an additional pattern was drawn on a steel wafer with 1 mm thickness. Then the obtained pattern was cut by laser cutter. Resulted mask was placed on the working area of the NC membrane and carbon ink was applied to give shape as the working (WE) and counter electrode (CE). Additionally, we used Ag/AgCl ink to create the reference electrode (RE). Conductive pads were created by using copper wire. Before attaching the copper wire, wax printed NC membrane with electrodes was backed at 100 °C for 5 min to let carbon ink fix on the NC membrane surface and also the wax melt and interpenetrate through the NC membrane. The electrode design is schematized in Fig. S2. The storage stability of the paper electrodes was evaluated and the results are given in Fig. S3.

2.5.Preparation of AuNPs modified electrodes

AuNPs were deposited onto PE using chronoamperometry in 15 mM $HAuCl_4$ gold precursor aqueous solution. 5 μL of $HAuCl_4$ solution was dropped onto the PE and -0.3 V constant potential is applied during 600 s.

2.6.Probe immobilization on AuNPs modified electrodes

5 μL of thiol liked miRNA-155 DNA probe is dropped and allowed to immobilize onto the AuNP-PE during 10 min. Then, washed with PBS (pH 7.40).

2.7.Hybridization of probe and miRNA-155

5 μL of miRNA-155 target in various concentrations were applied to the electrode and washed with PBS (pH 7.40) after 5 min of incubation at room temperature.

2.8.Impedimetric measurement

20 μL of 0.1 M KCl solution containing 5 mM $[Fe(CN)_6]^{3-/4-}$ was dropped into the paper electrochemical cell through the channel.

3. Results and Discussion

3.1. Microscobic and electrochemical characterization of modified/unmodified electrodes

Scanning electrone microscpy (SEM) was used to characterize AuNPs modified paper based electrode. AuNPs were deposited on the working electrode surface by electrochemical reduction. While Fig. 1a,b show bare carbon paste PE at different magnification levels, Fig. 1c,d show AuNPs deposited on PE surface. AuNPs were homogeneously distributed on the PE surface as shown in Fig. 1c,d. The average diameter of AuNPs was calculated as 262 ± 30 nm.

Figure 1

In order to explore the electrochemical behavior of PE and AuNPs modified PE, cyclic voltammetry was performed. The anodic and cathodic currents; I_a, I_c, relative charge; Q (C), and the surface areas of the PE and AuNPs modified PE are shown in Table S1. The I_c and I_a of AuNPs modified PE (Fig. 2-I) were much larger than I_c and I_a of PE (Fig. 2-I). There results confirmed that the accelerated electron transfer occured by means of AuNPs [38]. The electroactive surface areas (A) of PE and AuNPs-PE were calculated [39] as 0.019 cm^2 and 0.037 cm^2 respectively. In comparison to the calculated surface area of PE, an increase about 91 % at surface area of AuNP-PE was obtained by means of conductive behavior of AuNPs [38].

Figure 2.

EIS was also performed in order to obtain detailed information regarding modification process since measurements carried out in the absence/presence of AuNP modification (Fig. 2-II). The change in the value of R_{ct} was associated with the modification processes of PE surface. The measured R_{ct} of unmodified PE was 7122.75 ± 440 Ohm (RSD %, 6.18 %, n=4). After modification of AuNP, the R_{ct} exhibited a substantial decrease (161 folds) and measured as 44.12 ± 3.76 Ohm (RSD %, 8.53 %, n=4). This observation indicates that the AuNPs with high

conductivity acted as a conductive layer for the charge transfer. These results are in good consistent with the CV results presented in this present study.

Analytical performance

The analytical performance of AuNP-PEs is tested through the detection of miRNA-155 target at different concentrations. Shown in Fig. 3, with the increase of miRNA-155 target concentration, the R_{ct} value increases over a wide concentration range of 0 – 1.5 µg/mL and then decrease in the presence of 2 µg/mL miRNA-155 target as shown in Fig. S4. The linear regression equation was R_{ct} *(Ohm) = 16.57 [miRNA-155] (*µg/mL*) + 8.02* with a correlation coefficient of 0.98 and the limit of detection (LOD) was calculated as 0.25 µg/mL (33.8 nM) [40].

Figure 3.

3.2.Selectivity of the AuNPs modified electrodes

To evaluate the specificity of the AuNP-PE, the interference effect of the noncomplementary and single base mismatched strands were also investigated. The efficiency of hybridization (HE %) is used as the evidence of the hybridization effectiveness [41] and the average R_{ct} values with the HE % can be seen in Table S2. HE % is accepted as 100 % for hybridization between probe and miRNA 155. The results clearly reveal that the AuNP-PE showed a sensitive behavior to its complementary target.

Figure 4.

3.3.Analytical performance in serum media

In the present study, the analytical performance of AuNP-PEs was tested in serum media (Fig. 5). When the hybridization was performed in case of the increasing concentration of miRNA-155 target, the R_{ct} value increased among the concentration range of 0 – 4 µg/mL, then decreased in the presence of 6 µg/mL miRNA-155 target (Fig. S5). The linear regression equation was R_{ct} *(Ohm) = 11.15 [miRNA-155] (*µg/mL*) + 36* with R^2*=0.98* and the LOD was calculated as 0.69 µg/mL (93.4 nM).

Figure 5.

3.4.Selectivity in serum media

The specificity of the AuNP-PE in serum media was then tested (Fig. S6). The HE %s were calculated as 13 % and 16 %, in case of possible hybridization/interaction between probe and NC, MM, respectively. On the other hand, HE %s were 65 % and 41 %, respectively after hybridization of probe and miR-155 target in the presence of NC or MM (Table S3).

According to our results related to the selectivity test in buffer and serum media, it can be said that the proposed AuNP-PEs exhibited more selective behavior in complex serum medium than buffer. These results showed that AuNP-PEs are able to perform microRNA detection in a sensitive and selective way even in a complex medium as serum.

Storage stability of the paper electrodes was evaluated. Fig. S3 shows the stability of the electrodes during 60 days of storage in dark conditions and without other special care.

Several reports related to the electrochemical detection of miRNAs were summarized in Table S4 [29,41-61]. In the present work, the impedimetric miRNA-155 analysis was accomplished in relatively shorter time (i.e 15 min) compared to earlier works shown in Table S4. The detection limit was found respectively to be 33.8 nM in PBS and 93.4 nM in FBS:PBS (1:1) diluted solution, which was comparable to earlier works [42-48] (Table S4).

4. Conclusion

The gold nanoparticles (AuNPs) modified paper based electrode assemble system (AuNP-PE) was designed for the first time in this present study, and successfully applied to perform fast, sensitive, selective and quantitative miRNA-155 detection. Our results showed that the stability of these electrodes during 60 days of storage in dark conditions and without other special care.

Since this protocol exhibits more selective behavior in complex serum medium than buffer, it is obvious that AuNP-PEs could be able to perform selectively nucleic acid detection including miRNA, SNP, etc. even in a complex medium as serum.

AuNPs-PEs can be assesable as a promising platform for analysis in real serum samples for early diagnosis of cancer. It is important to mention that usage of a paper based microfluidic chip, except for isolation of target analyte from solution, provides us to benefit from the advantages of miniaturized systems comparison to the conventional batch technique.

Conflicts of interest

There are no conflicts of interest to declare

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The list of figure captions:

Figure 1. SEM images of (a,b) unmodified paper electrode and (c,d) AuNPs modified paper electrode at magnification of 20000× and 50000x.

Figure 2. The concept of AuNPs modified paper electrode sensing system. Left, the sketch of the paper electrode sensing system. AuNP deposition is performed by chronoamperometry in 15 mM $HAuCl_4$ aqueous solution by applying -0.3 V constant potential during 600 s. **(I)** CVs of PE and AuNP-PE. Supporting electrolyte solution is 0.1 M KCl containing 50 mM $[Fe(CN)_6]^{3-}$. **(II)** Nyquist diagrams of PE and AuNP-PE. Supporting electrolyte solution is 0.1 M KCl containing 5 mM $[Fe(CN)_6]^{3-/4-}$.

Figure 3. (A) Nyquist diagrams obtained before / after hybridization of probe with 0.5, 1, 1.5, 2 µg/mL miRNA 155 target. **(B)** The calibration graph obtained after hybridization between 0.5 µg/mL miRNA 155 DNA probe and miRNA 155 target with its various concentrations from 0 to 1.5 µg/mL (n=3).

Figure 4. Selectivity of AuNP-PE. Histograms representing the average R_{ct} values obtained by (a) miRNA 155 probe/AuNP-PE, hybridization between probe and (b) miRNA 155, (c) non-complementary (NC), (d) single-base mismatched strand (MM), and mixture samples with (e) miRNA 155 and NC or (f) miRNA 155 and MM strand (n=3).

Figure 5. (A) Nyquist diagrams obtained before / after hybridization of probe with 2, 4, 6 µg/mL miRNA 155 in 1:400 diluted FBS medium. **(B)** The calibration graph based on average R_{ct} values obtained after hybridization between probe and miRNA 155 with its various concentrations from 0 to 4 µg/mL in 1:400 diluted FBS medium (n=3).

Figures

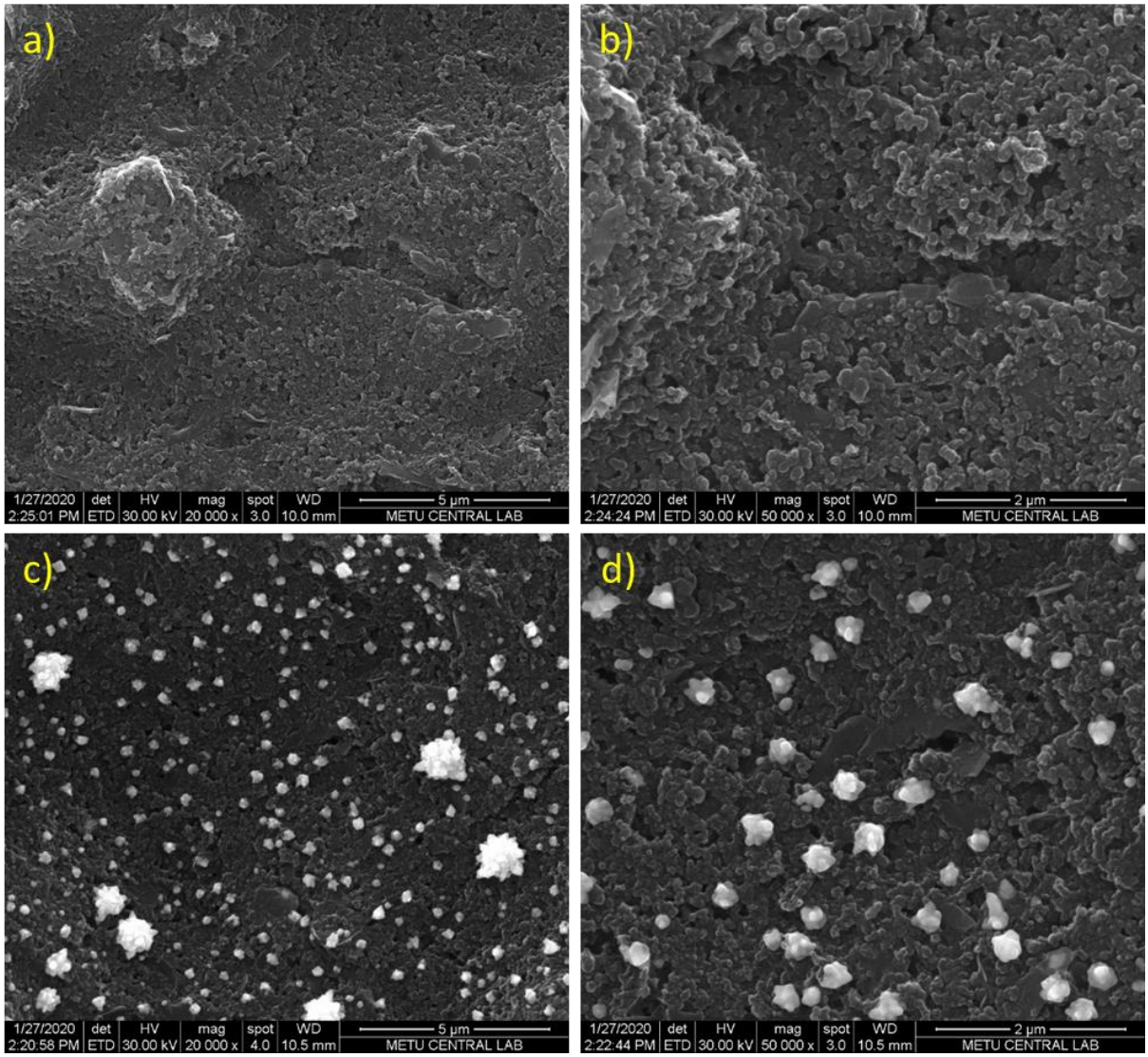


Figure 1.

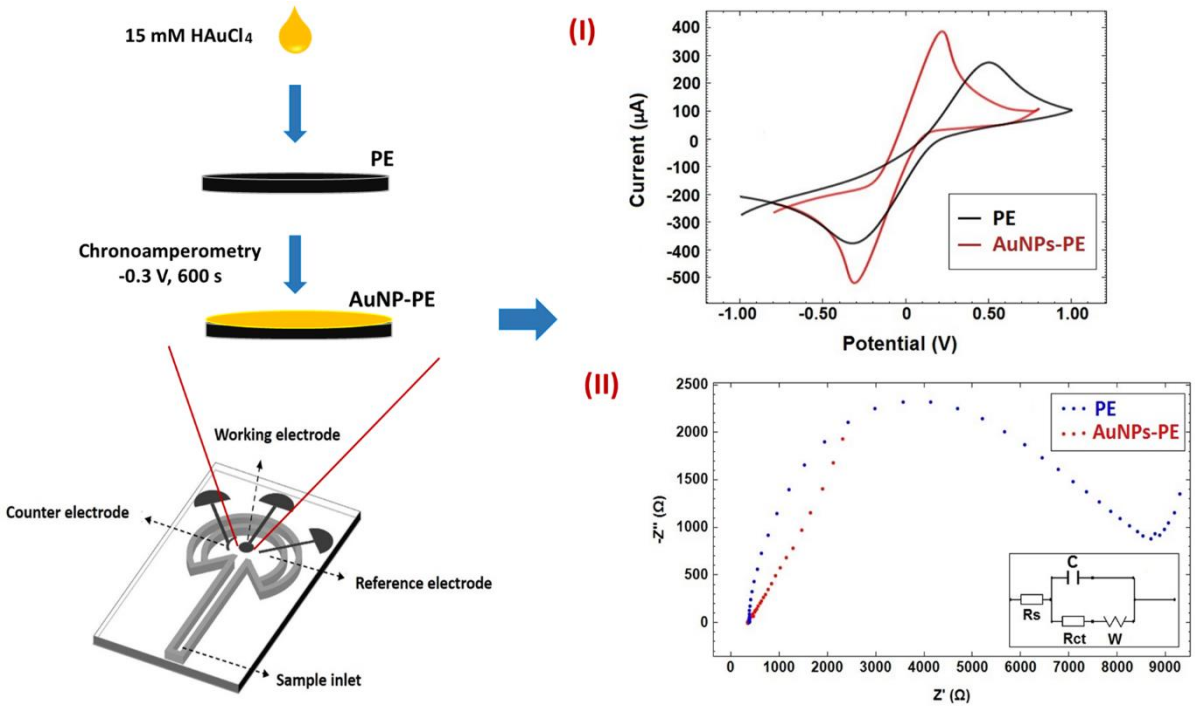
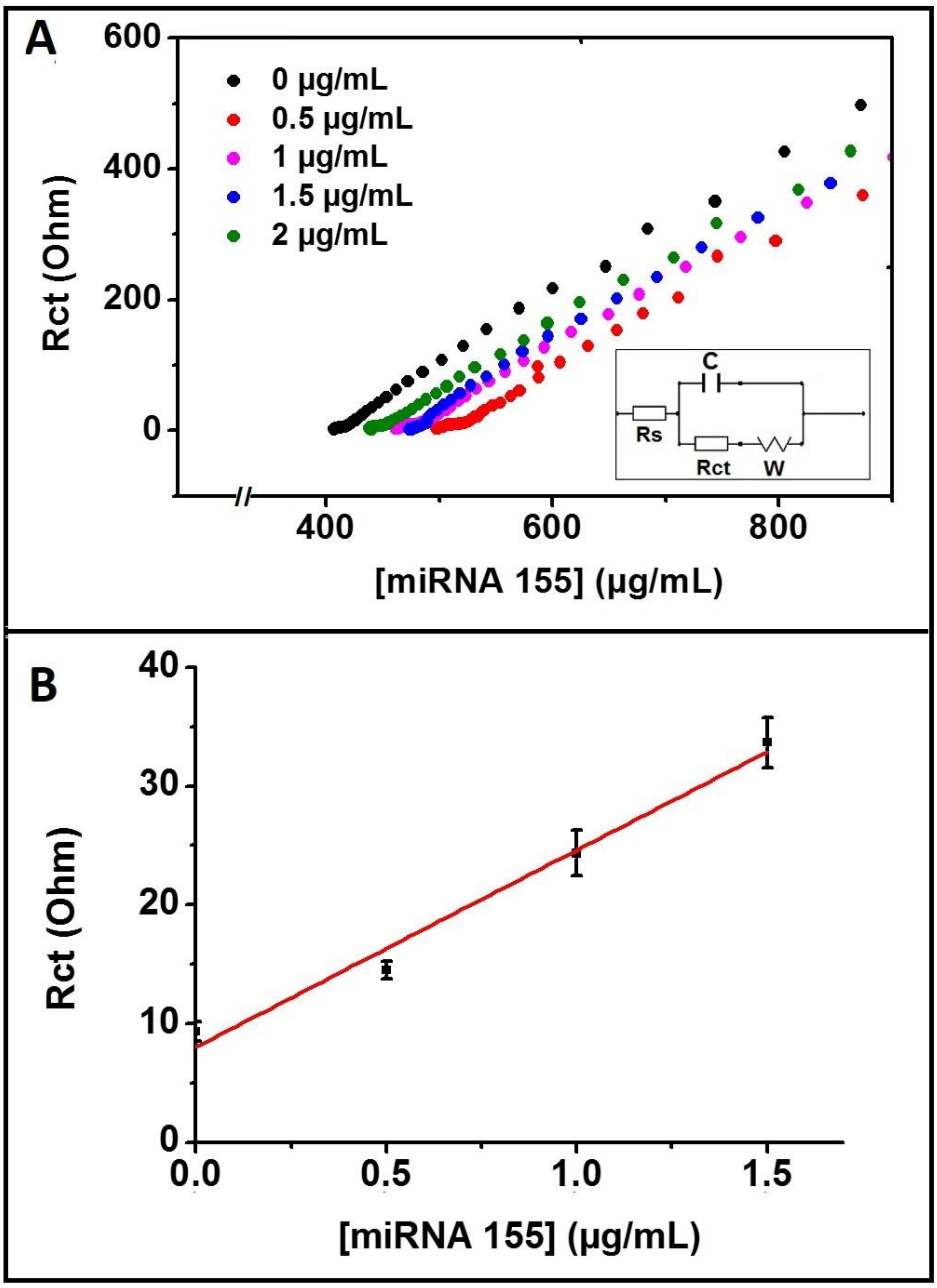

15 mM HAuCl4
PE
Chronoamperometry
-0.3 V, 600 s
AuNP-PE
Working electrode
Counter electrode
Reference electrode
Sample inlet
(I)
Current (μA)
Potential (V)
PE
AuNPs-PE
(II)
-Z'' (Ω)
Z' (Ω)
C
Rs
Rct
W

Figure 2.



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498 **Figure 3.**

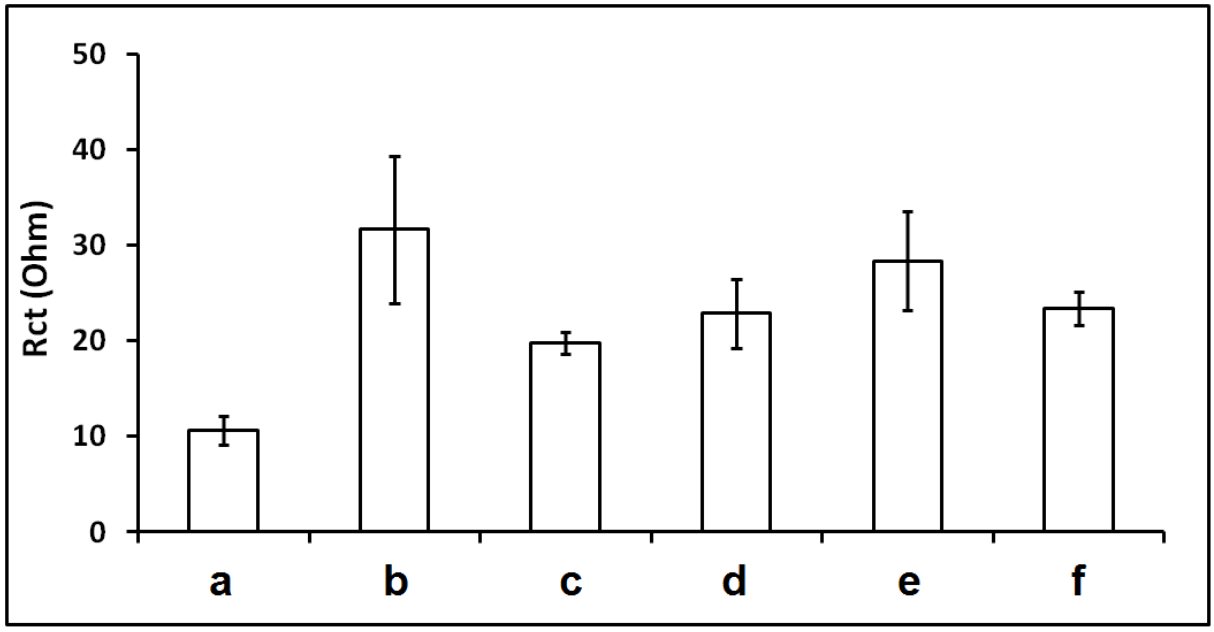
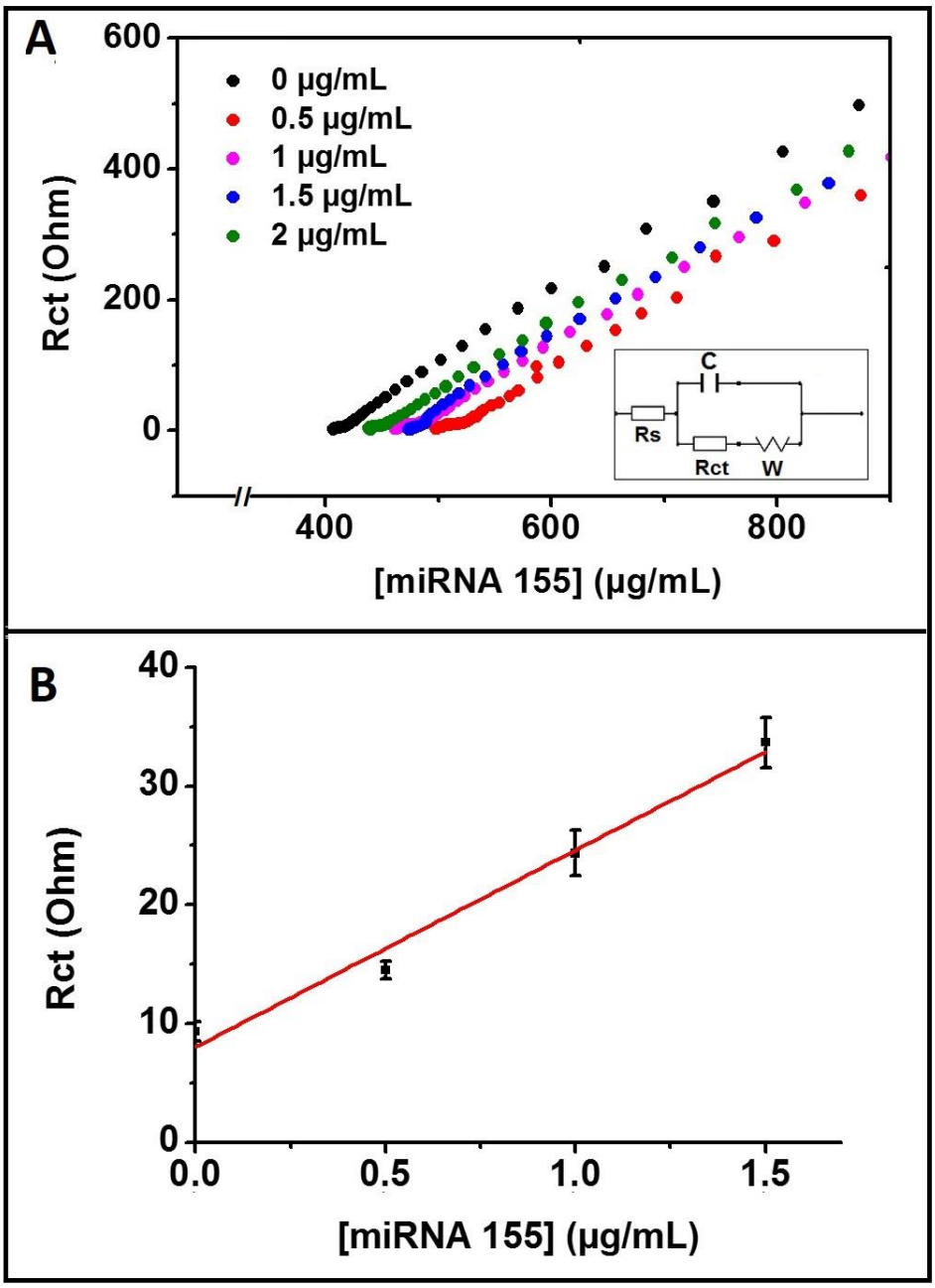


Figure 4.



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502 **Figure 5.**

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