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Duplex-specific nuclease-based electrochemical biosensor for the detection of microRNAs by conversion of homogeneous assay into surface-tethered electrochemical analysis

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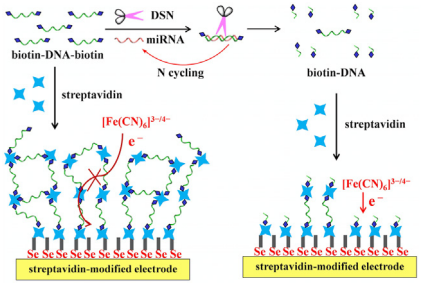
HIGHLIGHTS

- We proposed a new strategy for converting homogeneous assay into surface-tethered electrochemical analysis.
- Biotinylated detection probe triggered the in-situ assembly of streptavidin (SA) proteins.
- The method was used for the detection of microRNAs with satisfactory results.
- The signal was amplified by duplex-specific nuclease and (SA-biotin-DNA-biotin)$_n$ assemblies.
- The strategy can be used to design novel biosensors for the detection of other biomarkers.

GRAPHICAL ABSTRACT



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ABSTRACT

We proposed a simple and sensitive strategy for the detection of microRNAs (miRNAs) by converting homogeneous assay into surface-tethered electrochemical analysis. Specifically, the biotinylated detection probes (biotin-DNA-biotin) can trigger the *in-situ* assembly of tetrameric streptavidin (SA) proteins on an electrode surface via the SA-biotin interactions. The (SA-biotin-DNA-biotin)$_n$ assemblies electrically insulated the electrode interface, thereby blocking the electron transfer of $[Fe(CN)_6]^{3-/4-}$. When the probe was hybridized with the target miRNA, it would be cleaved into small fragments (denoted as biotin-DNA) by duplex-specific nuclease (DSN). The released target miRNA can enter into the next hybridization-enzymolysis cycle, thus leading to the generation of considerable amounts of biotin-DNA fragments. The released biotin-DNA competed with the detection probe to bind SA, thus limiting the *in-situ* formation of (SA-biotin-DNA-biotin)$_n$ assemblies. The surface-tethered electrochemical analysis by the dual signal amplification of DSN and (SA-biotin-DNA-biotin)$_n$ assemblies has been used for the determination of miRNAs in cell lysate with a satisfactory result. The method showed a detection limit down to 10 aM. The "one-step" immobilization-free strategy can be used to design novel biosensors for the detection of other biomarkers.

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1. Introduction

MicroRNAs (miRNAs), a group of endogenous, non-coding RNAs, plays an important role in physiological and pathological processes. Their abnormal expression has been closely related to many diseases, including cancers, neurodegenerative diseases, immune system dysfunction and so on [1,2]. Recently, miRNAs have been regarded as the effective biomarkers and targets for diagnosis, progression, and therapy of many diseases. However, detection of miRNAs is facing great challenges because of their low content, high homology and poor stability [3,4]. At present, the commonly used strategies for miRNA quantification, including Northern blotting, microarray and qRT-PCR, show the disadvantages of poor sensitivity, labor-intensive steps, time-consuming process and expensive reagents [5–7]. Thus, it is necessary to explore a simple, sensitive and high-throughput strategy for the quantification of miRNAs.

In recent years, various enzymatic reactions have been integrated in the design of newly strategies for miRNA quantification, such as exonuclease, nicking endonuclease, polymerase and duplex-specific nuclease (DSN) [8–11]. Among them, the DSN-assisted signal amplification has attracted extensive attention because the enzyme exhibits high stability and excellent ability to discriminate the perfectly and imperfectly matched heteroduplexes, even one-base mismatched duplexes [12–14]. After enzymatic cleavage of DNA in the DNA-RNA heteroduplexes, the released miRNAs can be dissociated and enter into the next hybridization-enzymolysis cycle, thus leading to the release of a large numbers of signal molecules labeled on the single-strand DNA (ssDNA). According to the difference of signal molecules and detection techniques, many DSN-based homogeneous and heterogeneous assays have been developed, including fluorescence, colorimetry, electrochemistry, electrochemiluminescence, surface-enhanced Raman scattering (SERS), mass spectrometry and so on [15–26]. The heterogeneous assays have the advantages of small sample size and high sensitivity and selectivity since a large number of probes are immobilized at a discrete location on the solid surface. In combination with other signal-amplified methods, such as enzymes, nanomaterials, quantum dots and DNA-mediated self-assembly, the DSN-assisted assays have achieved the detection of ultralow concentration of miRNAs [27–30]. The heterogeneous assays are sensitive but still limited by one or more factors [31–33]. For example, the steric hindrance effect from solid surface may decrease the hybridization efficiency and reduce the enzymolysis rate. Additionally, the immobilization and multistep washing processes are laborious and time-consuming, and the probe-modified solid substrates are unstable for long-time storage. Thus, it is of importance to seek novel strategies to remedy the shortcomings of DSN-based heterogeneous assays.

For DSN-based homogeneous analysis, DNA probes and miRNAs are present in the aqueous phase. Among various homogeneous methods, aggregation-based assays have received considerable attention because of their simple operation principle and detection procedures [34–41]. For example, DNA probes can be absorbed on the surface of bare gold nanoparticles (AuNPs) through the Au—N interactions, thus inhibiting salt-induced aggregation of AuNPs. Once the DNA probes were hybridized with target miRNAs, they would be digested by DSN and lose the ability to protect AuNPs from aggregation [34,39]. The “dispersion-to-aggregation” state of AuNPs accompanied by a “red-to-blue” color change allows for the naked-eye readout of miRNAs. However, the aggregation-based liquid-phase assays exhibit low sensitivity and poor anti-interference for analysis of miRNAs in biological fluids. Inspired by the “dispersion-to-aggregation” strategy, herein, we proposed that the surface-tethered analysis adapted from the aggregation-based homogeneous assay can be developed, thus infusing an existing principle into another field. To explore the feasibility of our proposal, streptavidin (SA), a tetramer protein with four biotin-binding sites, was used as the junction of aggregates. Electrochemical impedance spectroscopy (EIS), a powerful tool for characterization of surface processes and properties, was used to monitor the signal change. The detection principle of this design is illustrated in Scheme 1. The biotinylated detection probe (biotin-DNA-biotin) can trigger the assembly of SA proteins in solution via the strong SA-biotin interaction. When the electrode was modified with SA, the probe-triggered assembly of SA proteins could be facilely initiated on a solid-liquid surface by the same interactions. Specifically, the biotin-DNA-biotin probes were firstly caught by the SA-modified electrode through the SA-biotin interactions. Then, SA proteins in the solution were recruited on the electrode interface by the same interactions. The circular recruitment of biotin-DNA-biotin and SA from the solution to the electrode surface resulted in the *in-situ* formation of $(SA\text{-biotin-DNA-biotin})_n$ networks. This insulated the electrode interface and thus blocked the electron transfer of $[Fe(CN)_6]^{3-/4-}$. When the biotin-DNA-biotin probe in the solution was hybridized with the target miRNA and then cleaved into small fragments (denoted as biotin-DNA) by DSN, the probe-triggered assembly of SA would be inhibited. Meanwhile, the released biotin-DNA fragments competed with biotin-DNA-biotin to bind SA both on electrode interface and in solution, thus limiting the *in-situ* formation of $(SA\text{-biotin-DNA-biotin})_n$ assemblies. Consequently, a surface-tethered DSN-based electrochemical analysis has been developed for the detection of miRNAs. The “one-step” immobilization-free method is simple and does not require the use of expensive reagents or nanomaterials for signal amplification.

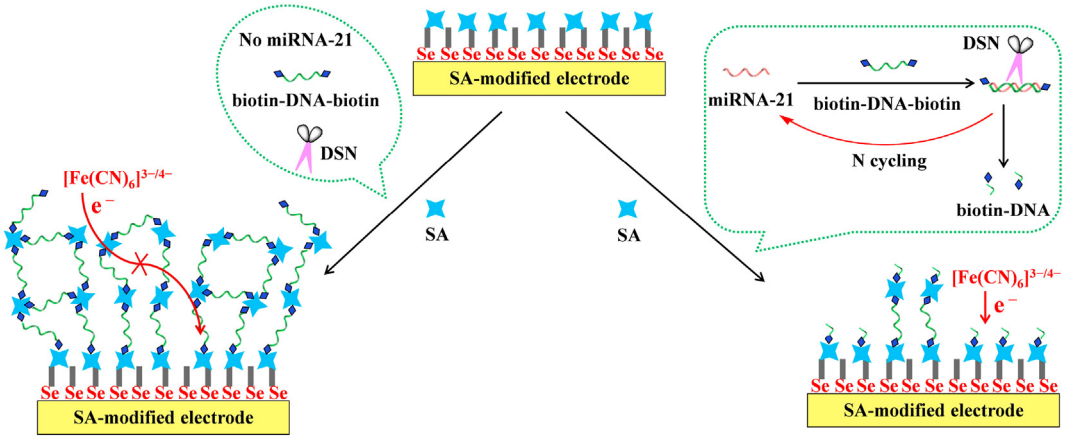
2. Experimental section

2.1. Chemicals and reagents

Biotinylated DNA, target miRNA-21 and the mismatching miRNAs were ordered from Sangon Biotech. Co., Ltd. (Shanghai). Their sequences are as follows: biotin-DNA-biotin, biotin-TCA ACA TCA GTC TGA TAA GCT A-biotin; biotin-DNA, biotin-TCA ACA TCA G; miRNA-21, UAG CUU AUC AGA CUG AUG UUG A; one-base mismatch, UAG CUU AUC GGA CUG AUG UUG A; three-base mismatch, UUG CUU AUC GGA CUG AUC UUG A; non-complementary, GUA AGG CAU CUG ACC GAA GGC A. DSN was ordered from Evrogen Joint Stock Company (Moscow, Russia). Recombinant SA, biotin, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), ethylene glycol-bis(aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA), phenylmethylsulfonyl fluoride (PMSF), tris(hydroxymethyl) aminomethane (Tris) were provided by Sangon Biotech. Co., Ltd. (Shanghai). L-Selenocystine was obtained from RedChemical Technology (Shanghai) Co., Ltd. (Shanghai). The SA-coated magnetic beads were acquired from Thermo Fisher Scientific (Shanghai, China). Other reagents were provided by Aladdin Reagent Co., Ltd (Shanghai). The miRNAs samples were prepared freshly with diethylpyrocarbonate-treated RNase-free ultrapure water.

2.2. Characterization of the assemblies

The morphology of SA in the absence and presence of biotinylated DNA was characterized by Atomic Force Microscopy (AFM) on a Dimension Edge microscope (Bruker Nano Inc., Santa Barbara, CA). Briefly, the SA powder was dissolved in 10 mM



Scheme 1. Schematic representation of the surface-tethered electrochemical analysis for miRNA detection based on the *in-situ* formation of (SA-biotin-DNA-biotin)$_n$ assemblies.

phosphate buffer (pH 7.4) with a concentration of 104 μg/mL (~2 μM). The biotinylated DNA was dissolved in the buffer to a concentration of 10 μM. Then, the mixture comprising of 12.5 μL of SA, 2.5 μL of biotinylated DNA and 35 μL of phosphate buffer was shaken at 37 °C for 5 min. After that, 10 μL of the mixed solution was put on the newly peeled mica slide to remain for 20 min. The slide was rinsed with water and then dried under a very gentle nitrogen stream. The surface morphology of the slide was characterized by AFM with the tapping mode.

2.3. Preparation of sensor electrode

The gold electrode with a diameter of 2 mm was polished with 0.05 μm alumina powder. After ultrasonic treatment in ethanol/water, the electrode was placed in sulfuric acid (0.5 M) for electrochemical scanning with a rate of 100 mV/s. The potential changed from −0.2 to 1.3 V. The SA proteins were immobilized on the selenocystine self-assembled monolayers (SAMs)-covered electrode surface through the amine coupling reaction. The selenocystine SAMs were used for the immobilization of SA because the Au−Se interfaces exhibit higher stability and fidelity than Au−S bonds, thus avoiding the signal distortion induced by biological thiols [42]. In brief, a cleaned gold electrode was incubated with 100 μM L-selenocystine for 12 h in 50 mM Tris buffer (pH 7.4) containing 1 mM TCEP. The L-selenocystine SAMs were then activated by the mixture comprising of 0.2 M EDC and 0.1 M NHS. After that, the electrode was incubated with 10 μg/mL SA in 10 mM phosphate buffer (pH 7.4) for 4 h and then rinshed with the buffer. The unreacted activated carboxyl groups on the SAMs were blocked by soaking the electrode in 1 mM ethanolamine for 30 min. Finally, the SA-modified sensing electrode was thoroughly rinsed and used for the sample detection.

2.4. Electrochemical detection

10 μL of biotin-DNA-biotin solution at a given concentration was mixed with 10 μL of miRNA sample in the DSN reaction buffer (10 mM Tris, pH 8.0, 0.1 M NaCl, 1 mM $MgCl_2$). After incubation for a given time at 50 °C, the SA-modified electrode was exposed to the mixture. Then, 20 μL of SA solution at an optimized concentration was added to the mixture for 30-min incubation. After being thoroughly rinsed with the buffer and ultrapure water, the electrode was immersed in the solution of 5 mM $[Fe(CN)_6]^{3-}$ or $[Fe(CN)_6]^{3-/4-}$ (1:1) containing 0.1 M KCl for electrochemical measurements. The differential pulse voltammetry and electrochemical impedance spectroscopy (EIS) were collected on a CHI 660 electrochemical workstation (CH Instruments, Shanghai, China). A platinum wire and a Ag/AgCl electrode were used as the reference and auxiliary electrode, respectively.

2.5. Detection of miRNAs in cell lysates

The cell lysates were collected with the previous reported procedures. Briefly, the cells were cultured in DMEM medium at 37 °C in a humidified atmosphere (95% air and 5% CO_2). After being cultured for five days, the cells are collected, washed, and lysed with lysis buffer. Then, 2 mL of lysates were centrifuged at 4 °C for 20 min with a speed of 12,000 rpm. The supernatant was diluted to different multiples and then mixed with the DSN reaction buffer containing a given concentration of biotin-DNA-biotin. After incubation for 2 h at 50 °C, the SA-modified electrode was incubated with 20 μL of the mixture for 5 min, followed by adding 20 μL of 0.2 μM SA solution. Other procedures for the detection of miRNAs in the cell lysates followed those aforementioned.

3. Results and discussion

3.1. Characterization of the assemblies

The detection principle of the method is based on the probe-triggered assembly of tetramer SA proteins via the SA-biotin interaction. AFM is a powerful technique for direct characterization of microstructural of biological samples. To investigate the probe-triggered assembly of SA proteins, the morphology of SA in the absence and presence of biotin-DNA-biotin was characterized by AFM. As depicted in Fig. 1A, the proteins with a height of 1−2 nm were dispersed on the mica surface (panel a), which is consistent with the previous result [43]. The width is much larger than the height due to the tip dilation effect [44,45]. After addition of equivalent biotin-DNA-biotin to the SA solution, network assemblies were observed on the mica surface (panel b). When the concentration of biotin-DNA-biotin was twice that of SA, most of SA proteins were clustered together (panel c). However, addition of biotin-DNA to the SA solution did not induce the formation of network assemblies (panel d). The results demonstrated that the biotin-DNA-biotin probe can trigger the assembly of tetramer SA proteins.

Fig. 1B depicts the Niquist EIS responses of the SA-modified electrode before and after exposure to different samples. The diameter of the semicircle equals to the electron-transfer resistance

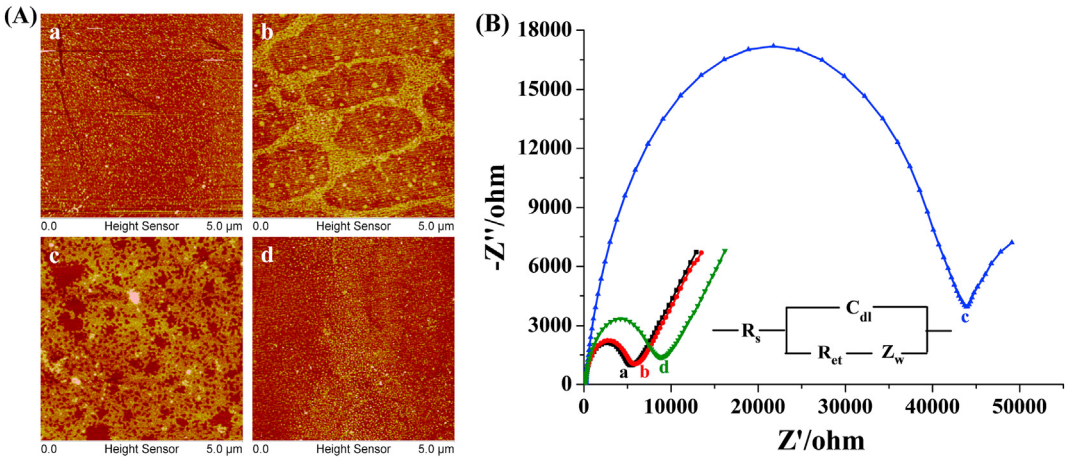


Fig. 1. (A) AFM images of 0.5 μM SA in the absence (panel a) and presence of 0.5 μM (panel b) or 1 μM biotin-DNA-biotin (panel c), and 1 μM biotin-DNA (panel d). (B) EIS responses of the SA-covered electrode before (curve a) and after incubation with biotin-DNA-biotin (curve b) or the biotin-DNA-biotin/SA mixture (curve c). Curve d corresponds to that by treating the SA-modified electrode with biotin-DNA-biotin, rinsing buffer and SA.

(R_{et}) at the sensor interface. Incubation of the SA-modified electrode with biotin-DNA-biotin induced a negligible increase in the impedance (c.f. curves a and b). Interestingly, when the SA-modified electrode was exposed to the mixture of biotin-DNA-biotin and SA (curve c), the impedance increased significantly. The R_{et} was remarkably higher than that achieved by two-step method: treating the SA-modified electrode with biotin-DNA-biotin, rinsing buffer and then SA (curve d). There was no apparent difference in the R_{et} value when the sensor was incubated with SA alone (data not shown). Thus, the SA protein was attached on the probe-treated interface through the SA-biotin interaction. These results suggested that the *in-situ* performed (SA-biotin-DNA-biotin)$_n$ assemblies can prevent the access of $[Fe(CN)_6]^{3-/4-}$ to the sensor interface more effectively than the single SA-biotin-DNA-biotin conjugate, thus significantly enhancing the impedance signal.

3.2. Optimization of experimental conditions

In this design, biotin-DNA-biotin was used as the detection probe and the linker for SA assembly. Thus, the effect of its concentration on the impedance change was firstly studied. As a result, the R_{et} increased with the increase of probe concentration below 0.5 μM (Fig. 2A). However, when the concentration was further raised, the R_{et} began to decrease. This is acceptable since the biotin-binding sites of tetramer SA protein in the solution will be saturated by excessive biotin-DNA-biotin, thereby blocking the *in-situ* formaiton of SA assemblies on the electrode interface. For this view, the molar ratio of biotin-DNA-biotin to SA remained at 2:1 in the following experiments. At the optimized molar ratio, the dependence of R_{et} on SA concentration was studied. As a result, the R_{et} increased with increasing SA concentration (Fig. 2B). Herein, biotin-DNA-biotin and SA at a compromised concentration was used for the detection of target miRNA-21.

3.3. Feasibility of the method

The EIS measurements were carried out to investigate the feasibility of this method for miRNA-21 detection. As shown in Fig. 3A, the sensor electrode exhibited a high impedance when it was incubated with the mixture of miRNA-21/biotin-DNA-biotin/SA (curve a) or DSN/biotin-DNA-biotin/SA (curve b). However, a smaller impedance was obtained when it was exposed to the DSN/miRNA-21/biotin-DNA-biotin/SA mixture (curve a). To prove that the signal change was caused by the DSN-assisted cleavage of biotin-DNA-biotin, the effects of enzymolysis time and DSN concentration on the impedance have been investigated. It was observed that the R_{et} decreased with the increase of enzymolysis time and DSN concentration. Thus, higher DSN and longer enzymolysis time can promote the hydrolysis of much more biotin-DNA-biotin probes. The resulting biotin-DNA fragments will compete with biotin-DNA-biotin to bind SA both on electrode surface and in the solution, thereby blocking the *in-situ* formation of (SA-biotin-DNA-biotin)$_n$ assemblies on the electrode surface.

3.4. Sensitivity

With the optimized experimental parameters, miRNA-21 samples at different concentrations were tested. As shown in Fig. 4A, the impedance decreased gradually with the increase of miRNA-21 concentration. The result indicated that the method can be used to determine miRNA-21 at a low concentration. Usually, the "signal-on" biosensor has a higher sensitivity than the "signal-off" one owing to the lower background signal. Besides EIS, the electron transfer of ferricyanide on the electrode surface was also monitored by differential pulse voltammetry (DPV) (Fig. 4B). It can be seen that the (SA-biotin-DNA-biotin)$_n$ assemblies performed on the electrode surface hindered the reduction of $[Fe(CN)_6]^{3-}$. However, the DSN-assisted cleavage of biotin-DNA-biotin depressed the assembly of SA, thus facilitating the reduction of $[Fe(CN)_6]^{3-}$ and allowing for the "signal-on" detection of miRNA-21. As depicted in Fig. 4C and D, the EIS and DPV signals increased with the increasing level of miRNA-21. The method showed a good linear relationship from 0.01 to 2.5 fM. The linear equations were expressed as $R_{et} = 36{,}255$ [miRNA-21] (fM) − 9493 or $I(\mu A) = 1.67$[miRNA-21] (fM) + 1.46. The detection limit was estimated to be 10 aM based on the slope and the standard deviation of blank response. The value is comparable to (or even lower than) that obtained by the DSN-assisted heterogeneous electrochemical analysis with the signal amplification of enzyme labels, nanomaterials and/or redox cycling (Table 1). The high sensitivity can be attributed to the poor conductivity of (SA-biotin-DNA-biotin)$_n$ assemblies and the competition reaction between the hydrolysis products (biotin-DNA) and the detection probes to bind SA proteins both on the electrode interface and in the solution. More importantly, this work proposed a novel concept for converting a homogeneous assay into a surface-tethered electrochemical analysis. The "one-step" immobilization-free method is simple and may be used to design novel biosensors for the detection of various biomarkers.

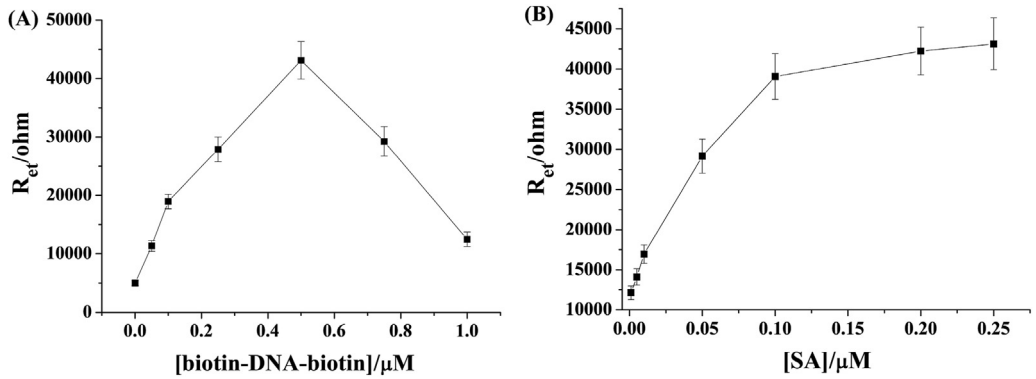


Fig. 2. Effect of biotin-DNA-biotin (A) and SA (B) concentration on the R_{et} value. In panel A, the final concentration of SA was 0.25 μM. In panel B, the molar ratio of biotin-DNA-biotin to SA was 2:1.

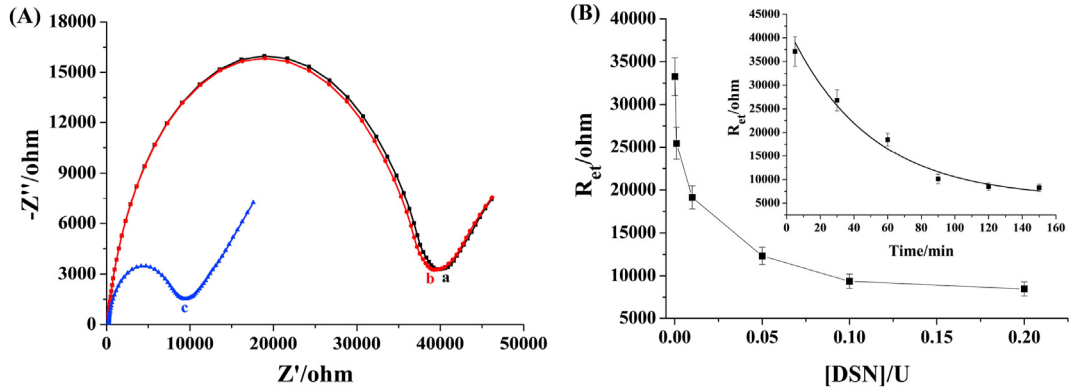


Fig. 3. (A) EIS responses of the SA-modified electrode after incubation with the mixture of miRNA-21/biotin-DNA-biotin/SA (curve a), DSN/biotin-DNA-biotin/SA (curve b) and DSN/miRNA-21/biotin-DNA-biotin/SA (curve c). The concentrations of SA, biotin-DNA-biotin, miRNA-21 and DSN are 0.1 μM, 0.2 μM, 5 fM and 0.2 U, respectively. The enzymolysis time was 2 h. (B) Dependence of R_{et} on DSN concentration and enzymolysis time. The enzymolysis time was 2 h and the DSN concentration was 0.2 U in the orthogonal experiments.

3.5. Selectivity

The selectivity of the method was demonstrated by analyzing base-mismatched and non-complementary miRNAs at the concentration of 10-fold higher than the target. As shown in Fig. 5, the biosensor shows excellent selectivity to distinguish miRNA-21 from other miRNAs (bars 1–4), even the single-base-mismatched sequence. The good selectivity can be attributed to the elevated hybridization/enzymolysis temperature and the excellent ability of DSN to discriminate the perfectly and imperfectly matched duplexes. The surface-tethered analysis is based on the competition reaction between the released fragments (biotin-DNA) and the biotin-DNA-biotin probe to bind with SA both on the electrode interface and in solution. However, biotin existed in body fluids at nanomolar concentration may limit the applications of the method for in vitro diagnostic tests [62]. To avoid the interference of biotin, the sample can be pretreated by the commercial SA-coated magnetic beads. We found that the interference from biotin has been well eliminated by the pretreatment step (bars 5 and 6).

3.6. Analysis of cell lysates

To demonstrate the applicability of the method in biological samples, cellular miRNA-21 extracted from living MCF-7 and HeLa cells was detected. As shown in Fig. 6, the current increased with the increase of MCF-7 cell number. However, there was no significant increase in the peak current for HeLa cells. The result indicated that the level of miRNA-21 in MCF-7 cells is higher than that in HeLa cells. The result was in good agreement with that achieved by other methods [63,64]. Thus, the surface-tethered electrochemical analysis exhibited good reliability for practical applications.

4. Conclusion

We proposed a simple and sensitive platform for the detection of miRNAs by conversion of homogeneous assay into surface-tethered electrochemical analysis. The signal was amplified by the DSN-assisted target recycling and the probe-triggered assembly of tetramer SA proteins. The *in-situ* performed $(SA\text{-}biotin\text{-}DNA\text{-}biotin)_n$ assemblies prevented the electron transfer owing to their poor conductivity and large molecular size. The detection limit of this method is lower than that of DSN-assisted homogeneous assays and is comparable to that of DSN-assisted surface-tethered electrochemical analysis with the enzymatic and/or nanomaterials-based signal amplification. The method allowed the detection of miRNA-21 in cell lysate. Taking the advantages of high sensitivity, simple operation, and straightforward detection principle, the method opens a promising avenue for the design of novel biosensing palt-forms and likely lead to many biological and clinical applications.

CRediT authorship contribution statement

Lin Liu: Conceptualization, Writing - original draft, Project administration. **Dehua Deng:** Methodology, Investigation.

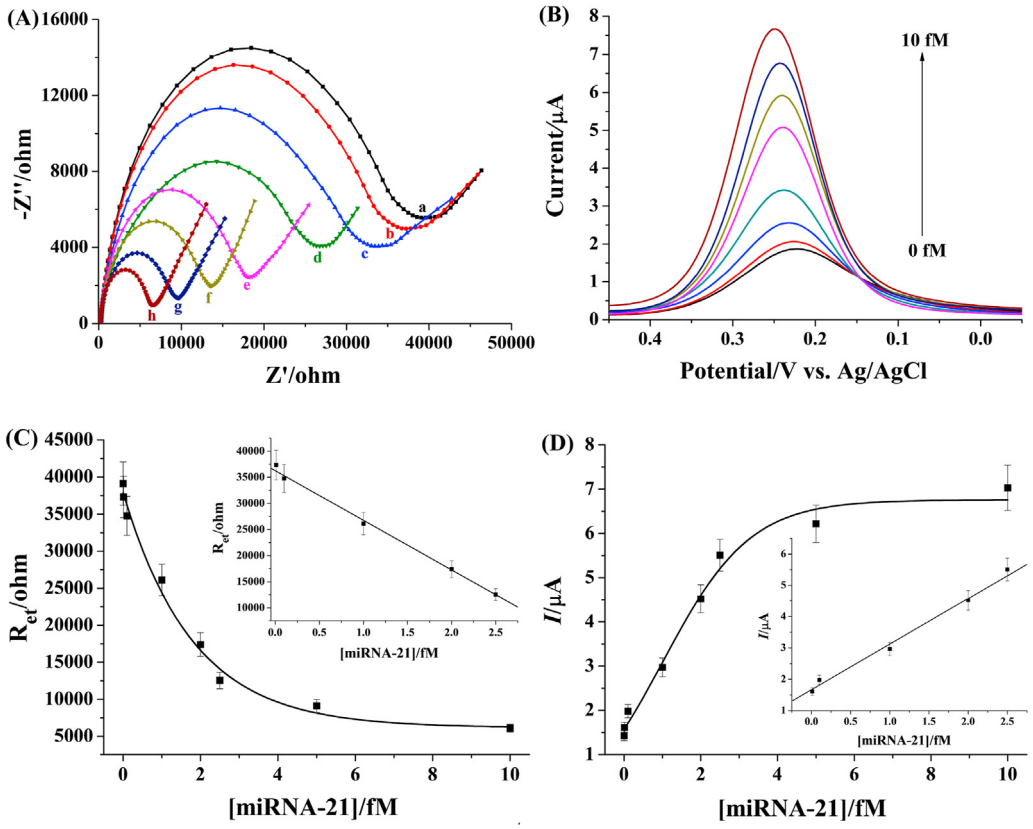


Fig. 4. Representative EIS (A) and DPV (B) responses and calibration curves (C, D) for analyzing different concentrations of miRNA-21 (0, 0.01, 0.1, 1, 2, 2.5, 5 and 10 fM). The insets shows the linear segments of the calibration curves. The enzymolysis time was 2 h and the DSN concentration was 0.1 U.

Table 1
Analytical performances of the DSN-assisted electrochemical methods for miRNA detection.

Method	Signal amplification	Detection limit	Linear range	Ref.
EIS	–	1 fM	2 fM – 2 pM	[46]
EIS	CuCo–CeO_2	33 aM	0.1 fM – 10 nM	[47]
DPV	TdT	15 aM	50 aM–50 pM	[48]
DPV	CHA/AuNPs	–	25.1 aM	[49]
DPV	ALP + redox reaction	34 aM	100 aM–10 nM	[50]
DPV	ALP + redox cycling	86 aM	0.1 fM – 0.1pM	[51]
DPV	ALP + redox cycling	10 aM	0.1 fM – 100 pM	[52]
DPV	CdTe QDs	1.2 aM	5 aM–5 fM	[53]
DPV	MB/rGO	0.01 fM	0.05–5 fM	[54]
DPV	AuNPs/Thi + HCR	11 aM	0.1 fM – 0.1 nM	[55]
SWV	AuNPs/CNNS	2.9 fM	10 fM – 1 nM	[56]
SWV	AuNPs@Dox	0.17 pM	1 pM–10 nM	[57]
CC	DNA-AuNPs	0.05 fM	0.1 fM –100 pM	[58]
CC	DNA-AuNPs	6.8 aM	10 aM–10 pM	[59]
i-t	AuNPs/HRP	43.3 aM	0.1 fM – 100 pM	[60]
i-t	DNAzyme	8 aM	50 aM –500 aM	[61]
DPV	SA	10 aM	10 aM–2.5 fM	this work

Abbreviation: SWV, square wave voltammetry; CC, chronocoulometry; i-t, amperometry; TdT, terminal deoxynucleotidyl transferase; CHA, catalytic hairpin assembly; CNNS, carbon nitride nanosheet; HRP, horseradish peroxidase; ALP, alkaline phosphatase; QDs, quantum dots; AuNPs@Dox, doxorubicin-loaded gold nanoparticles; MB/rGO, methylene blue (MB)/reduced graphene oxide; HCR, hybridization chain reaction.

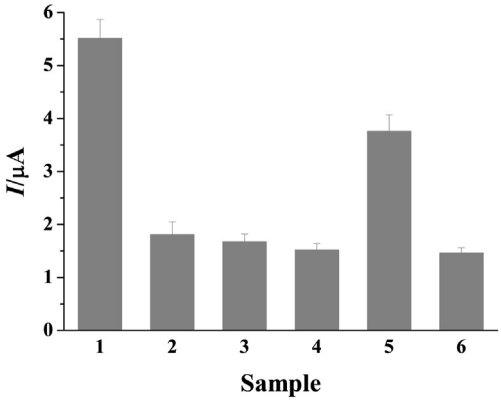


Fig. 5. (A) Selectivity of this method. Bar 1, miRNA-21; bar 2, single-base mismatch; bar 3, three-base mismatch; bar 4, noncomplementary; bar 5, 1 nM biotin; bar 6, 1 nM biotin pretreated by SA-coated magnetic beads. The concentration of miRNA-21 was 2.5 fM and that of the mismatched sequences was 25 fM.

Daohong Wu: Investigation. **Weilin Hou:** Investigation. **Lu Wang:** Investigation. **Ning Li:** Investigation. **Zhifang Sun:** Formal analysis, Writing - review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

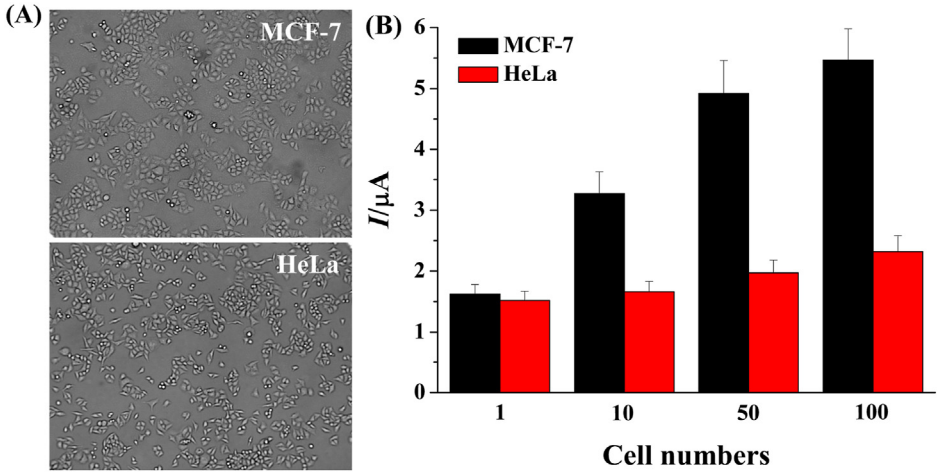


Fig. 6. (A) Confocal images of living MCF-7 and HeLa cells. (B) Dependence of peak current on the concentration of MCF-7 and HeLa cells.

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