Microchimica Acta (2020) 187:4
https://doi.org/10.1007/s00604-019-4011-7

ORIGINAL PAPER

DNA-templated copper nanoparticles as signalling probe for electrochemical determination of microRNA-222

Ya Wang[1] · Wenwen Meng[1] · Xi Chen[1] · Yuzhong Zhang[1]

Received: 8 July 2019 / Accepted: 8 November 2019

Abstract

An ultrasensitive electrochemical biosensor is described for the determination of microRNAs. It is based on the use of DNA-templated copper nanoparticles (Cu NPs) as signalling probe. MicroRNA-222 was selected as the model analyte. The probe was obtained from two different oligonucleotides (containing complementary bases) via hybridization chain reaction to form long DNA concatemers as template. The Cu NPs were formed by reaction of ascorbate with copper sulfate. The biosensor was fabricated as follows: (a) Capture probe (cDNA) with a thiolated group was immobilized on reduced graphene oxide modified with gold nanoparticles (rGO/Au NPs), (b) materials was placed on a glassy carbon electrode (GCE); (c) the modified electrode (cDNA/rGO/Au NPs/GCE) was sequentially hybridized with microRNA-222 and signal probe; this results in the formation of a sandwich structure of cDNA-microRNA-signal probe on surface of the modified electrode. Differential pulse voltammetry was employed to record the electrochemical response of biosensor in pH 6.0 solution. As a result, a sensitive oxidation current with a peak potential at 0.10 V (vs. SCE) was obtained corresponding to Cu NPs. The experimental conditions were optimized. Under optimal conditions, the biosensor exhibited wide linear response range (0.5 fM to 70 nM) and low limit of detection (0.03 fM; at $S/N = 3$). The assay possesses high selectivity and can discriminate analyte microRNA from single-base mismatched microRNA.

Keywords MicroRNA-222 · DNA concatemers · Copper nanoparticles · Biosensor · Differential pulse voltammetry

Introduction

MicroRNAs, a large class of small non-coding RNAs, play important regulatory roles in many biological processes such as cellular differentiation and cell apoptosis [1]. More evidences show the dysregulation of expression of microRNA is correlated to the development of many diseases including cancer and cardiovascular diseases [2, 3]. For example, higher level of microRNA-21 in serum is related to breast and liver [4]; higher level of microRNA-101 is related to hepatocellular carcinoma [5]. The level of microRNA-222 expression is related to colorectal, gastric and breast cancers [6]. Therefore, microRNAs have been regarded as clinically promising diseases biomarkers. Conventional methods for microRNA detection are quantitative reverse transcription polymerase chain reaction (qRT-PCR), oligonucleotide microarray and Northern blotting [7]. However, the qRT-PCR detection requires the design primers to label locked nucleic acid (LNA), Northern blotting requires large amount of sample (5–25 mg) and suffers from sample degradation from RNases and low sensitivity. Microarray technology suffers from the complicated procedures of fluorescent labeling of microRNAs. Hence, it is important to develop a sensitive technology for microRNA detection. Because of microRNAs possess short sequence (18–22 nt), low expression level, and highly similar sequence among family members, therefore it is challenge to develop an analytical method for microRNA detection with high sensitivity and high selectivity. During past two decades, various methods for microRNA detection have been reported including fluorescence [8], colorimetry [9], surface plasmon resonance SPR

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00604-019-4011-7) contains supplementary material, which is available to authorized users.

✉ Yuzhong Zhang
zhyz65@mail.ahnu.edu.cn

[1] College of Chemistry and Materials Science, Anhui Key Laboratory of Chem-Biosensing, Anhui Normal University, Wuhu 241002, People's Republic of China

Published online: 03 December 2019

[10] and electrochemistry [11–20] and several reviews have been published [7, 21, 22]. The fluorescent method needs duplex specific nuclease to cleavage molecular beacon (MB)-microRNA duplex, target microRNA was subsequently be released to hybridize with another MB, resulting in signal amplification to obtain high sensitivity. Miao's [23] and Zuo's [24] groups have all reported a sensitive electrochemical method for microRNA detection based on hybridization chain reaction (HCR) amplifying sensing signal. In Miao's work, tetrahedral DNA with the recognition hairpin was designed and immobilized on electrode surface, gold nanoparticles (Au NPs) labeled with complementary sequence and two different hairpins DNA labeled with silver nanoparticles (Ag NPs) were employed. In presence of target microRNA, hairpin on the tetrahedral DNA can be opened to capture DNA with complementary sequence, resulting in HCR occurring and lots of Ag NPs loaded on DNA concatemers. Hence, the electrochemical signal from Ag NPs was clearly amplified. Miao's groups also employed tetrahedral DNA with the recognition hairpin and the difference was that enzymes (HRP) were used to catalyze the 3,3′,5,5′-tetramethylbenzidine reduction, resulting in clearly enhanced electrochemical signal. Liu's group have reported an enzyme-free electrochemical detection of microRNA-21 using HCR amplification strategy [25], CdTe quantum dots (QD) were labeled at auxiliary stands, when HCR occurs, HCR products loaded lots of CdTe QDs, as a result, the signal intensity of Cd^{2+} were clearly enhanced. Recently, dsDNA-templated copper nanoparticles (Cu NPs) were synthesized and the Cu NPs showed sensitive fluorescent [26–28] or electrochemical response [29]. For instance, Dong's group reported a fluorescent sensor for aptamer detection based on double-strand DNA-templated Cu NPs [27]. Zhang and his coworkers reported a sensitive electrochemical CRP immunosensor based on dsDNA-templated Cu NPs as signal probe (the limit of detection was 0.33 fg mL^{-1}) [29]. Recently progress in copper nanocluster-based fluorescent probe has been reviewed [30]. In present work, we described a ultrasensitive electrochemical biosensor for the microRNA detection based on the HCR producing long DNA concatemers as templates to synthesis Cu NPs as signal probe. Graphene decorated with Au NPs was used as carries for improving immobilization amounts of capture DNA, and the microRNA-222 was selected for model analyte. In contrast to other HCR amplification technology, signal molecules do not to be labeled at terminus of single strands DNA, or to be intercalated into the groove of DNA concatemer. Thus, the experimental method has following advantages: (i) The synthesis of dsDNA-templated Cu NPs was simple. (ii) HCR was gentle and produce long concatemers. Hence, the microRNA biosensor exhibited wide linear response rang (0.5 fM to 70 nM) and the low limit of detection (0.03 fM).

Experimental

Reagents

L-cysteine (L-cys), ascorbic acid (AA), Bovine serum albumin (BSA), anhydrous copper sulfate ($CuSO_4$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (http://www.reagent168.cn). Chloroauric acid ($HAuCl_4{\cdot}4H_2O$) was purchased from Shanghai Chemical Reagent Co., Ltd. (http://ccn.mofcom.gov.cn). Reduced graphene oxide (purity ≥98%) (rGO) was purchased from Sinocarbon Materials Technology Co., Ltd. (http://www.sinocarbon-cas.com). All oligonucleotides sequences were designed by us and synthetized by Sangon Inc. (http://www.sangon.com), and their sequences are listed as follows.

Capture probe (S_0): 5′- SH-$(CH_2)_6$-GAC ACC CAG TAG C -3′
microRNA-222: 5′- AGC UAC AUC UGG CUA CUG GGU -3′
Probe 1 (S_1): 5′- CAG ATG TAG CT G ATC TGC CTA −3′
Probe 2 (S_2): 5′- AGC TAC ATC TGT AGG CAG ATC -3′
Probe 3 (S_3): 5′-TAA CGT CGA GC T AGG CAG ATC -3′
Single base mismatched sequence: 5′- AGC UAC AUC UGG CUX CUG GGU -3′
(X stands for C, G, U)
Noncomplementary sequence: 5′- CUA AGG UCG ACC GAC GCU UAC -3′

The following phosphate buffers (PB) solution were employed over all experiment: 0.01 M PB (pH 7.4) was used for dissolving all oligonucleotides. 0.10 M PB (pH 6.0) containing 0.10 M NaCl (saline) was used for differential pulse voltammetry (DPV) detection solution. 0.01 M PB (pH 7.4) containing 0.10 M NaCl was used hybridization solution. 0.10 M PB containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$ + 0.10 M KCl was used for cyclic voltammetry (CV) measurement, and 0.10 M PB containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$ + 0.10 M KCl was used for electrochemical impedance spectra (EIS) detection. The serum samples were kindly provided from the Anhui Normal University Hospital (Wuhu, China) with ethical approval. All solutions were treated by DEPC (containing no Rnase) and twice-quartz-distilled water was used in this study.

Apparatus

CHI650C electrochemical analyzer (Chenhua, Shanghai, China) was used for various electrochemical measurement including EIS, CV and DPV. A conventional three electrode

system was employed which the bare or the modified glassy carbon electrode (GCE, 3.0 mm in diameter) was used for working electrode, a platinum wire was for auxiliary electrode, and saturated calomel electrode (SCE) was for reference electrode. All potentials were referenced to the SCE. The morphologies of the rGO and Au NPs were taken using scanning electron microscopy (SEM) (Hitachi S-8100, Japan) and the morphology of the DNA-templated Cu NPs was recorded from transmission electron microscopy (TEM, Hitachi, Japan). Fluorescence spectra were recorded on F-4500 fluorescence spectrophotometer (Hitachi, Japan).

Electrochemical measurement

Various electrochemical measurements were performed on CHI650C electrochemical workstation. The assembled processes of microRNA sensor were validated by CV and EIS in 5.0 mL 1.0 mM $[Fe(CN)_6]^{3-/4-}$ or 5.0 mM $[Fe(CN)_6]^{3-/4-}$. DPV was employed to record the electrochemical response of Cu NPs in 5.0 mL pH 6.0 PB. Before experiment, the detection solution was passed into high-purity nitrogen (N_2) for 10 min and N_2 atmosphere was maintained during measurement. The DPV parameters are listed as follows: Potential range, −0.3 V to 0.35 V; Pulse amplitude, 0.05 V; Pulse width, 0.08 s; Sample interval, 0.002 s. EIS parameters: DC potential, 0.18 V; Frequency range, 0.1 to 100 kHz; AC potential amplitude, 5 mV.

Preparation of signal probe

The signal probe was obtained according to our previously reported [29]: Briefly, first, 300 μL of 1.0 μM S_1 was mixed with 300 μL of 1.0 μM S_2 and reacted for 2.5 h at 37 °C. The S_1 and S_2 hybridized continuously to produce long DNA concatemers during this process. Afterwards, 30 μL AA (50 mM), 30 μL $CuSO_4$ (10 mM) was sequentially injected into the DNA concatemers solution above and kept it for 40 min at 37 °C. Thus, Cu NPs was formed due to AA reduce Cu^{2+}, and inserted into the grooves of DNA concatemers. After centrifugal separation, and the signal probe was obtained and kept it in refrigerator.

Fabrication of the microRNA sensor

The sensing protocol of the microRNA detection was illustrated in Scheme 1. The bare GCE was first polished and actived according to literature previously [31] with various grain size of alumina slurries (0.3 μm, 0.05 μm) and washed ultrasonically in water and ethanol for 3 min. Then, 5.0 μL of 1.0 mg mL^{-1} rGO was dropped on the surface of the pretreated GCE and natural dried at room temperature, followed by it was electrodeposited 20 s at −0.20 V in 1.0 mg mL^{-1} $HAuCl_4$ solution. Thus, Au NPs were deposited on the surface of rGO. Subsequently, 10.0 μL of 500 nM capture probe (S_0) was dropped onto the surface of Au NPs decorated rGO and kept it for 12 h in refrigerator. The modified electrode was then treated with MCH for 1 h to block out the active site of electrode surface and to make capture DNA upright. The modified electrode was denoted as cDNA/Au NPs/GCE.

Procedure for microRNA detection

The serum samples, kindly provided from the Anhui Normal University Hospital (Wuhu, China), was centrifugal separated. The upper solution was collected. The collected serum solution was 10-fold diluted with 0.01 M PB (pH 7.4) (eg, 10 μL serum solution was injected 90 μL PB). Then diluted serum solution was used to electrochemical measurement.

The modified electrode (cDNA/Au NPs/GCE) was incubated with 10 μL of different concentrations of microRNA or serum samples for 50 min at 37 °C and 10 μL of signal probe (dsDNA-templated Cu NPs) for 150 min at 37 °C, respectively. As a result, signal probe was linked to the electrode surface via hybridization reaction. Finally, it was transferred to electrolytic cell containing 5.0 mL detection solution for the electrochemical measurement using DPV technique. A DPV oxidation peak at peak potential of 0.10 V (vs. SCE) corresponding to Cu NPs was appeared. The peak intensity is related to original microRNA level.

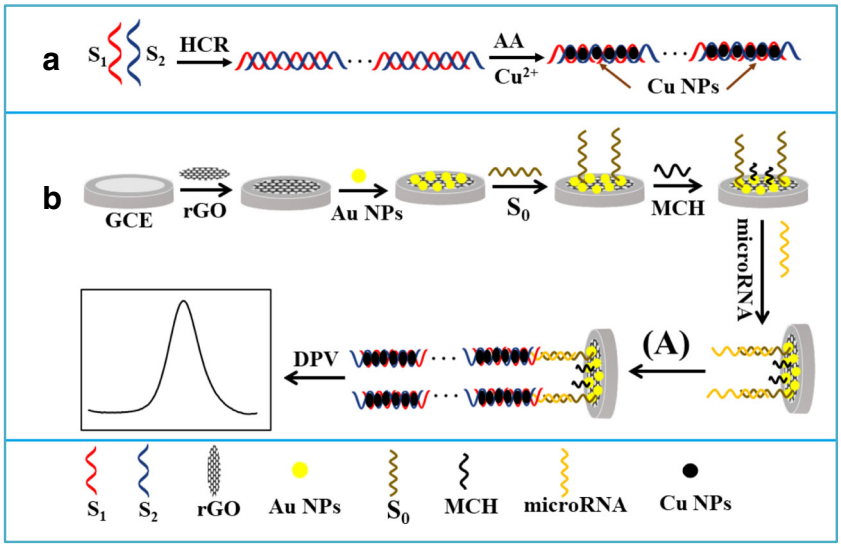
Results and discussion

Choice of materials

As is well known, graphene possess larger surface area and excellent conductivity, and Au NPs have good biocompatibility, therefore graphene-Au NPs nanocomposites have been widely used in sensing field for improving sensing performance [31]. In this study, we employed the graphene-Au NPs nanocomposites for improving immobilization amount of capture probe, and dsDNA-templated Cu NPs as signal probe, to fabricate sandwich type electrochemical biosensor for microRNA detection. As a result, the biosensor showed high sensitivity.

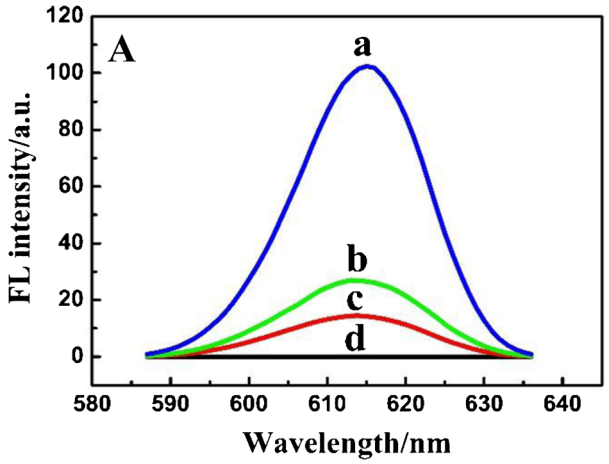
Characterization of signal probe

In order to investigate the formation of signal probe, fluorescence and TEM technique are employed. Two different single strands DNA (S_1 and S_2) are designed to produce longer concatemers: S_1 have 10 base (underline) complementary with 10 base in S_2 (underline) and other 11 base complementary with 11 base in S_2 (not underline). Two single strands DNA arises continuously HCR in hybridization solution to produce longer concatemers. For literature [26], the sizes of



Scheme 1 Schematic displays the preparation process of the microRNA sensor

Cu NPs are dependent on the base pair numbers of dsDNA templates, ration of Cu^{2+} and AA, and longer dsDNA-templates can lead to amount of Cu NPs production. The formation of dsDNA-templated Cu NPs can be investigated using fluorescence method. As shown in Fig. 1A, the solution containing AA, Cu^{2+} and dsDNA concatemers template shows the highest fluorescence intensity at 616 nm (curve a), while no obvious fluorescence emission at 616 nm is observed when AA was absent (curve d) due to the lack of the reductant, only short dsDNA-templated Cu NPs shows weak fluorescence intensity (b). TEM of DNA-templated Cu NPs are shown Fig. 1B. Cu NPs (black dot) are clearly observed, and the size of Cu NPs is about 5 nm in diameters, indicating that the signal probe was formed.



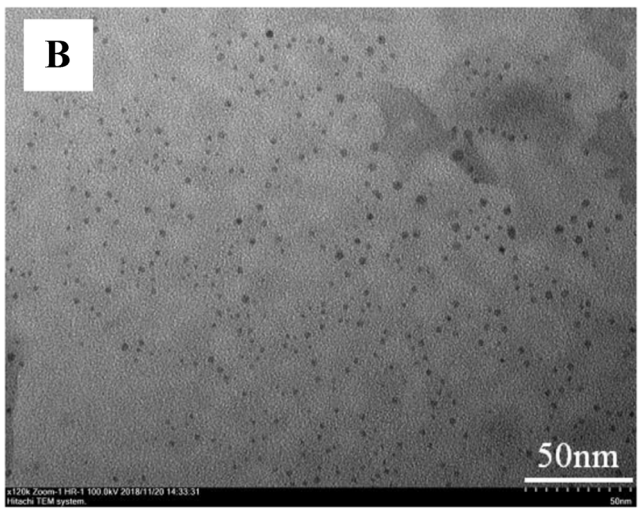
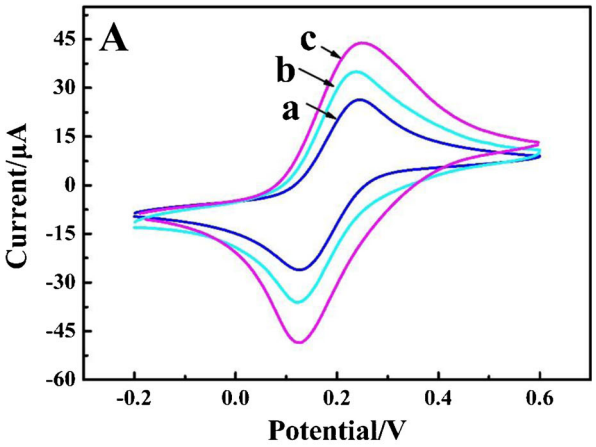
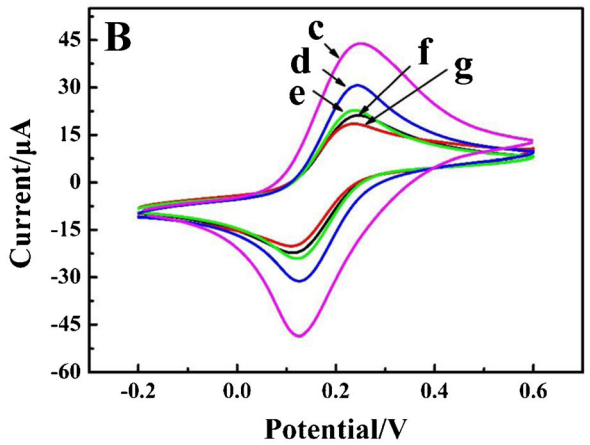


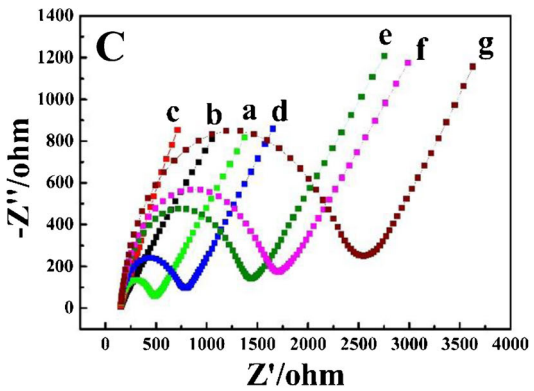
Fig. 1 **A** Fluorescence spectra obtained at various solution (a) long DNA concatemers, AA and Cu^{2+}, (b) short dsDNA, AA and Cu^{2+} (c) single DNA, AA, Cu^{2+} (d) long DNA concatemers, Cu^{2+} (**B**) TEM image of Cu NPs loaded on long DNA concatemers

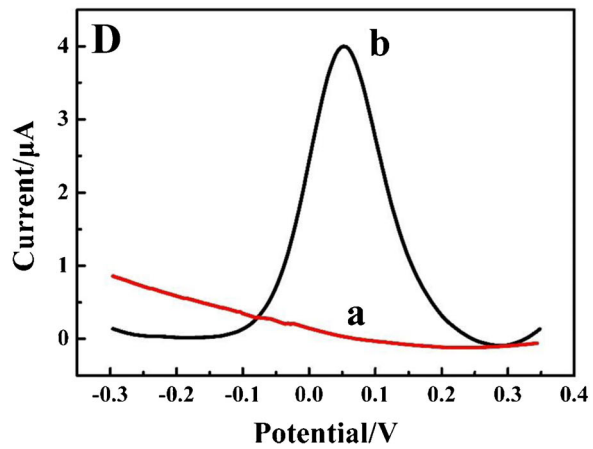
Electrochemical investigation

The Au NPs deposited on the surface of rGO was first investigated with SEM (Seen in Fig. S1), it can be seen that rGO presented thin wrinkled structure (Seen in Fig. S1A) and its surface was uniformly dispersed a layer of Au NPs (Seen in Fig. S1B). The effective surface area of electrode modified with rGO and Au NPs was 0.21 cm^2 according to Randle-Sevcik equation ($i_p = 2.69 \times 10^5 An^{3/2} D_0^{1/2} V^{1/2} C_0$, $D_0 = 7.6 \times 10^{-6}$ $cm^2 \cdot s^{-1}$, $n = 1$, $C_0 = 1.0$ mM $[Fe(CN)_6]^{3-/4-}$). Figure 2A, B shows the CVs of $[Fe(CN)_6]^{3-/4-}$ at differential assembling process of sensor. When capture probe, MCH, microRNA and signal probe were assembled on the surface of Au NPs/rGO, respectively. The peak currents of $[Fe(CN)_6]^{3-/4-}$ are decreased continuously. The suitable explanation is that the negatively charged capture probe, microRNA and signal probe repels the negatively charged redox species, $[Fe(CN)_6]^{3-/4-}$ and decrease the electron transfer rate of redox probes. Figure 2C shows the EIS curves obtained at









◀ **Fig. 2** **A** and (**B**) CVs of the different assembled steps of the microRNA in 1.0 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.1 M KCl. Curves: (a) GCE; (b) rGO/GCE; (c) Au NPs/rGO/GCE; (d) cDNA/Au NPs/rGO/GCE; (e) MCH/cDNA/Au NPs/rGO/GCE; (f) microRNA/MCH/cDNA/Au NPs/rGO/GCE; (g) signal probes/microRNA/MCH/cDNA/Au NPs/rGO/GCE. (**C**) Nyquist plots of 5.0 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.1 M KCl at the different assembled steps of the microRNA sensor. Curves: (a) GCE; (b) rGO/GCE; (c) Au NPs/rGO/GCE; (d) cDNA/Au NPs/rGO/GCE; (e) MCH/cDNA/AuNPs/rGO/GCE; (f) microRNA/MCH/cDNA/AuNPs/rGO/GCE; (g) signal probes/microRNA/MCH/cDNA/Au NPs/rGO/GCE. (**D**) DPVs of biosensor recorded in absence (a) and presence of signal probe (b). Conditions: C_{cDNA} = 500 nM; $C_{microRNA}$ = 1.0 nM

differential assembling steps of sensor (Seen Fig. 2C). The electrode modified with Au NPs and rGO exhibits an almost straight line (Seen curve c). After capture probe and MCH were sequentially assembled on the surface of Au NPs, the EIS cures present a small semicircle and the diameter of semicircle are increased (the diameter of semicircle represents the electron transfer resistance (R_{et})), indicating the R_{et} is clearly increased. Along with target microRNA, signal probe was assembled on the electrode surface, respectively. The semicircles are continuously enlarger and R_{et} continuously increased. After signal probe was linked to the electrode surface, a clearly oxidation peak corresponding to Cu NPs was observed in DPVs, which the peak potential was about 0.10 V (Seen black cure b in Fig. 2D), indicating the designed protocol was successful.

Optimization of experimental conditions

The following parameters were optimized: (a) A sample pH value; (b) Incubation time (c) Hybridization time. Respective text and Figures on optimization are given in the Electronic supporting Material (Seen Fig. S2). In short, the following experimental conditions were found to give best results. (a) Best sample pH value, 6.0; (b) Best incubation time of signal probe and target microRNA, 60 min; The incubation time of capture probe-target micro RNA, 50 min; Hybridization time of S_1-S_2, 150 min.

Signal amplification

Here, we designed two different signal probes for this investigation: one was short dsDNA-templated Cu NPs (S_1-S_3 hybridization for double strand DNA formation), another was long DNA concatemers-templated Cu NPs (S_1-S_2 continuously hybridization for long DNA concatemers formation). Two biosensors were employed to detect 1.0 nM of microRNA and lined to different signal probes. Their electrochemical response was recorded and shown in Fig. 3. As can be seen, the larger peak signal is obtained in presence of long DNA concatemers-templated Cu NPs (Seen cure b), and the signal intensity is 10-fold higher than that of short dsDNA-templated

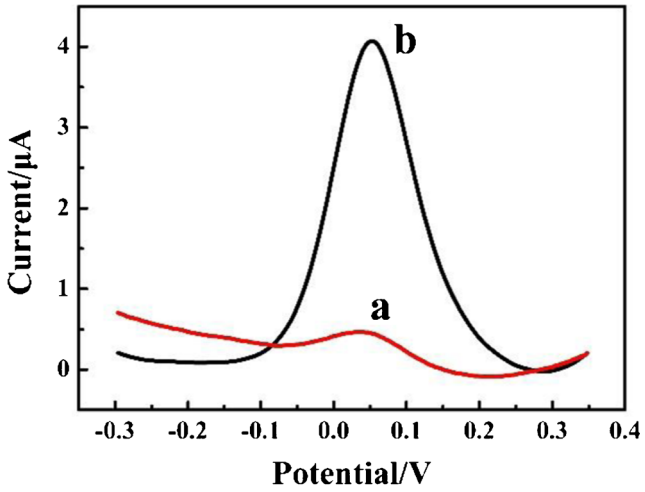


Fig. 3 DPV responses of microRNA sensor in presence of short dsDNA (a) and long DNA concatemers-templated Cu NPs (b)

Cu NPs. The signal intensity enhanced is attributed to long DNA concatemers loading numerous of Cu NPs.

Analytical performance

The analytical performance of microRNA sensor was evaluated by series concentrations of microRNA under the optimized conditions, and the results are shown in Fig. 4A. The peak currents at peak potential of about 0.10 V (vs. SCE) are increased as the concentrations of microRNA increased, and a good calibration plot of peak current vs the logarithmic of the concentration of microRNA was observed (Seen Fig. 4B). The linear range is within 0.50 fM to 70 nM along with the linear regression equation was $\Delta I\ (\mu A) = 3.964 + 0.3937\ \lg C$ (C, nM) ($R^2 = 0.9985$), and the limit of detection obtained was 0.03 fM (at $S/N = 3$). In contrast to previous reported work about microRNA detection in Table 1, the analytical performance of microRNA sensor is lower than that of literature [23, 24] and higher than that of other literature in Table 1, indicating the microRNA sensor have good analytical performance including wider response range and low limit of detection.

Selectivity

The selectivity of sensor is an important parameter. In design, we selected similarly sequence microRNA with microRNA-222 for this investigation. These microRNA are only single base (C, G, U) mismatched with microRNA-222 sequences and noncomplementary sequence microRNA with microRNA-222. if the sensor can distinguish single base mismatched microRNA-222 sequence, it is that the sensor possess good selectivity. In experiment, the concentration of five different kinds of microRNA-222 sequences was 1.0 nM. The results are shown in Fig. 5 with histograms. As can be seen, the signal intensity from microRNA-222 is the largest, and

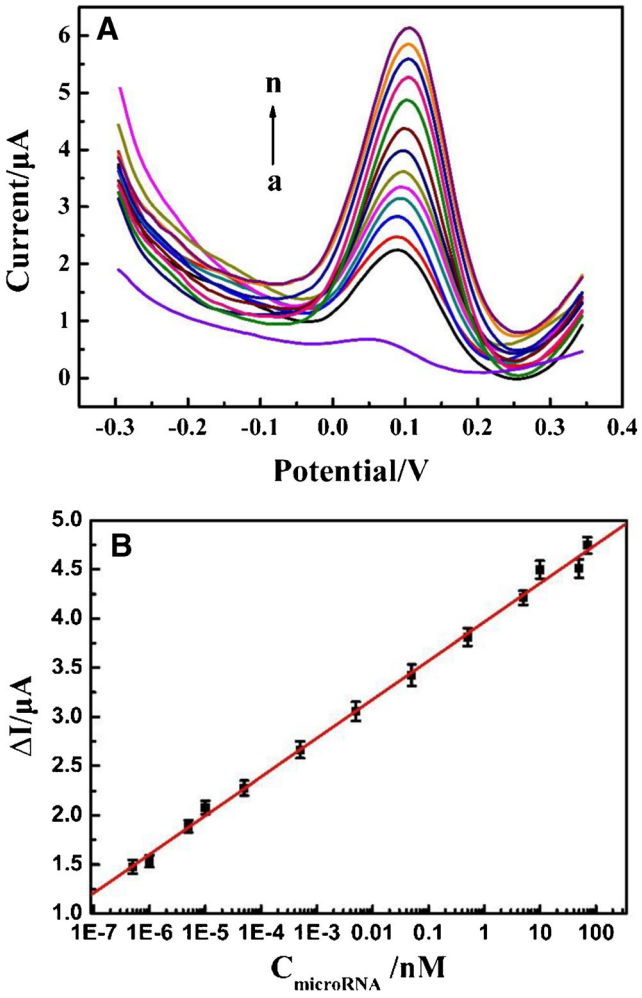


Fig. 4 **A** DPVs of microRNA sensor in various microRNA concentrations in 0.10 M pH 6.0 PB: (a) 0 M; (b) 0.5 fM; (c) 1.0 fM; (d) 5.0 fM; (e) 10.0 fM; (f) 50 fM; (g) 500 fM; (h) 5 pM; (i) 50 pM; (j) 500 pM; (k) 5.0 nM; (l) 10.0 nM; (m) 50 nM; (n) 70 nM. **B** Calibration plots of peak current vs the logarithm of microRNA concentration

signal intensities from single base mismatched sequences (B C D, gray bar) are lower, and only obtain about 30% that of microRNA-222, and the non-complementary sequence has the lowest signal intensity (only about 5% signal intensity of microRNA-222), indicating that the microRNA sensor possesses good selectivity which can discriminates the single base mismatched microRNA-222.

Reproducibility

The reproducibility of microRNA sensor was evaluated by using the same sensor to analysis parallel three different concentrations of microRNA (1.0 nM, 5.0 nM, 10 nM) five times. The variation coefficient obtained according to different concentration were 3.58%, 3.11% and 4.23%, respectively. Further, five sensors fabricated under the same conditions were employed to test three different concentrations of

Table 1 Comparison of analytical performance of different microRNA electrochemical biosensors

Detection method	Used material	Time	Target	Linear range	Detection limit	Ref.
Electrochemical	WO_3-GO	2.5 h	microRNA-21	0.1 fM-100 pM	0.05 fM	[13]
Electrochemical	dsDNA templated Cu NCs	1.5 h	microRNA-101	25 fM-300 fM	8.2 fM	[14]
Electrochemical	$MoSe_2$-AuNPs	3.5 h	microRNA-21	10 fM-1 nM	0.17 fM	[15]
Electrochemical	DA-Au NPs	1.5 h	microRNA-21	0.1 pM-10 pM	45 fM	[16]
Electrochemical	GQDs	4 h	microRNA-155	1 fM-100 pM	0.14 fM	[19]
Electrochemical	AuNPs-GO	4 h	microRNA-155	10 fM-1 nM	3.3 fM	[20]
Electrochemical	HCR and Au NPs	4 h	microRNA-21	10 aM-100 pM	2 aM	[23]
Electrochemical	HCR and HRP	3 h	microRNA-122b	10 aM-1 pM	10 aM	[24]
Electrochemical	CdTe QDs	3.5 h	microRNA-21	0.1 fM-0.1 nM	33 aM	[25]
Electrochemical	enzyme and molecular beacon	1.5 h	microRNA-222	50 pM-10 nM	40 pM	[32]
Electrochemical	magnetic bead	10 h	microRNA-222	25 fM-2.5 pM	12 fM	[33]
Electrochemical	Au-PtBNPs	0.5 h	microRNA-21	1 fM-1.0 μM	1 fM	[34]
Electrochemical	PB-GO	1.5 h	microRNA-122	10 fM-10 nM	1.5 fM	[35]
Electrochemical	Ag NPs	1.5 h	microRNA-21	0.1 fM-50 fM	20 aM	[36]
Electrochemical	dsDNA templated Cu NPs	2 h	microRNA-222	0.5 fM-70 nM	30 aM	This work

QDs: graphene quantum dot; HCR: hybridization chain reaction; NPs: nanoparticles; NCs: nanoclusters; DA-Au NPs: dopamine (DA)-capped AuNPs; Au-PtBNPs: gold platinum bimetallic nanoparticles; PB-GO: graphene oxide with in-situ grown prussian blue

microRNA (1.0 nM, 5.0 nM, 10 nM). The variation coefficient corresponding to different concentrations of microRNA were 5.41%, 4.97%, 4.3%, respectively, indicating that the microRNA sensor possessed good reproducibility.

Analytical application

In order to investigate the reliability and accuracy of the microRNA sensor in clinic sample, recovery experiments were performed by spiking various concentration of microRNA-222 (5, 10 nM) into the 10-fold diluted healthy human serum samples. This preparation of serum samples is seen in Procedure for microRNA detection. DPV technique was employed to record electrochemical signal of biosensor, and the level of sample was measured using working cures method, each sample was detected three times ($n = 3$) and results are shown in Table 2. The recoveries are within 98.4% ~ 102.2% and RSDs are within 3.3% ~ 6.2%. This datum indicates the microsensor can analysis the microRNA in serum sample.

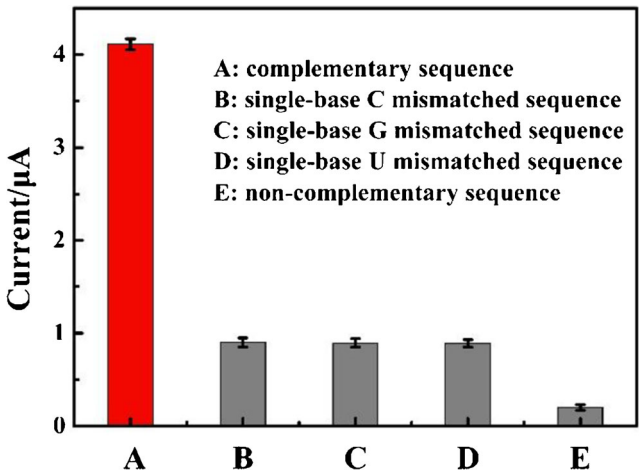


Fig. 5 Selectivity of biosensor:(**a**) complementary sequence; **b** single-base C mismatched sequence; **c** single-base G mismatched sequence; **d** single-base U mismatched sequence; **e** noncomplementary sequence

Conclusions

A sensitive electrochemical biosensor for microRNA-222 detection was fabricated. The dsDNA-templated Cu NPs as signalling probes and amplify sensing signal. The microRNA sensor displays wider response range and low limit of detection. Further, the microRNA biosensor can be applied to the detection of microRNA in serum.

Table 2 Determination results of the serum sample using the microRNA sensor ($n = 3$)

Sample	Spiked (nM)	Proposed immunosensor (nM)	Recovery (%)	RSD (%)
1	5	$5.09^a \pm 0.20^b$	101.8	3.93
	10	$10.11^a \pm 0.37^b$	101.1	3.66
2	5	$5.35^a \pm 0.33^b$	101.0	6.17
	10	$9.93^a \pm 0.55^b$	99.3	5.54
3	5	$4.92^a \pm 0.16^b$	98.4	3.25
	10	$10.22^a \pm 0.52^b$	102.2	5.08

[a] Average value of three determination results

[b] Standard deviation

Acknowledgements This work was financially supported by the National Natural Science Foundation of China (No. 20675002).

References

1. Png KJ, Halberg N, Yoshida M, Tavazoie SF (2012) A microRNA regulon that mediates endothelial recruitment and metastasis by cancer cells. Nature 481:190–194
2. Meng XD, Zhang K, Dai WH, Cao Y, Yang F, Dong HF, Zhang XJ (2018) Multiplex microRNA imaging in living cells using DNA-capped-au assembled hydrogels. Chem Sci 9:7419–7425
3. Volinia S, Calin GA, Liu CG, Ambs S, Cimmion A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM (2006) A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A 103:2257–2261
4. Wang F, Zheng Z, Guo J, Ding X (2010) Correlation and quantitation of microRNA aberrant expression in tissues and sera from patients with breast tumor. Gynecol Oncol 119:586–593
5. Degliangeli F, Kshirsagar P, Brunetti V, Pompa PP, Fiammengo R (2014) Absolute and direct microRNA quantification using DNA–gold nanoparticle probes. J Am Chem Soc 136:2264–2267
6. Zhang CZ, Han L, Zhang AL, Fu YC, Yue X, Wang GX, Jia ZF, Pu PY, Zhang QY, Kang CS (2010) MicroRNA-221 and microRNA-222 regulate gastric carcinoma cell proliferation and radioresistance by targeting PTEN. BMC Cancer 10:367–377
7. Kilic T, Erdem A, Ozsoz M, Carrara S (2018) microRNA biosensors: opportunities and challenges among conventional and commercially available techniques. Biosens Bioelectron 99:525–546
8. Yang L, Liu CH, Ren W, Li ZP (2012) Graphene surface-anchored fluorescence sensor for sensitive detection of microRNA coupled with enzyme-free signal amplification of hybridization chain reaction. ACS Appl Mater Interfaces 4:6450–6453
9. Zhang YL, Li ZP, Cheng YQ, Lv XC (2009) Colorimetric detection of microRNA and RNase H activity in homogeneous solution with cationic polythiophene derivative. Chem Commun 22:3172–3174
10. Fang SP, Lee HJ, Wark AW, Corn RM (2006) Attomole microarray detection of microRNAs by nanoparticle-amplified SPR imaging measurements of surface polyadenylation reactions. J Am Chem Soc 128:14044–14046
11. Gao ZQ, Yu YH (2007) A microRNA biosensor based on direct chemical ligation and electrochemically amplified detection. Sensors Actuators B Chem 121:552–559
12. Zouari M, Campuzano S, Pingarrón JM, Raouafi N (2017) Competitive RNA-RNA hybridization-based integrated nanostructured-disposable electrode for highly sensitive determination of microRNAs in cancer cells. Biosens Bioelectron 91:40–45
13. Shuai HL, Huang KJ, Xing LL, Chen YX (2016) Ultrasensitive electrochemical sensing platform for microRNA based on tungsten oxide-graphene composites coupling with catalyzed hairpin assembly target recycling and enzyme signal amplification. Biosens Bioelectron 86:337–345
14. Wang ZY, Si L, Bao JC, Dai ZH (2015) A reusable microRNA sensor based on the electrocatalytic property of heteroduplex-templated copper nanoclusters. Chem Commun 51:6305–6307
15. Wang YH, Huang KJ, Wu X, Ma YY, Song DL, Du CY, Chang SH (2018) Ultrasensitive supersandwich-type biosensor for enzyme-free amplified microRNA detection based on N-doped graphene/au nanoparticles and hemin/G-quadruplexes. J Mater Chem B 6:2134–2142
16. Xia N, Zhang LP, Wang GF, Feng QQ, Liu L (2013) Label-free and sensitive strategy for microRNAs detection based on the formation of boronate ester bonds and the dual-amplification of gold nanoparticles. Biosens Bioelectron 47:461–466
17. Liao YH, Huang R, Ma ZK, Wu YX, Zhou XM, Xiung D (2014) Target-triggered enzyme-free amplification strategy for sensitive detection of microRNA in tumor cells and tissues. Anal Chem 86:4596–4604
18. Lv WF, Zhao JM, Situ B, Li B, Ma W, Liu JM, Wu ZX, Wang W, Yan XH, Zheng L (2016) A target-triggered dual amplification strategy for sensitive detection of microRNA. Biosens Bioelectron 83:250–255
19. Hu TX, Zhang L, Wen W, Zhang XH, Wang SF (2016) Enzyme catalytic amplification of microRNA-155 detection with graphene quantum dot-based electrochemical biosensor. Biosens Bioelectron 77:451–456
20. Wu XY, Chai YQ, Yuan R, Zhuo Y, Chen Y (2014) Dual signal amplification strategy for enzyme-free electrochemical detection of microRNAs. Sens. Actuators B 203:296–302
21. Chen YX, Huang KJ, Niu KX (2018) Recent advances in signal amplification strategy based on oligonucleotide and nanomaterials for microRNA detection a review. Biosens Bioelectron 99:612–624
22. Wang YH, He LL, Huang KJ, Chen YX, Wang SY, Liu ZH, Li D (2019) Recent advances in nanomaterial-based electrochemical and optical sensing platforms for microRNA assays. Analyst 144:2849–2866
23. Miao P, Tang YG, Yin J (2015) MicroRNA detection based on analyte triggered nanoparticle localization on a tetrahedral DNA modified electrode followed by hybridization chain reaction dual amplification. Chem Commun 51:15603–15740
24. Ge ZL, Lin MH, Wang P, Pei H, Yan J, Shi JY, Huang Q, He DN, Fan CH, Zuo XL (2014) Hybridization chain reaction amplification of microRNA detection with a tetrahedral DNA nanostructure-based electrochemical biosensor. Anal Chem 86:2124–2130
25. Liu HY, Bei XQ, Xia QT, Fu Y, Zhang S, Liu MC, Fan K, Zhang MZ, Yang Y (2016) Enzyme-free electrochemical detection of microRNA-21 using immobilized hairpin probes and a target-triggered hybridization chain reaction amplification strategy. Microchim Acta 183:297–304
26. Rotaru A, Dutta S, Jentzsch E, Gothelf K, Mokhir A (2010) Selective dsDNA-templated formation of copper nanoparticles in solution. Angew Chem Int Ed Eng 49:5665–5667
27. Zhou Z, Du Y, Dong S (2011) Double strand DNA-templated formation of copper nanoparticles as fluorescent probe for label-free aptamer sensor. Anal Chem 83:5122–5127
28. Xu FZ, Shi H, He XX, Wang KM, He DG, Guo QP, Qing ZH, Yan LA, Ye XS, Li D, Tang JL (2014) Concatemeric dsDNA-templated copper nanoparticles strategy with improved sensitivity and stability based on rolling circle replication and its application in microRNA detection. Anal Chem 86:6976–6982
29. Zhang JJ, Zhang WJ, Guo JJ, Wang JC, Zhang YZ (2017) Electrochemical detection of C-reactive protein using copper nanoparticles and hybridization chain reaction amplifying signal. Anal Biochem 539:1–7
30. Qing TP, Zhang KW, Qing ZH, Wang X, Long CC, Zhang P, Hu HZ, Bo F (2019) Recent progress in copper nanocluster-based fluorescent probing: a review. Microchim Acta 186:670–690. https://doi.org/10.1007/s00604-019-3747-4
31. Wang J, Shi AQ, Fang X, Han XW, Zhang YZ (2014) Ultrasensitive electrochemical supersandwich DNA biosensorusing a glassy carbon electrode modified with gold particle deco-rated sheets of graphene oxide. Microchim Acta 181:935–940

32. Wang MG, Shen B, Yuan R, Cheng W, Xu HB, Ding SJ (2015) An electrochemical biosensor for highly sensitive determination of microRNA based on enzymatic and molecular beacon mediated strand displacement amplification. J. Electroanal Chem 756:147–152
33. Bettazzi F, Asl EH, Esposito CL, Quintavalle C, Formisano N, Laschi S, Catuogno S, Marrazza MIG, Mascini M, Cerchia L, Franciscis VD, Condorelli G, Palchetti I (2013) Electrochemical detection of microRNA-222 by use of a magnetic bead-based bioassay. Anal Bioanal Chem 405:1025–1034
34. Bharti A, Agnihotri N, Prabhakar N (2019) A voltammetric hybridization assay for microRNA-21 using carboxylated graphene oxide decorated with gold-platinum bimetallic nanoparticles. Microchim Acta 186:185–196. https://doi.org/10.1007/s00604-019-3302-3
35. Wang F, Chu Y, Ai Y, Chen L, Gao F (2019) Graphene oxide with in-situ grown prussian blue as an electrochemical probe for microRNA-122. Microchim Acta 186:116–125. https://doi.org/10.1007/s00604-018-3204-9
36. Liu LL, Chang Y, Xia N, Peng PZ, Zhang LP, Jiang MG, Zhang JB, Liu L (2017) Simple, sensitive and label-free electrochemical detection of microRNAs based on the in situ formation of silver nanoparticles aggregates for signal amplification. Biosens Bioelectron 94:235–242