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DNA Tetrahedral Nanostructure-based Electrochemical miRNA Biosensor for Simultaneous Detection of Multiple miRNAs in Pancreatic Carcinoma

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Abstract

Specific and sensitive biomarker detection is essential to early cancer diagnosis. In this study, we demonstrate an ultrasensitive electrochemical biosensor with the ability to detect multiple pancreatic carcinoma (PC) related microRNA biomarkers. By employing DNA tetrahedral nanostructure capture probes to enhance the detection sensitivity as well as a disposable 16-channel screen-printed gold electrode (SPGE) detection platform to enhance the detection efficiency, we were able to simultaneously detect four PC-related miRNAs: miRNA21, miRNA155, miRNA196a and miRNA210. The detection sensitivity reached to as low as 10 fM. We then profiled the serum levels of the four miRNAs for PC patients and healthy individuals with our multiplexing electrochemical biosensor. Through the combined analyses of the four miRNAs, our results showed that PC patients could be discriminated from healthy

controls with fairly high sensitivity. This multiplexing PCR-free miRNA detection sensor shows promising applications in early diagnosis of PC disease.

Keywords: Pancreatic carcinoma; Biomarker; Multiple miRNA detection; Electrochemical biosensor; DNA tetrahedral nanostructures

1. INTRODUCTION

Pancreatic carcinoma (PC) is a lethal malignancy and becomes a major clinical challenge[1-2]. It ranks as the fourth leading death cause of cancer globally due to its aggressive nature, late clinical presentation, lack of biomarkers for early diagnosis and limited efficacy of existing treatment[3]. As currently used biomarkers are not specific and sensitive enough to diagnose pancreatic carcinoma at early stages, researchers are making every effort on discovering specific biomarkers for early diagnosis of pancreatic carcinoma.

MicroRNAs (MiRNAs) are non-coding and short RNA molecules with 19-24 nucleotides in length. MiRNAs are closely related to the regulation of gene expression at posttranscriptional level and play significant roles in many physiological and pathological conditions[4-5]. Extensive efforts have been done on miRNA research to understand the gene regulation mechanism. It has become evident that miRNAs are extensively involved in cell and tumor growth regulation in the last few years[6-7]. Studies have shown that the profiling expression of four miRNAs (miRNA21, miRNA155, miRNA196a and miRNA210) in plasma can differentiate PC patients from healthy controls[6,8-10]. Wang *et al* have evaluated the expression levels of the four miRNA in plasma by quantitative polymerase chain reaction (qPCR) method[10]. Nevertheless, qPCR method requires specialized reagents, expensive instruments, sophisticated readout system and skilled operators, which greatly increase the complexity and experimental cost. Therefore, convenient and cheap miRNAs analysis for pancreatic carcinoma with high specificity and sensitivity are desired for routine miRNAs analysis.

Electrochemical biosensor is considered to be the most promising device for point-of-care testing (POCT) because its' advantage of sensitive, rapid, simple, efficient, etc. [11-16]. Moreover, the equipment is low cost, little energy consumption and

easy to miniaturization or integration[17-19]. Therefore, miRNA analysis by electrochemical technique has drawn great attention recently[12, 20-22]. Sandwich type assay is one of the most popular strategies used in various electrochemical miRNA biosensors[23-28]. Our previous reports have shown that thiolated DNA tetrahedral nanostructures could be attached onto gold electrode surface as capture probes, which could provide solution-like environment, increase biomolecule target accessibility and reduce surface crowding effect[26, 28-40]. Hence, the DNA tetrahedron capture probe could greatly enhance the electrochemical miRNA or DNA biosensor's sensitivity.

In this work, we employed DNA tetrahedral nanostructure capture probe-based electrochemical approach for PCR-free multiplexed detection of miRNA21, miRNA155, miRNA196a and miRNA210, which are proved to be PC-related miRNAs. Importantly, we assembled the DNA tetrahedral nanostructure-based sandwich type assay onto a disposable 16-channel screen-printed gold electrode (SPGE) detection platform. As far as we know, such PCR-free and sensitive detection platform to perform simultaneous detection of multiple miRNAs has never been reported. By taking advantages of both tetrahedral nanostructure-based electrochemical miRNA assay and the SPGE platform, we realized simultaneous sensitive detection of the four PC-related miRNAs. Furthermore, we profiled serum levels of the four miRNA for 8 PC patients and 8 healthy controls with this ultrasensitive multiplexed electrochemical miRNA biosensor.

2. EXPERIMENTAL PART

2.1 Materials and Apparatus

The single-stranded DNAs (sequences shown in table 1) were synthesized and purified from Shanghai Sangon Biological Engineering Technology and Services Co. Ltd (Shanghai, China). The miRNAs (sequences shown in table 2) were purchased from Invitrogen Co. Ltd (shanghai, China).

Table 1 Synthetic oligonucleotides used in this study.

Probe name	Sequence (5'to3')
T-21	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTAAAAAA AAAAATCAACATCAG

T-155	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTAAAAAA AAAAA ACC CCT ATC A
T-196a	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTAAAAAA AAAAA CCC AAC AAC A
T-210	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTAAAAAA AAAAA C AGT GTG CGG
T-B	SH-TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATAC
T-C	SH-TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTC
T-D	SH-TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCAT
swRP-21	TCTGATAAGCTA-biotin
swRP-155	CG ATT AGC ATT AA-biotin
swRP-196a	TGAAACTA CCT A-biotin
swRP-210	TGGGCAGGGGCT-biotin
SS-capture-21	SH-TTTTTATCAACATCAG

Table 2 Sequences of mature human miRNAs in this study

miRNA name	Sequence (5'to3')	Accession
miRNA21	UAGCUUAUCAGACUGAUGUUGA	MIMAT0000076
miRNA155	UUAAUGCUAAUCGUGAUAGGGGU	MIMAT0000646
miRNA196a	UAGGUAGUUUCAUGUUGUUGGG	MIMAT0000226
miRNA210	AGCCCCUGCCCACCGCACACUG	MIMAT0026475

Total RNA extraction kit was purchased from QIAGEN (Germany). Poly-HRP40 (Streptavidin-poly-HRP40) was purchased from Fitzgerald Industries (North Acton, MA, USA). The ready-to-use TMB substrate solution (TMB = 3, 3, 5, 5 tetramethylbenzidine; K-blue low activity substrate; H_2O_2 included) was purchased from Neogen(Lansing, USA). Tris base (tris-(hydroxymethyl) aminomethane), TCEP (tris(2-carboxyethyl) phosphine hydrochloride), DEPC (diethyl pyrocarbonate) and EDTA (ethylenediaminetetraacetic acid) were purchased from Sigma-Aldrich (St.

Louis, USA). The other chemicals involved were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). All chemicals were of analytical grade and used when received.

Serum samples from 8 pancreatic carcinoma patients were obtained from Fudan University Shanghai Cancer Center. Control serum samples were obtained from 8 healthy volunteers. The mean age for the patients and healthy volunteers at time of recruitment, were 55 ± 17 years old and 43 ± 8 years old, respectively. Informed written consent was obtained from every individual who participated in the study according to the ethics committee vote of Fudan University Shanghai Cancer Center.

The buffer solutions used were prepared as follows: TCEP solution: 30mM in water; TM buffer (DNA tetrahedron capture probe forming buffer): 20 mM tris buffer and 50 mM $MgCl_2$, pH 8.0; Hybridization-buffer: 10 mM Na_2HPO_4, 10 mM KH_2PO_4, 1 M NaCl, 20 mM $MgCl_2$; Washing-buffer: 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 137 mM NaCl and 7 mM KCl, pH7.4; Poly-HRP40 was diluted with 0.5% casein solution by 1000 times. All solutions were prepared with MilliQ water (resistivity 18 MΩ.cm) from a Millipore ultrapure water purifications system.

2.2 MicroRNAs extraction

Circulating miRNAs were extracted from serum samples using miRNeasy RNA isolation kits following the Qiagen's instructions. The standard protocol was modified on the basis of Exiqon's application note of "RNA Purification from Blood Plasma & Serum". Briefly, 200 μL of serum was added to 1 mL of Qiazol solution. Vortex the mixture briefly and incubate it at RT (room temperature) for 5 minutes to dissociate nucleoprotein complexes. Then add 200 μL of chloroform and vortex the mixture vigorously for 15 seconds. After centrifuging at 13 000 ×g at 4℃ for 15 minutes, purification of RNA in upper phase and the precipitation was performed according to the recommended protocol. The extracted miRNAs solution was stored in a −20 ℃ freezer.

2.3 Preparation of electrochemical miRNAs sensor

The capture tetrahedral structured probes were prepared as reported process[14]: 1 μl of T-21(or T-155, T-196a, T-210), T-B, T-C and T-D (50 μM) were mixed with 41 μl of TM buffer and 5 μl of TCEP solution (30 mM). The mixture was heated to 95℃, lasted for 3 minutes, then cooled to 4℃ over 30 seconds with a BioRad thermal cycler PTC-100 equipment. A final concentration of 1 uM solution was obtained. The successful synthesis of DNA tetrahedral nanostructures was confirmed by PAGE (Native polyacrylamide gel electrophoresis) (Figure S-1). 1uM of SS-capture-21 was used as capture probe instead for the control experiment.

The 2 mm-diameter gold electrodes were cleaned according to the previously reported procedures [29]. Then 3 μl of DNA tetrahedron capture probe solution was placed on the cleaned gold electrode surface and incubated for 16 hours at RT for immobilization. Different concentration of target miRNA solutions were mixed with 100 nM signal probe (swRP-21, swRP-155, swRP-196a, swRP-210) in Hybridization-buffer. The mixture was incubated for 5 minutes at 80 ℃ and then cooled to RT. Next, rinsed the DNA tetrahedron capture probe modified electrodes with Washing-buffer and dried with nitrogen gas. Then the dried electrodes were incubated in 100 uL of the prepared target solution in a RNase-free tube for 5 hours at 4 ℃ for hybridization. Rinsed the electrodes again with Washing-buffer and dried with nitrogen. Later, 3 uL of diluted Poly-HRP40 solution was injected onto each gold electrode for 15 minutes at 4℃. Lastly, the electrodes were rinsed thoroughly again with Washing-buffer then subjected to the following electrochemical detection.

2.4 Electrochemical measurements

The traditional electrochemical configuration (three-electrode system) was used in our detection with a platinum wire as counter electrode and an Ag/AgCl (3M KCl) as reference electrode. Electrochemical work station modeled CHI 760E was used for the collection of electrochemical signal. Cyclic voltammetry (CV) was performed at a scan rate of 0.1 Vs^{-1}. The amperometric current *vs.* time was measured by fixing the potential at 0.1V (*vs.* Ag/AgCl). The signal value was collected at 100 seconds after the PolyHRP40 redox reaction reaching the steady state.

2.5 Fabrication of 16 channel SPGE array

The 16 channel disposable SPGE (photograph shown in Figure S-2, purchased from GeneFluidics, Monterey Park, CA) was immersed in isopropanol for 1 minute and rinsed thoroughly by Milli-Q water before use. After blew-dry with nitrogen, 5 μl of DNA tetrahedron capture probe solution was injected on a gold working electrode and incubated at RT overnight. The after procedures were similar with normal gold electrode-based electrochemical miRNAs sensor as described before, except that the SPGE could not be immersed in the target and signal probe mixture. We tried two methods of adding target miRNA and signal probe. In method 1, various concentration of target miRNA solutions were first added on the SPGE and incubated at 4 ℃ for around 5 hours and then the SPGE was rinsed by Washing-buffer and dried with nitrogen. After, 100 nM of signal probe solution was added and incubated again at 4 ℃ for around 5 hours and the SPGE was rinsed by Washing-buffer and dried with nitrogen. In method 2, target miRNA solutions with various concentrations were mixed together with 100 nM of signal probe (swRP-21, swRP-155, swRP-196a, swRP-210) in Hybridization-buffer. The mixtures were incubated at 80℃ for 5 minutes and cooled to RT. Then the mixture was injected onto the working electrode and incubated for 5 hours at 4℃. The after procedures of fabricating miRNA array on SPGE were the same as normal electrochemical miRNA sensor. CV was carried out with a potential range of -0.25~0.45 V at a scan rate of 0.1V s^{-1}. And the amperometric electro reduction current was measured fixing the potential at -0.2V (*vs.* Ag/AgCl) at 60 seconds.

3. RESULTS AND DISCUSSION

3.1 Strategy of analysis

Three components hybridization strategy was used in this study (Figure 1). The capture probe was appended to one vertex of the DNA tetrahedron. The oligonucleotides' sequences design strategy for forming tetrahedron structure was the same as the previously reported[14]. Briefly, four single stranded DNA were used to

assemble tetrahedral structured probes, three of which (T-B, T-C and T-D) were thiolated at their 5'-terminal. And the fourth strand (T-21, T-155, T-196a, or T-210) had extended sequence which is complementary to part of the corresponding miRNA target. The signal probes were biotin-tagged DNA strands (probe swRP-21, swRP-155, swRP-196a, swRP-210) and were complementary to the other part of the miRNA target. Thus, if the target miRNA existed, the capture and report probes would hybridize with the target miRNA and form a stable duplex. Furthermore, the Poly-HRP40 could be captured on the gold electrodes by the biotin-tagged report probe, catalyzing the reduction of hydrogen peroxide (H_2O_2), and thus generated quantitative amperometric signal in the presence of TMB substrate.

The DNA tetrahedral nanostructure based electrochemical miRNA biosensor has been reported to provide a sensitive method for quantitative analysis of miRNAs[30]. Compared to conventional miRNA detection method, our method showed some combined advantages. Firstly, DNA capture probes in our method could reduce inter-strand interactions because they are uniformly placed on spatial isolated DNA tetrahedron nanostructures, which could enhance the hybridization efficiency. Secondly, the background signal could be reduced as the DNA tetrahedron-modified electrode surface is protein-resistant which leading to a high signal to background ratio. We have compared DNA tetrahedral nanostructure based sensor with SSDNA-based sensor. And we found that DNA tetrahedral nanostructure based sensor was considerably more sensitive than ssDNA/MCH-based biosensors (8.8 fold) (Figure S-3).

We further employed 16-channel SPGE as multiplexed detection platform to carry out simultaneous detections of the four PC-related miRNAs. The same sandwich configuration strategy was used here, which involved the DNA tetrahedral nanostructure capture probe and biotin-tagged single stranded oligonucleotide signal probe to form a duplex with the target miRNAs.

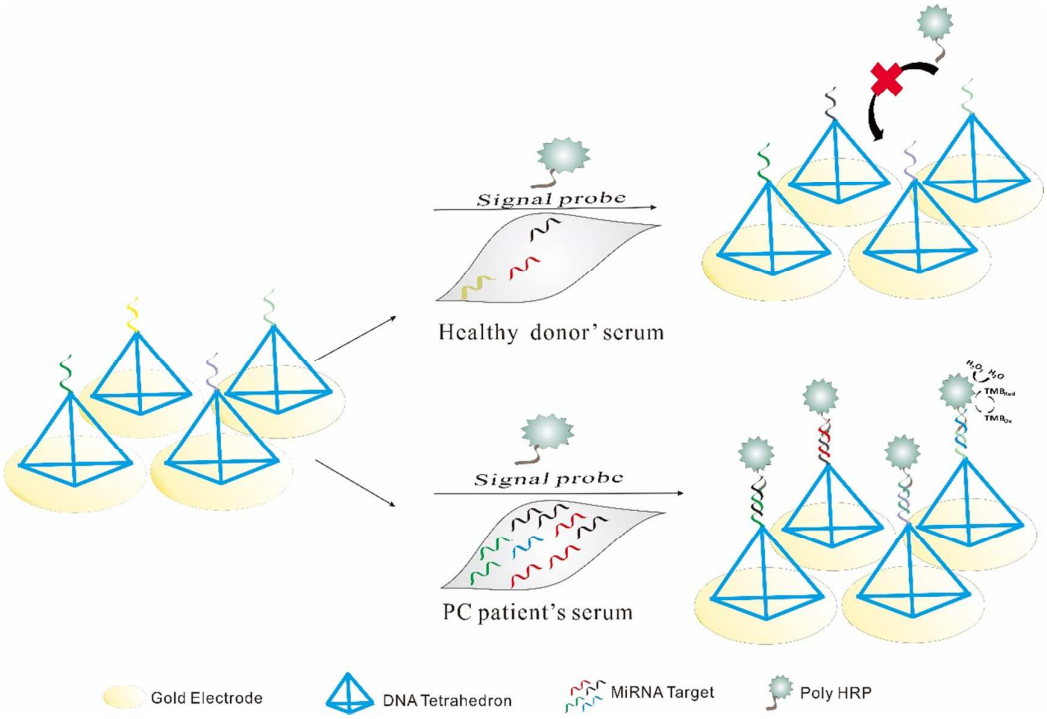

Figure 1. Scheme of DNA tetrahedral nanostructure-based electrochemical miRNA biosensor for simultaneous detection of four PC-related miRNAs. The thiolated 3D DNA tetrahedral nanostructures were immobilized on gold electrodes for capturing of target miRNA. The biotin-tagged signal probes were hybridized when the target miRNA were present, resulting in the binding of poly-HRP40 onto gold electrode surface that could generate catalytic amperometric readout.

3.2 Detection performance of target miRNAs

In this study, we detected a panel of four PC associated miRNAs: miRNA21, miRNA155, miRNA196a and miRNA210. These four miRNAs have been reported to be overexpressed in pancreatic ductal adenocarcinoma[10].

CV were employed to characterize electrochemical process of our electrochemical miRNA biosensor for the multiplexing detection of miRNA targets. Cyclic voltammograms were recorded between a potential range of 0~0.7 V (*vs.* Ag/AgCl) for DNA tetrahedron capture probe-based electrochemical biosensor（shown in Figure 2A, 2B, 2C and 2D）. Two pairs of representative redox peaks were observed in the absence of miRNA target. The peaks were ascribed to two-electron oxidation and

reduction reactions of TMB substrate. When the target miRNA was present, a pair of asymmetric redox peaks appeared as the reduction peak located at ~0.2 V increased apparently. The peak was assigned to the reduction of H_2O_2 catalyzed by polyHRP40 with TMB serving as electron mediator. This indicated that both miRNA target and the polyHRP binding report probe were fastened by the DNA tetrahedron capture probe and formed the sandwich configuration successfully on the gold electrode surface.

Amperometry was employed to measure our miRNA biosensor quantitatively. When the potential was fixed at 100 mV(*vs.* Ag/AgCl), a decay curve was instantly observed for reduction current *vs.* time and reached a steady-state current within around 100 seconds. The background signal was observed as low as ~246±62 nA when target miRNA21 was absent. In contrast, the amperometric current of 1090±20 nA was observed when 1 pM of miRNA21 target was present. Impressively, as low as 10 fM of the target miRNA21 could be detected. Moreover, we found that the electrochemical current increased as the concentration of miRNA target increased, which was across a wide response region of 10 fM~1 nM (Figure 2E). A regression equation of $Y=0.25-0.04X+0.06X^2$(R^2=0.990) was obtained for range from 10 fM to 1 nM, where x was the target concentration in fM and y was the amperometric signal value in uA. Our miRNA sensor with such a wide dynamic range shows great potential in analyzing target miRNAs that exist quite diversely in cells.

The same CV and amperometry method was used in detection of other three kinds of miRNA (miNRA155, miRNA196a and miRNA210). For miRNA155, the background current was ~ 256±19nA and the amperometric current was ~1118±44 nA for 1 pM of target. The dynamic range was even larger than miRNA21, which is from 10fM to 10 nM with a fitting curve equation of $Y=0.24-0.03X+0.07X^2$ (R^2=0.989) (Figure 2F). The dynamic range for miRNA196a was also from 10fM to 10 nM, with a fitting curve equation of $Y=0.34-0.03X+0.05X^2$ (R^2=0.985) (figure 2G). The background current and the amperometric current of 1 pM target were ~352±7 nA and ~1010±28 nA, respectively. And for miRNA210, the range was the same as miRNA155 and miRNA196a with a fitting curve equation of $Y=0.78-0.07X+0.07X^2$(R^2=0.990)

(figure 2H). The background current and the amperometric current of 1 pM target was ~205±46nA and ~1440±98 nA, respectively.

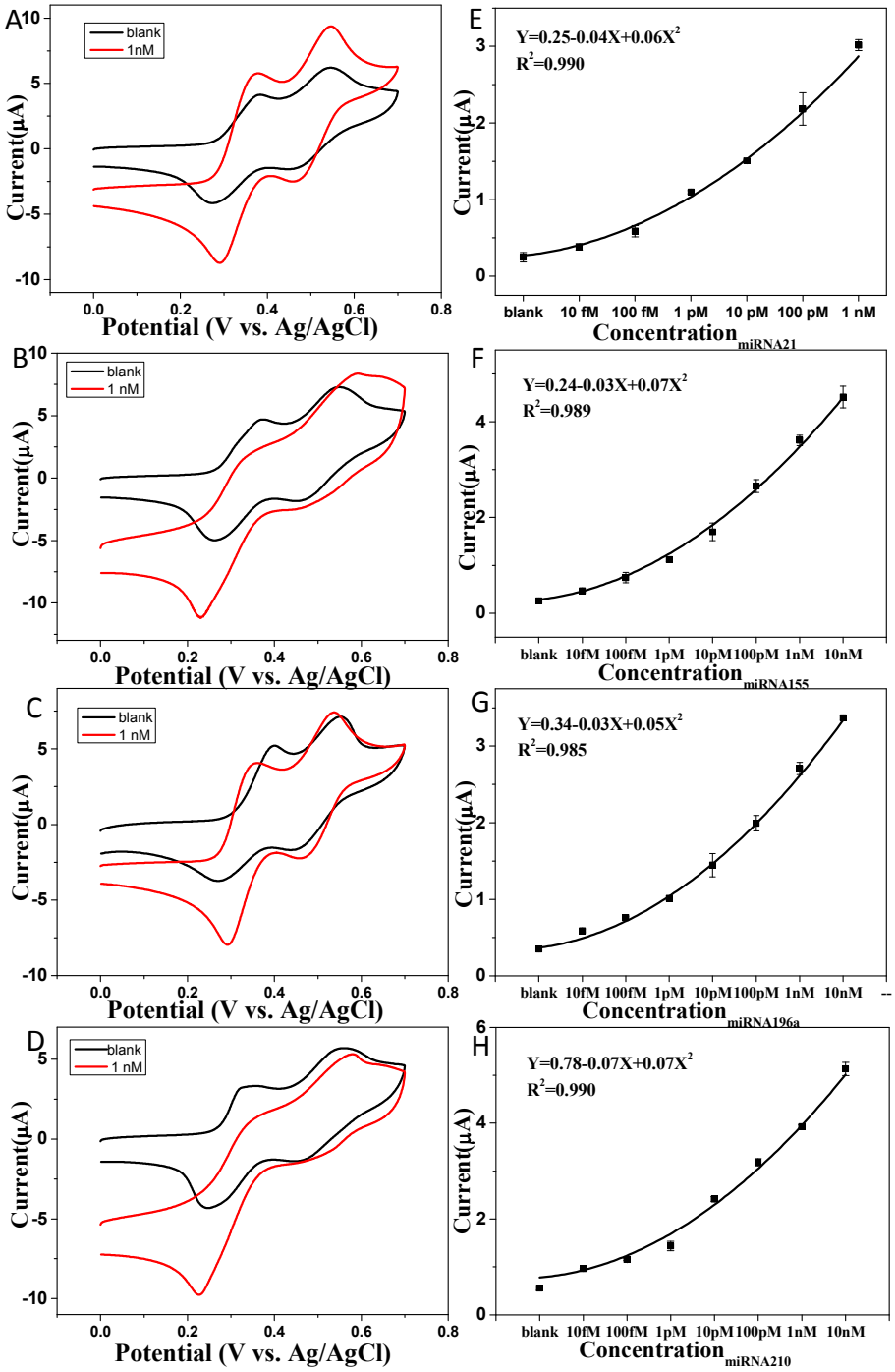

Figure 2. Electrochemical analysis of DNA tetrahedral structure capture probe-based miRNA

sensor. (A)(B)(C)(D) CV for DNA tetrahedron capture probe based miRNA sensors when target miRNA21(A), miRNA155(B), miRNA196a(C) and miRNA210(D) were absent (black) and were present(1 nM) (red).Scan rate: 0.1 V s^{-1}. A pair of well-defined redox peaks of TMB was observed, and the reduction peak increased which showed the successful formation of sandwich structure. (E)(F)(G)(H) Plots of amperometric signal *vs.* concentration of target miRNA using DNA tetrahedron capture probe-based miRNA biosensor. The amperometric current increased with the miRNA target concentration. Calibration curves for different concentration of target miRNA were obtained. The range was from 10fM to 1nM, and the fit equation was $Y=0.25-0.04X+0.06X^2$(R^2=0.990) for target miRNA21 (E), range 10fM to 10 nM and fit equation $Y=0.24-0.03X+0.07X^2$ (R^2=0.989) for target miRNA155 (F), range 10fM to 10 nM and fit equation $Y=0.34-0.03X+0.05X^2$ (R^2=0.985) for miRNA196a(G), range 10fM to 10 nM and fit equation $Y=0.78-0.07X+0.07X^2$(R^2=0.990) for target miRNA21(H). Error bar represents standard deviation of over three independent experiments. Here, the potential was set at 0.1 V (*vs.* Ag/AgCl) and the amperometric signal was recorded at 100s.

3.3 Optimization of the conditions for sensor preparation

To enhance the sensitivity, DNA hybridization efficiency is one of the key factors. We thus optimized the hybridization procedure during the preparation of the electrochemical miRNA sensor. Wen *et al*[30] has given a procedure of sandwich structure formation for the normal gold electrode, which we have followed most of the steps for our PC-related miRNA sensor. However, the SPGE could not be immersed in different target solutions at the same time. We thus changed the immersing step by adding the signal probe and target miRNA directly onto the capture probe-modified working electrode. We investigated two different methods of forming the "sandwich" structure. In method 1, the target miRNA and the signal probe was added onto the DNA tetrahedron-modified SPGE step by step. In method 2, the target miRNA21 and the signal probe was mixed beforehand and the mixture was injected onto the DNA tetrahedron-modified SPGE. We observed a significantly higher amperometric current response for 1 nM target of miRNA21 in method 2(Figure 3), which clearly showed the superiority of mixing the target and the signal

probe before injected onto the electrodes. We ascribe great importance to enhance the hybridization efficiency by mixing the target and the signal probe in solution, which could overcome the steric hindrance for hybridization of signal probe. Moreover, by mixing the target and the signal probe in solution, we were able to reduce the incubation time. Thus we determined method 2 for our following experiments.

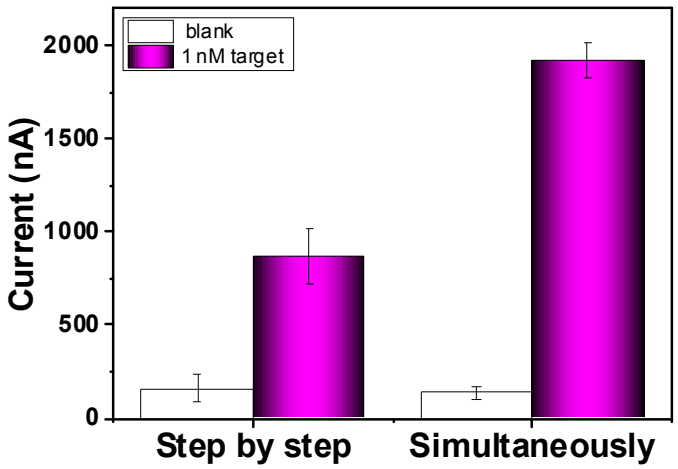

Figure 3. Comparison of different assembling methods for formation of "sandwich" structure. Amperometric current responses for no target (unfilled) and 1 nM target miRNA21(filled) is shown.

3.3 Simultaneous detection of multiple miRNAs in serum sample of pancreatic cancer patients

Serum has the chemical composition similar to plasma but without coagulation proteins or other factors, which make serum the most preferred part of blood used in checking blood groups and diagnosis of diseases[31]. In order to demonstrate the application of our biosensor on analyzing target miRNAs in real clinical samples, we tested the human serum samples from 8 diagnosed pancreatic cancer patients and 8 healthy donors using our SPGE-based sensor.

We tested at least three times for each serum sample by using our SPGE-based sensor. A box plot analysis of current-time responses of miRNA21, miRNA155, miRNA196a

and miRNA210 in serums of healthy donors and pancreatic cancer patients (Figure 4). First we observed small variations of current-time responses (80 to 98 nA) for the four miRNAs from serum samples of healthy donors. Then we found that the expression levels of all four kinds of miRNAs in PC patients' serum were apparently higher than those detected in healthy people, suggesting up regulated expression of miRNA21, miRNA155, miRNA196a and miRNA210 in PC patients. The mean fold changes reflected segregation between PC samples and normal healthy controls. The mean expression level of miRNA21 was increased in serum of PC patients compared with that of healthy donors by a factor of 2.6 ($p<0.05$). It was similar to the reported value of 2~4 fold change in PC samples versus healthy controls[32]. We noticed that the levels of miRNA21 and miRNA155 were spread over a narrow range in both healthy donors and PC patients' samples. The mean current response of miRNA155 was increased by a factor of 2.3 in PC samples compared with healthy controls ($p<0.05$). On the other hand, fold changes of 2.2 and 3.5 were determined for miRNA196a and miRNA210 respectively; while a broader range in PC patient samples in these two cases were observed. The detail data of fold changes of miRNAs expression in PC patients and in healthy donors is listed in Table 3.

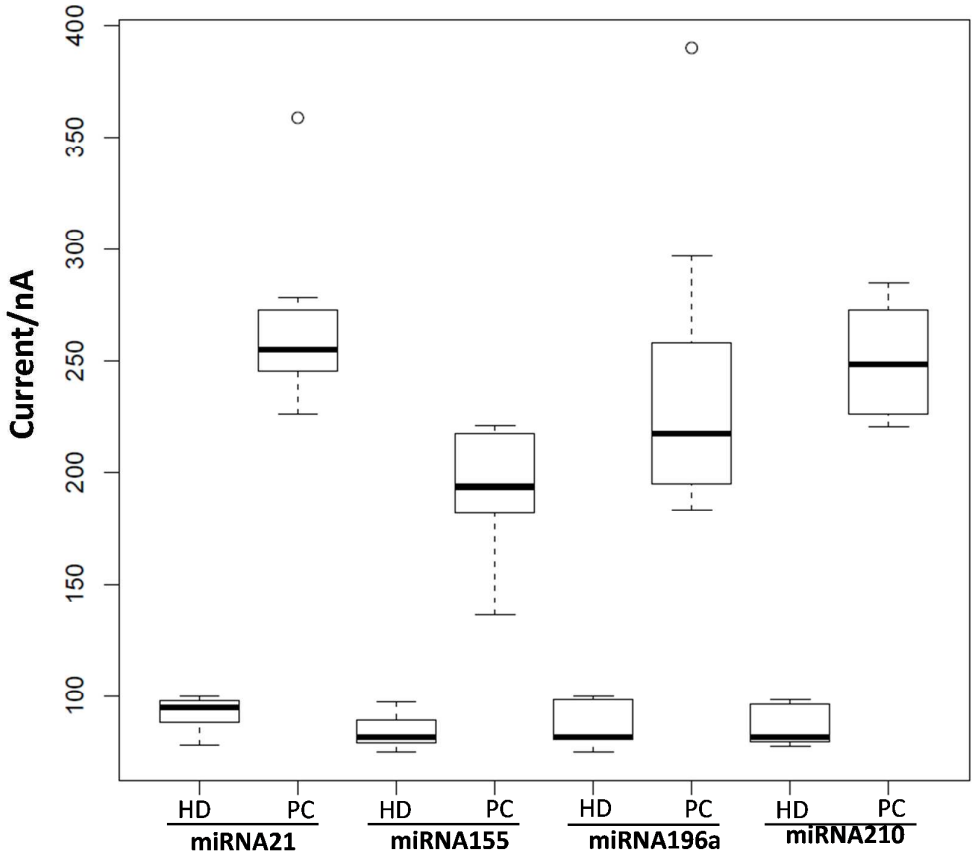

Figure 4. Box plot analysis of current-time responses of miRNA21, miRNA155, miRNA196a and miRNA210 in serum of HD and PC. HD, healthy donor; PC, pancreatic cancer patient.

Table 3 MiRNAs expressed in PC serum sample.

miRNA name	Regulation	Fold change
miRNA21	up	2.6
miRNA155	up	2.3
miRNA196a	up	2.2
miRNA210	up	3.5

4. CONCLUSION

We demonstrated a PCR-free electrochemical biosensor for the detection of multiple target miRNAs. We realized simultaneous detection of four PC related microRNA

(miRNA21, miRNA155, miRNA196a and miRNA210) biomarkers in a cheap and simple way by employing a disposable 16-channel SPGE detection platform to enhance the detection efficiency. In addition, DNA tetrahedral nanostructure was employed to ensure the high sensitivity of the analysis. The detection limit reached to 10 fM with a large response range of at least 7 orders. The disposable 16 channel SPGE was used as the multiplexing detection platform, which was considerably simple and effective. This approach was tested successfully for the evaluation of PC-related miRNAs in human serum samples. Our results showed that combined analyses of the four miRNAs by our sensor could discriminate PC samples from healthy controls with fairly good sensitivity, which greatly satisfy the high standard for POCT.

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Supporting Information

Native PAGE image of DNA tetrahedral nanostructures, the photograph of 16-channel screen-printed gold electrode, and comparison of single

stranded probe and DNA tetrahedron-based miRNA sensor.

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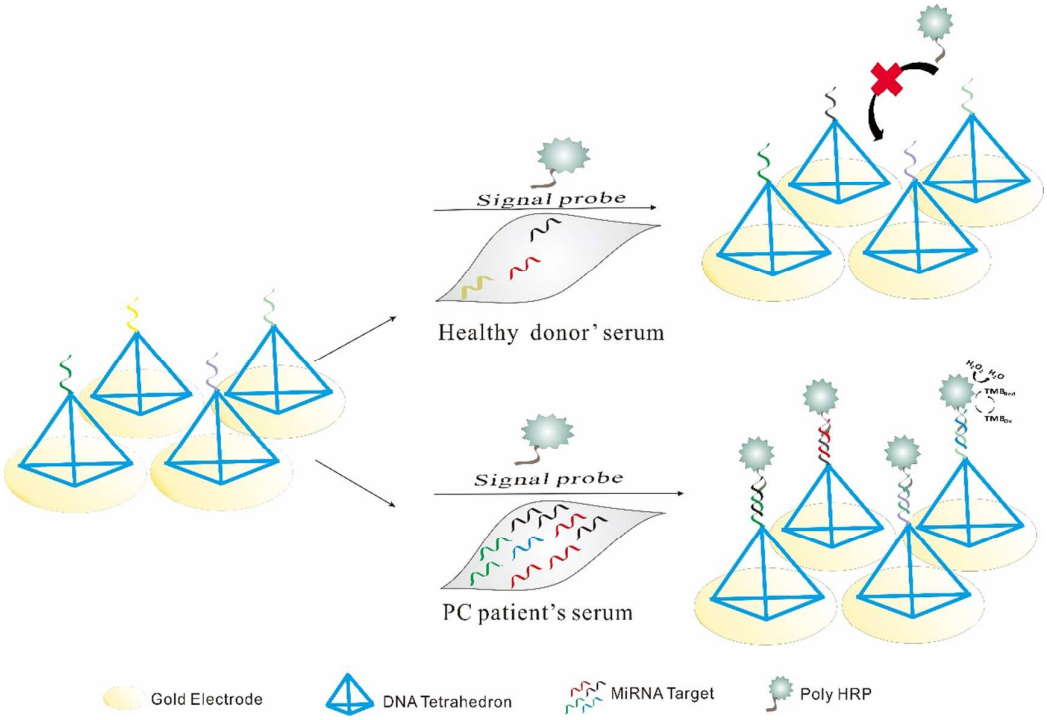
Signal probe
Healthy donor' serum
Signal probe
PC patient's serum
Gold Electrode
DNA Tetrahedron
MiRNA Target
Poly HRP