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# Paper-based electrode assemble for impedimetric detection of miRNA

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## Supplementary Material

### *Sequences of synthetic oligonucleotides:*

tiyol link miRNA-155 DNA probe

5'- SH-ACC CCT ATC ACG ATT AGC ATT AA-3'

miRNA-155 RNA target

5'- UUA AUG CUA AUC GUG AUA GGG GU-3'

miRNA-155 Non-complementary (NC)

5'-UGG CAG UGU CUU AGC UGG UUG U-3'

miRNA-155 Mismatch (MM)

5'-UUA AUG CUA AUC GUC AUA GGG GU-3'

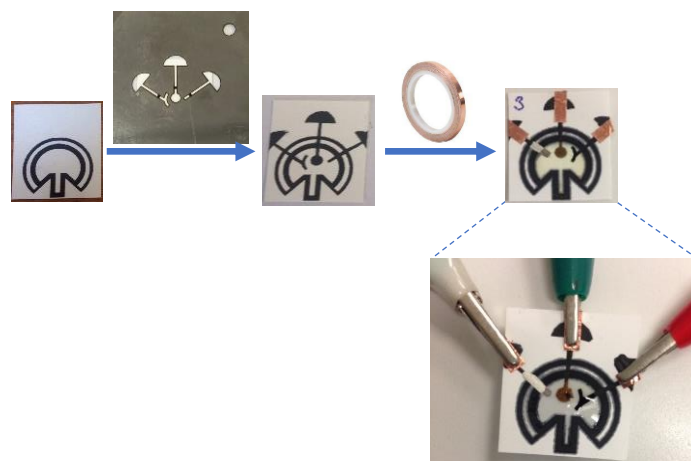
DNA probe and miRNA target stock solutions was prepared in Tris-EDTA buffer solution (pH 8.00) and kept frozen. The diluted solutions were prepared in PBS (pH 7.40).

### *Impedimetric measurements*

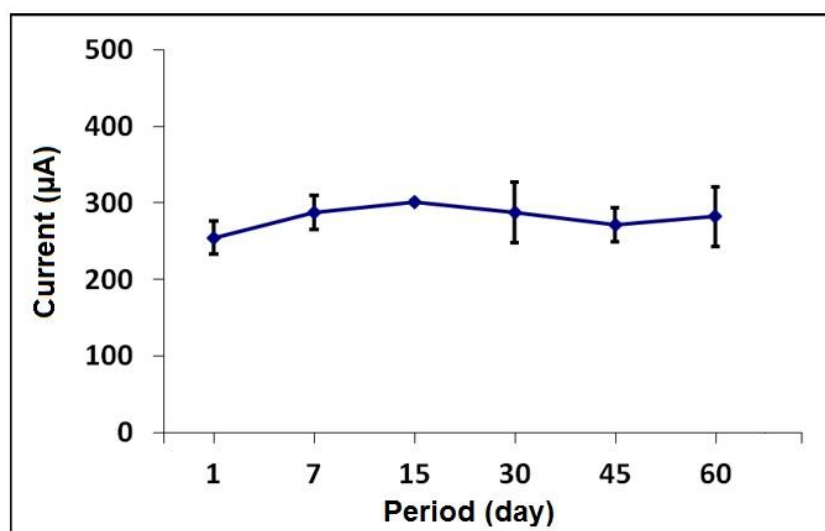
The Randles circuit which consist of the solution resistance ( $R_s$ ), the capacitance ( $Q$ ), the charge transfer resistance ( $R_{ct}$ ) and Warburg impedance ( $W$ ), was used as equivalent circuit model used for fitting of the impedance data. The equivalent circuit model, which was given as inset in all Nyquist diagrams, used for fitting of the impedance data.



**Fig. S1.** The fabrication process of the paper electrode.



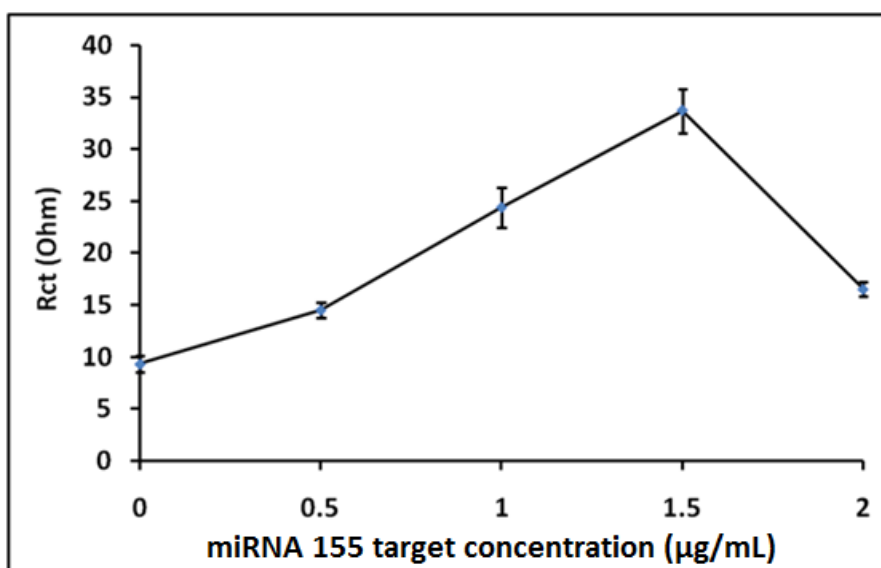
**Fig. S2.** The fabrication process of the paper electrode.



**Fig. S3.** Storage stability of the paper electrodes. The oxidation signal obtained by CV measurement in 50 mM  $K_3[Fe(CN)_6]$  solution that prepared in 0.1 M KCl ( $n=3$ ).

**Table S1.** The average values ( $n=4$ ) of anodic peaks ( $I_a$ ), cathodic peaks ( $I_c$ ), relative anodic charge ( $Q_a$ ), relative cathodic charge ( $Q_c$ ) and calculated surface area were measured by CV technique for PE and AuNP-PWE.

Electrodes	$I_a$ ( $\mu A$ )	$I_c$ ( $\mu A$ )	$Q_a$ (mC)	$Q_c$ (mC)	A ( $cm^2$ )
PE	234.65 ± 12.51 (RSD%, 5.33%)	239.58 ± 15.36 (RSD%, 6.41%)	2.23	3.10	0.0198
AuNP-PE	447.53 ± 45.44 (RSD%, 10.15%)	430.10 ± 28.68 (RSD%, 6.67%)	2.56	4.66	0.0379

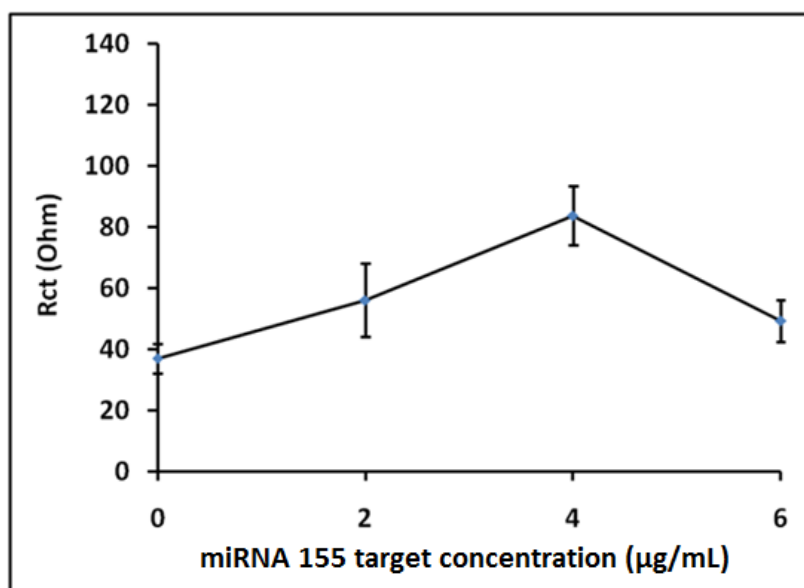


**Fig. S4.** Line graph representing the  $R_{ct}$  values recorded by the hybridization of 0.5  $\mu\text{g/mL}$  miRNA 155 DNA probe and miRNA 155 target with its various concentrations from 0 to 2  $\mu\text{g/mL}$  ( $n=3$ ).

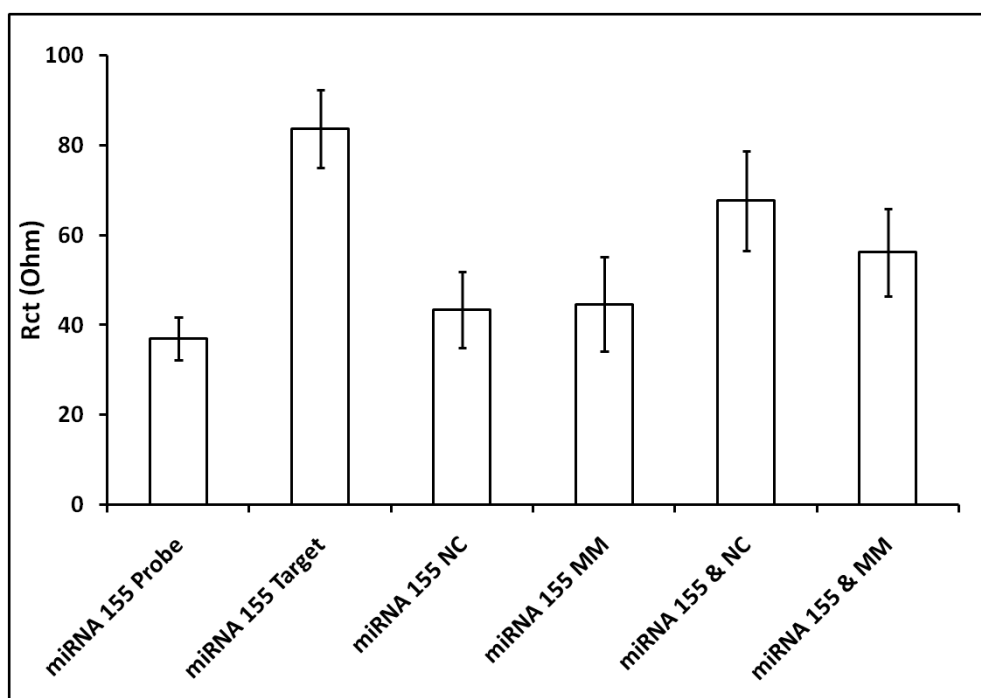
**Table S2.** The  $R_{ct}$  values ( $n=3$ ) recorded by AuNP-PE after hybridization of probe with miRNA-155, NC, MM, or mixture samples and hybridization efficiency (HE %) calculated according to the  $R_{ct}$  values obtained after hybridization.

	Average $R_{ct}$ values ( $n=3$ )	HE %
<b>miRNA 155</b>	$31.63 \pm 7.74$ Ohm (RSD %, 24.46%)	100*
<b>NC</b>	$19.80 \pm 1.15$ Ohm (RSD %, 5,82%)	43
<b>MM</b>	$22.90 \pm 3.61$ Ohm (RSD %,15,74)	58
<b>miRNA 155 : NC mix</b>	$28.35 \pm 5.20$ Ohm (RSD %, 18,35%)	84
<b>miRNA 155 : MM mix</b>	$23.40 \pm 1.76$ Ohm (RSD %, 7,51%)	60

\*Accepted case



**Fig. S5.** Line graph representing the average  $R_{ct}$  values recorded by AuNP-PEs in case of hybridization of probe and miRNA 155 with its various concentrations from 0 to 6  $\mu\text{g/mL}$  in 1:400 diluted FBS medium.



**Fig. S6.** Selectivity of AuNP-PE in FBS medium. Histograms representing the average  $R_{ct}$  values obtained by miRNA 155 probe/AuNP-PE, hybridization between probe and miRNA 155, non-complementary (NC), single-base mismatched strand (MM), and mixture samples with miRNA 155 and NC or MM (n=3).

**Table S3.** The  $R_{ct}$  values (n=3) recorded by AuNP-PE after hybridization of probe with miRNA-155, NC, MM, or mixture samples in 1:400 diluted FBS medium and hybridization efficiency (HE %) calculated according to the  $R_{ct}$  values obtained after hybridization.

	Average $R_{ct}$ values (n=3)	HE %
miRNA 155 target	83.75 ± 8.67 Ohm (RSD %, 10.36%)	100*
NC	43.43 ± 8.45 Ohm (RSD %, 19.46)	13
MM	44.63 ± 10.50 Ohm (RSD %, 23.52%)	16
miRNA 155 : NC mix	67.70 ± 11.10 Ohm (RSD %, 16.40%)	65
miRNA 155 : MM mix	56.20 ± 9.77 Ohm (RSD %, 17.39%)	41

\*Accepted case

**Table S4.** Comparison of different biosensors for determination of miRNA. **Abbreviations:** IL: ionic liquid, CA: covalent agent, PGE: pencil graphite electrode, CNF: carbon nanofiber, PPy: polypyrrole, GO: graphene oxide, MWCNT: multiwalled carbon nanotube, GCE: glassy carbon electrode, HMDE: hanging mercury drop electrode, AuE: gold electrode, CB: carbon black, ITO: indium tin oxide, GONRs: graphene oxide nanoribbons, SPE: screen printed electrode, DPV: differential pulse voltammetry, SWV: square wave voltammetry, ASV: stripping voltammetry, CC: chronocoulometry.

miRNA	Electrode	Method	Indicator	Analysis Time	Real Sample	DL	Reference
miRNA-34a	IL-CA-PGE	DPV	-	35 min	+	0.56 µg/mL (PBS) 0.40 µg/mL (FBS)	[42]
	GO/CA/PGE	EIS	-	2 h	+	1.12 µg/mL (PBS) 0.29 µg/mL (FBS)	[43]
	CNFs/SPE	DPV, EIS	-	50 min	-	10.98 µg/mL	[44]
	PPy-PGE	EIS	-	40 min	+	0.20 µg/mL	[45]
	GO/CA/PGE	DPV	-	3.5 h	-	7.52 µg/mL	[46]
	IL/CA/PGE	EIS	-	2.5 h	+	0.772 µg/mL	[47]
	GO/PGE	CV, EIS	-	1.5 h	+	1.9 µg/mL	[48]
	MWCNT-GCE	DPV	-	1 h	+	1 pM	[49]
miRNA-522	Os(VI)bipy-HMDE	DPV	+	1.5 h	-	2 nM	[50]
miRNA-21 and miRNA-141	MoS <sub>2</sub> /AuNPs/Ag NW paper electrode	SWV	+	17 h	+	0.1 fM	[31]
miRNA-21	MoS <sub>2</sub> -Thi – AuNPs nanocomposite/GCE	SWV	+	18 h	+	0.26 pM	[51]
	AuNPs/ITO	ASV	+	17 h	+	0.12 fM	[52]
	AuNPs/GCE	DPV	+	3.5 h	+	78 aM	[29]

	AuNPs@MoS <sub>2</sub> /GCE	DPV EIS	+	17 h	+	0.78 fM 0.45 fM	[53]
	MWCNTs@GONRs/AuNPs	DPV	+	14 h	+	0.034 fM	[54]
	AuE	DPV	+	14 h	+	0.04 fM	[55]
	AuNPs@MoS <sub>2</sub> (10k)/SPGE AuNPs@/SPGE	CC	+	22 h	+	100 aM 10 fM	[41]
let 7a	Gold disk electrode	DPV	+	25 h	-	1 nM	[56]
miRNA-145	AuE	EIS, SWV	+	18 h	-	0.37 fM	[57]
miRNA-125a	CB/PGE MWCNTs/PGE GO/PGE	EIS	-	2.5 min	+	0.01 nM 1 nM 0.1 nM	[58]
miRNA-155	AuE	SWV	+		+	0.13 fM	[59]
miRNA-122	GO/AuE	DPV	+	29 h	+	1.5 fM	[60]
miRNA-375	Au-SPE	SWV	+	4 h	+	11.7 aM	[61]
miRNA-155	AuNP-PE	EIS	-	15 min	+	33.8 nM (PBS) 93.4 nM (FBS)	This work