Supporting information

Material and methods

1. Reagent and instrument

Blood was drawn into EDTA tubes (1.5 mg/mL) from the antecubital vein of 10 healthy adult volunteers following written consent. The diabetic group for the current study consisted of 10 diabetic patients who did not take any medication recruited from The First Affiliated Hospital of Jinan University. RBCs were separated from whole blood by centrifugation (3000×g, 5 min) and then washed three times with Hanks' buffered saline solution (HBSS without Ca^{2+} or Mg^{2+}, Sigma) containing 0.2% bovine serum albumin (BSA, Sigma). The cells smears were compared to our micrograph bank consisting of hundreds of RBC micrographs and found to be representative of a typical RBC. No spontaneous fibrin fiber networks were found between these RBCs, suggesting that no inflammation is present. Poly-$_L$-lysine (PLL, Mw ≥ 300,000) was obtained from Sigma. Double-distilled water was used in all experiments.

The AFM system was Bio-Scope Catalyst (Bruker, German). The AFM probes were Tap 150Al-G Silicon probes, which purchased from Budget Sensors Company (Bulgaria).

2. Endothelial cells culture

Human umbilical vein endothelial cells (HUVECs) were obtained from Lonza (UK). HUVECs were cultured in flasks at 37 °C in a CO_2 (5%) incubator. Once the cells reached 80% confluence, they were sub-cultured in 35 mm petri dishes (Greiner) pre-coated with gelatin from fish skin (Sigma) and grown to confluence for the adhesion assay.

3. Quartz crystal microbalance with dissipation measurement

Quartz-crystal microbalance with dissipation instrument (QCM-D, Biolin Scientific/Q-Sense, KSV) was used to record changes in energy dissipation (ΔD) and resonant frequency (Δf) as a function of time. All measurements were done with optically polished gold deposited quartz crystals (AT-cut, 14 mm disks) with a fundamental resonant frequency of 5 MHz (QSX 301). The sensors were prepared as follows: QCM-D sensors were cleaned using a UV/ozone cleaner for 10 min. The sensors were then dipped in a mixture of 10 mL of pure water, 2 mL of ammonium hydroxide, and 2 mL of hydrogen peroxide at 75 °C for 5 min. Then the sensors were rinsed with pure water and sonicated for 2 min. Later, cleaned sensors were dried using a gentle stream of nitrogen. The cleaned sensors were then dipped in 5 mM 3-mercaptopropionic acid (3-MPA) overnight. 3-MPA-modified sensors were

rinsed in ethanol and pure water thoroughly and incubated with poly-$_L$-lysine for 1 h. Then the sensors were rinsed with water, dried, and used for culturing the cells. The sensor was then mounted in a flow module (Q-sense), and the assay buffer at 37 °C was injected. Once the assay buffer was exited through the outlet of the flow module, the flow stopped and the changes in frequency (Δf) and dissipation (ΔD) were recorded simultaneously. After obtaining the stable baselines, flow was resumed by changing the tube to 3×10^5 cells/mL ECs solutions that were pre-warmed to 37 °C. Flow changed to 2×10^5 cells/mL RBCs when the frequency and dissipation changes in a constant. Changes in frequency and dissipation were recorded simultaneously at 37 °C till to changes in a constant.

4. Electrochemistry measurement

Electrochemical measurements, including differential pulse voltammetry (DPV) analysis and cyclic voltammograms (CV), were performed with a CHI 660 D electrochemical analyzer (CHI, USA). The three-electrode system was composed of a platinum wire as an auxiliary electrode, saturated calomel electrode as a reference electrode and GCE electrode as a working electrode. The glassy carbon electrode (GCE) was carefully polished by 0.3 mm and 0.05 mm alumina. Then, it was sonicated successively with distilled water and ethanol for 5 min until a mirror-like surface was obtained. According to the previous work [1], 10 mL of 2 mg mL^{-1} PANI-NF suspension, dispersed in double-distilled water with the aid of ultrasonication, was dropped on the pretreated GCE surface and dried in a desiccator to obtain the GCE/PANI-NF, Then cultured with 3×10^5 cells/mL HUVECs for 2 h, after rinsed by PBS, further exposed to 2×10^5 cells/mL normal RBCs or type 2 diabetic RBCs at 37 °C for 2 h. Binding for electrostatic interaction thus yield the GCE/PANI-NF/HUVECs/RBC or GCE/PANI-NF/HUVECs/II-RBC. The obtained biosensors were used to the electrochemical measurements.

Results

At last, as we all know, electrochemistry biosensor has been applied in many cells analysis.[2, 3] Herein, we designed an electrochemistry biosensor to confirm our above findings that diabetic RBCs easy to adhere to ECs. Fig S1 describes the fabrication of the biosensor. After the electropolymerization of polyaniline (PANI-NF), the cells were modified on glassy carbon electrode (GCE) layer by layer. It can be seen from the cyclic voltammetric and differential pulse voltammetry responses in Fig. S2A and B, the peak current responses separated to a large extent after the immobilization of HUVECs, revealing that the adhered cells efficiently blocked the electron transfers of ferri-/ferrocyanide probe. Moreover, the negative effect of type 2 diabetic RBCs (II-RBC) on the electrode transfer was more obvious as the diabetic RBCs-bound electrode presented larger electron transfers resistance than normal RBCs (RBC) did, which demonstrate that it is easier for diabetic RBCs to be adhere to

HUVECs.

Reference

1. S. Zhang, L. Zhang, X. Zhang, P. Yang and J. Cai, *Analyst*, 2014, **139**, 3629-3635.
2. C. Hu, D.-P. Yang, Z. Wang, P. Huang, X. Wang, D. Chen, D. Cui, M. Yang and N. Jia, *Biosensors and Bioelectronics*, 2013, **41**, 656-662.
3. C. Hu, D.-P. Yang, Z. Wang, L. Yu, J. Zhang and N. Jia, *Analytical chemistry*, 2013, **85**, 5200-5206.

Figure caption

Fig. S1 Fabrication the RBCs adhesion modified biosenor.

Fig. S2 (A) The cyclic voltammetric and (B) differential pulse voltammetry responses of the electrochemistry biosensor.

Table S1 The selectivity of the QCM-D biosensor

Table S2 The information of the 10 healthy RBCs (H1 to H10) and diabetic RBCs sample (D1 to D10), including the obtained Young's modulus evaluated by AFM, the Δf and ΔD values when adhesion to HUVECs in stage III.

Table S3 Clinical data erythrocyte parameters of all patients and healthy volunteers

Figure

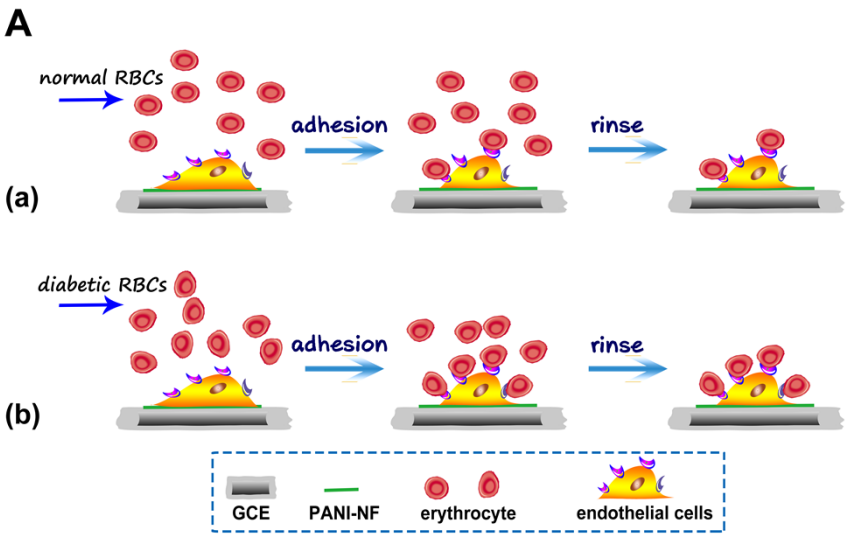

Fig. S1

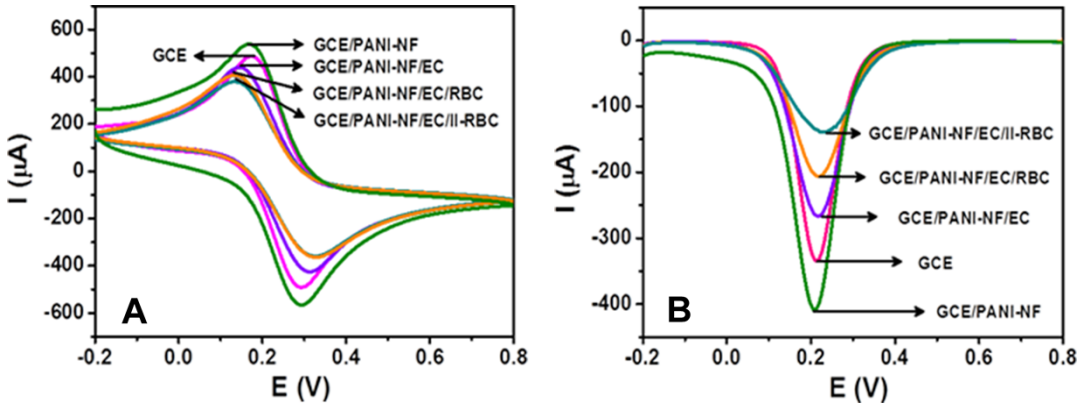

Fig. S2

solution	stage I		stage II		stage III	
	Δf (Hz)	ΔD ($\times 10^{-5}$)	Δf (Hz)	ΔD ($\times 10^{-5}$)	Δf (Hz)	ΔD($\times 10^{-5}$)
PBS	0 ± 23	0 ± 0.6	—	—	—	—
HUVEC	0 ± 21	0 ± 0.6	245 ± 50	5.2 ± 1.8	—	—
normal RBC	0 ± 24	0 ± 0.6	240 ± 48	5.1 ± 1.6	-393±43	16 ± 3.7
diabetic RBC	0 ± 20	0 ± 0.5	243 ± 50	5.2 ± 1.4	-568±58	7.9±0.46
white blood cell	0 ± 23	0 ± 0.7	245 ± 52	5.0 ± 1.9	-298±43	2.0 ± 3.7
blood platelet	0 ± 25	0 ± 0.6	251 ± 50	5.2 ± 1.8	-270±43	1.6 ± 2.5

Table S1

Number	**age**	**Young's modulus (Kpa)**	**Δf (Hz)**	**ΔD**($\times 10^{-5}$)
H1	24	0.20	50.67	10.24
H2	27	0.17	51.32	10.49
H3	35	0.24	51.24	13.18
H4	48	0.41	56.23	10.37
H5	57	0.48	53.88	14.62
H6	59	0.33	54.25	12.67
H7	68	0.43	53.54	13.29
H8	54	0.32	54.59	10.99
H9	59	0.29	55.28	10.12
H10	58	0.27	55.47	11.52
average value	—	**0.33**	**53.65**	**11.74**
D1	35	20.12	300.23	3.32
D2	47	24.37	318.43	2.78
D3	39	23.79	307.75	3.19
D4	53	24.87	314.79	3.08
D5	56	25.32	325.39	2.78
D6	59	25.19	315.23	2.98
D7	66	26.31	303.90	1.53
D8	64	25.47	319.99	2.58
D9	68	25.38	315.89	2.67
D10	72	27.52	328.68	2.76
average value	—	**24.83**	**315.03**	**2.77**

Table S2

Sample	Age	Sex	RBC ($\times 10^{12}$)	Hb (g/L)	MCV (fL)	MCH (pg/L)	MCHC (g/L)
H1	24	female	5.0	120	82	33	354.4
H2	27	male	4.4	133	84	31	341.2
H3	35	female	4.2	121	94	32	330.1
H4	48	female	4.7	146	93	34	326.7
H5	57	male	4.7	138	85	28	341.4
H6	59	male	4.9	127	88	29	332.5
H7	68	female	4.9	141	89	32	328.0
H8	54	male	4.5	154	90	21	343.3
H9	59	female	4.2	119	93	32	352.2
H10	58	male	3.9	144	81	29	332.2
D1	35	male	3.0	106	82	27	327.9
D2	47	female	2.7	102	81	31	342.5
D3	39	female	3.2	106	88	32	345.5
D4	53	male	2.1	100	89	30	327.9
D5	56	female	2.0	99	83	28	352.3
D6	59	male	2.2	100	90	27	346.1
D7	66	female	1.9	103	91	32	328.9
D8	64	male	2.5	98	92	34	354.2
D9	68	female	2.2	91	88	29	341.2
D10	72	male	1.7	86	82	31	347.5

Table S3