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Short communication

Paper-based analytical devices for electrochemical study of the breathing process of red blood cells

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ABSTRACT

Herein we utilized the filter paper to physically trap red blood cells (RBC) to observe the breathing process of red blood cells based on the permeability of the filter paper. By integrating double-sided conductive carbon tape as the working electrodes, the device could be applied to monitor electrochemical responses of RBC for up to hundreds of minutes. The differential pulse voltammetry (DPV) peak currents increased under oxygen while decreased under nitrogen, indicating that RBC could take in and release oxygen. Further studies demonstrated that the RBC suspension could more effectively take in oxygen than the solution of hemoglobin and the supernatant of RBC, suggesting the natural advantage of RBC on oxygen transportation. This study implied that simple paper-based analytical devices might be effectively applied in the study of gas-participating reactions and biochemical detections.

1. Introduction

Past years have witnessed numerous applications of cell-based biosensors in environmental monitoring [1–3], drug discovery [4], food safety [5] and biomedical assays [6–9]. Fluorescent [10], electrochemical [11], and colorimetric [12] approaches have been coupled for detection in cell-based biosensors. For cell-based electrochemical biosensors, it is critical for cells to attach on the surface of the working electrode to promise that most of target molecules from the cells could reach the working electrode. Benefited from their robust cell wall, bacterial cells could be well immobilized on the working electrode for applications. For biological cells without cell wall, a biorecognition interface is normally required on the working electrode based on modification [13–15]. For example, Ju's research group modified single-walled carbon nanotubes or nanorods with argininegly cine–aspartic acid–serine tetrapeptide to effectively capture cells through integrin receptors on cell surface for electrochemical cell sensing [16,17]. Overall, it is still difficult for electrochemical biosensor of mammalian cells because of their thinner and delicate membrane as well as physiological environments.

Paper-based analytical devices (PAD) have brought more opportunities for development of portable analytical devices and biosensors since Whitesides' research group introduced the technique of photolithography to pattern the filter paper [18,19]. The filter paper could be an ideal substrate for construction of portable and inexpensive analytical instruments because of its low-cost, absorbency and flex, porous structure [20–22]. The unique characteristics of filter paper made it possible for development of whole cell biosensors for portable semi-quantification of bacterial quorum signaling molecules [23]. In our previous reports, indium-tin-oxide (ITO) glass modified with gold nanorods was applied to fabricate disposable working electrodes in the paper analytical devices for cell analysis based on sensitive electrochemical detection of guanine and hydrogen dioxide [24,25]. It was found that simple and inexpensive paper-based analytical devices provided a convenient and effective solution for cell-based electrochemical biosensors because of following reasons: Firstly, biological cells could be well trapped by the fiber matrix of paper so that the immobilization of cells is convenient. Secondly, the buffer solution could diffuse on the paper orderly so that cells could be easily maintained in good conditions. Thirdly, the porous structure of paper allows outside materials, such as stimulants, to reach biological cells without disturbance so that responses of cells could be well studied.

Continuing our previous investigations, herein we applied paper-based electroanalytical devices to investigate the breathing process of red blood cells (RBC) based on permeability of filter

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paper to oxygen and nitrogen. Our study demonstrated that electrochemical responses of the RBC suspension could be significantly influenced by oxygen or nitrogen. Electrochemical responses of the RBC suspension, the RBC supernatant and the hemoglobin solution were compared under oxygen and nitrogen for several cycles. Our study implied that paper-based analytical devices could be a convenient platform for the study of gas-participating biosensing.

2. Materials and methods

2.1. Reagents and materials

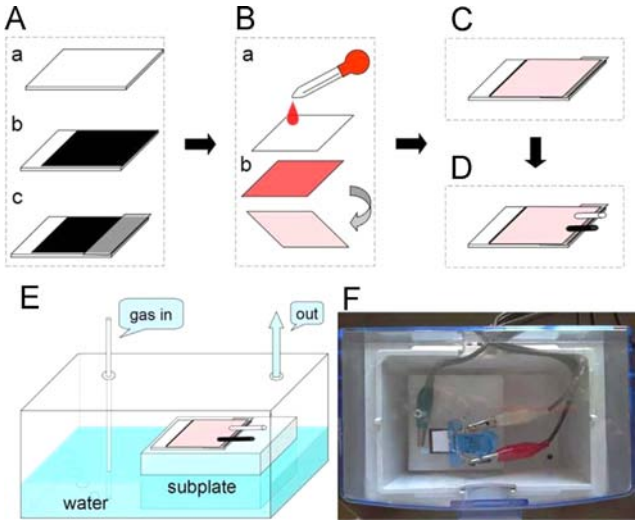
The chemicals ($Na_2HPO_4 \cdot 12H_2O$, KH_2PO_4, sucrose, pure oxygen and nitrogen) were of analytical grade. The solutions were prepared with the double distilled water collected from SZ-93A automatic double water distiller (Shanghai Yarong Biochemical Instruments, Shanghai, China). The qualitative filter paper (Whatman No.1) was obtained from Whatman International Ltd. (Maidstone, United Kingdom). The indium tin oxide (ITO) conductive glass (355.6 mm wide, 406.4 mm long and 1.1 mm thick, 10 Ω) was purchased from Nanbo Display Technology Co.Ltd. (Shenzhen, Guangdong, China). The conductive double-sided carbon adhesive tape (25 mm wide, 0.16 mm thick and 20 m long) was purchased from SPI Supplies (WestChester, PA, USA). The blood samples were collected from healthy volunteers. Oxygen and nitrogen with high purity were purchased from a local gas company.

2.2. Sample preparation

Fresh whole blood was collected from healthy volunteers. The fresh blood was mixed with the running buffer (10 mM Na_2HPO_4, 10 mM KH_2PO_4 and 250 mM $C_{12}H_{22}O_{10}$, pH = 6.8) with the volume ratio of 1:2 and then centrifuged at 1500 rpm for 10 min at 4 °C. The RBC were collected from the sediment after centrifuge and then washed and suspended in the running buffer for experiments. The RBC supernatant (the upper layer of the RBC suspension without any RBC) was collected from the centrifuged RBC suspension. The hemoglobin solution was prepared with the concentration of 3 mg/mL according to the same amount of hemoglobin in the RBC suspension.

2.3. Electrochemical detection

ITO glass with 2.5 cm long and 2.0 cm wide was successively washed with acetone, alcohol and distilled water in ultrasonic for 3 to 5 min before dried for use. As shown in Scheme 1, Carbon tape with the size of 2.0 cm wide and 2.0 cm long was attached on the ITO glass. A piece of transparent tape with 0.50 cm wide adhered on one side of carbon tape to provide area for placing counter electrode and reference electrode. The sample solution (the RBC suspension, RBC supernatant or the hemoglobin solution) with the volume of 100 μL was dropped on a piece of filter paper with 1.8 cm wide and 1.8 cm long. The filter paper with the sample solution was then turned upside down and put on the carbon tape for electrochemical detection. The paper-based analytical device was then put into a box with water for stable humidity. Meanwhile, oxygen or nitrogen could flow into the box for examination of oxygen carrying capability of the sample solution. Differential pulse voltammetry (DPV) was applied for all experiments with parameters as follows: potential range, −0.9 V to −0.1 V; potential increase, 0.004 V; pulse amplitude, 0.05 V; pulse width, 0.20 s, sample width, 0.067 s; pulse period, 0.50 s; and quite time, 2.0 s.



Scheme 1. Schematic of the paper-based analytical device for the study of breathing process of RBC. A) On the conductive surface of ITO glass (2.5 cm long and 2.0 cm wide) (a), a piece of double-sided conductive carbon tape (2.0 cm long and 2.0 wide) (b) was attached and then a piece of transparent tape was adhered on one side of the carbon tape (c) to provide the insulated area. B) On the filter paper (1.8 cm long and 1.8 cm wide) (a) the RBC suspension with the volume of 100 μL was dropped (b). The filter paper with RBC suspension was turned upside-down (c). C) The filter paper with RBC suspension was then put on the surface of the carbon tape supported on ITO glass. D) An Ag/AgCl wire and a platinum wire fixed by a clasp were then put on the filter paper at the insulated side of the carbon tape to form the paper-based analytical device. E) The detection system was put into a small box with two holes which allow oxygen or nitrogen to flow through. F) The image of the system.

3. Results and discussion

Scheme 1 illustrates the paper-based analytical device for electrochemical studies of the breathing process of RBC. The working electrode was fabricated with double sided conductive carbon tape supported by ITO glass for several-hour electrochemical detection [26]. The filter paper with the sample solution at the volume of 100 μL was applied on the carbon tape electrode. It needs to emphasize that the sample solution was in between the filter paper and the carbon tape electrode so that RBC could be physically trapped by the filter paper on the surface of the working electrode. The paper-based electroanalytical device was put into a box in which oxygen or nitrogen could flow through. During experiments, the humidity inside the box was maintained with water. Because of permeability of the filter paper, oxygen or nitrogen could pass through porous filter paper and reach RBC [27,28]. DPV was then applied to observe the breathing process of RBC based on electrochemical responses [29].

Fig. 1A shows electrochemical responses of the RBC suspension and the influence of filtration with a 0.22 μm membrane. It could be found that no signals could be found for the filtrated RBC suspension. By comparison, there was a DPV peak at the potential of −0.52 V for the normal RBC suspension. When nitrogen was applied in the box (Fig. 1B), the DPV peak currents decreased with the time going on. The signals increased with the increase time under oxygen (Fig. 1C). The DPV peak heights at different time points were then collected to show their trend for three cycles of nitrogen and oxygen (Fig. 1D). It could be interestingly observed that the highest DPV peak current appeared in the first cycle and it decreased gradually for following cycles. It needs to emphasize that the decrease of the highest responses after each cycle might be because of voltammetry involving more or less complicated pathways, such as the reported influences of radicals generated by pure oxygen [30]. As shown in Fig. 1E, a DPV peak at the potential of ~0.55 V could be observed for the RBC supernatant. Interestingly, no signal could be found after the

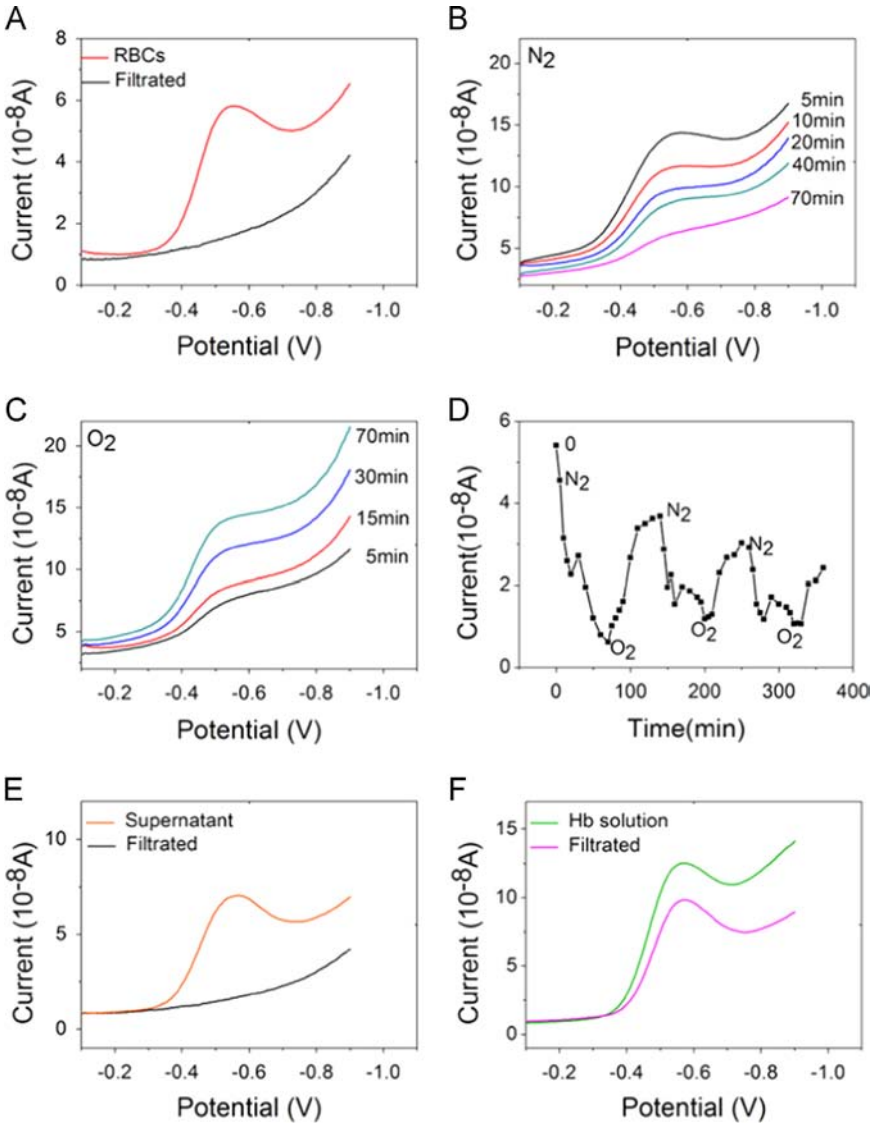


Fig. 1. The DPV curves of the normal and filtrated RBC suspension with 0.22 μm membrane (A). The dependence of DPV curves under nitrogen (B) or oxygen via time (C). The dependence of DPV peak currents on time under nitrogen or oxygen (D). Electrochemical responses of the RBC supernatant and the influence of filtration with 0.22 μm membrane (E). Electrochemical responses of the hemoglobin solution and the influence of filtration with 0.22 μm membrane (F).

supernatant was filtered with a 0.22 μm membrane. Fig. 1F shows that there was a DPV peak at the potential of ~0.55 V for the hemoglobin solution and filtration could only decrease the peak height.

Since the hemoglobin solution had electrochemical responses in our design, the DPV signals could be attributed to electrochemical reduction of oxygen combined with hemoglobin. It has been reported that oxyhemoglobin (oxygenated hemoglobin) could be electrochemically reduced at the bare glassy carbon electrode [31]. Their results revealed that the potentials and the peak current of the electrochemical reduction were pH-dependent because it was proposed that H^+ ions took part in the electrochemical reduction of oxyhemoglobin. Based on the pH value of 6.8 for our running buffer, the DPV peak current and potential agreed well with the reported trend [31]. Therefore, it is mostly possible that the electrochemical responses of the hemoglobin solution originated from oxyhemoglobin. Because of the same DPV potential, the electrochemical signals of the RBC supernatant could also be attributed to reduction of oxyhemoglobin-like materials. Although electrochemical reduction of solved oxygen has been reported before [32,33], no peaks could be found in our study when the PBS solution was applied without RBC. Since the filtrated RBC supernatant had no signals, it is believed that in the RBC supernatant oxyhemoglobin might combine together or with other molecules. For the same reason, the electrochemical responses of the RBC suspension could also be explained. The influence of oxygen and nitrogen on those responses could provide the supporting evidence.

Fig. 2 illustrates the influence of nitrogen and oxygen on electrochemical responses of the RBC suspension, the RBC supernatant and the hemoglobin solution for two cycles. For better comparison, all the DPV peak currents were normalized. It could be observed that after the first cycle electrochemical responses could reach 84% for the RBC suspension while 44% for the RBC supernatant and 29% for the hemoglobin solution. In addition, in the first cycle of oxygen, the DPV peak currents only increased in the first 10 min for the RBC supernatant and the hemoglobin solution. After the second cycle, the electrochemical responses decreased to be 32%, 22% and 15%, respectively, for the RBC suspension, the RBC supernatant and the hemoglobin solution. Such results indicated that the RBC suspension is more suitable for transportation of oxygen. The reason might be that the molecules of hemoglobin inside RBC are under better physiological environments.

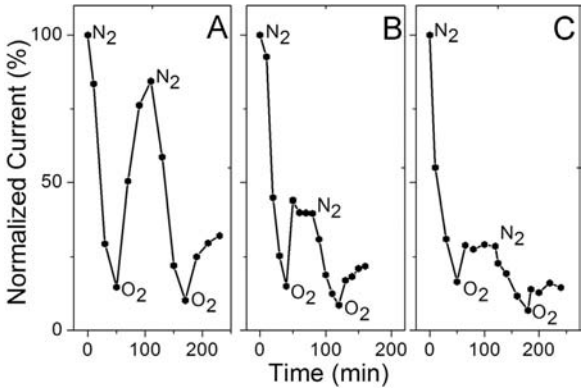


Fig. 2. The dependence of normalized DPV peak currents of the RBC suspension (A), the RBC supernatant (B) and the hemoglobin solution (C) on time under nitrogen and oxygen.

4. Conclusion

Overall, our approach provides a simple and convenient solution for cell-based electrochemical biosensors by using filter paper to physically trap RBC and allow the oxygen to reach RBC. Such design makes it possible to study the breathing process of the RBC suspension, the RBC supernatant and the hemoglobin solution. Their electrochemical responses could possibly be ascribed to reduction of oxyhemoglobin although more investigation is necessary on the RBC supernatant. Our experimental results indicated that the RBC suspension could breathe oxygen more efficiently, indicating their natural priority on transporting oxygen. Our current and previous investigations demonstrated that paper-based analytical devices could be an effective and facile platform for the electrochemical study of biological cells under stimulations with the forms of solution or gas.

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