ELECTROANALYTICAL

Prototypical Nonelectrochemical Method for Surface Regeneration of an Integrated Electrode in a PDMS Microfluidic Chip

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Abstract: A prototypical method for surface regeneration of an integrated electrode in a microfluidic chip is demonstrated. A platinum wire as working electrode was mounted in a polydimethylsiloxane chip vertical to the chip through the channel. The regeneration of the electrode was easily achieved by drawing the platinum wire out for 5 mm, because the area exposed to the channel or the stream would be altered. With continuous motion, the wire electrode can maintain a fresh surface just like a dropping mercury electrode. The current–time curve and open circuit potential (OCP) of dopamine solution show the performance of this prototypical system.

Received 18 November 2008; accepted 19 May 2009.

This research was supported by funding from the National Basic Research Program (973 Program) of China (2007CB714505) and the Ministry of Education of China (No. 2008030102).

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INTRODUCTION

Electrochemical (EC) detection integrated in microfluidic chips has developed rapidly in recent years (Wang 2005; Mora et al. 2008; Vandaveer et al. 2004; Xu, Wang, and Chen 2007). Although laser-induced fluorescence (LIF) is the most prevalent detection method for microfluidic devices and miniature LIF configuration has been developed (Yao et al. 2006, 2005; Ren et al. 2007, 2009), EC detection still has inherent advantages, such as miniaturized detectors and control instruments. There are other advantages of EC, including high sensitivity (approaching that of LIF), no need for derivatization, and less cost. Until now, there have been three methods to integrate an electrode with a microfluidic chip:

1. The electrode (band) is fabricated in the chip by standard MEMS (micro-electro-mechanical systems) processes (Woolley 1998; Martin 2000; Yan 2003; Joo 2007; Wang 1999). It is beneficial that the microfabrication of the electrode is compatible with the production of microchip.
2. The electrode (wire, strip) is mounted perpendicularly to the flow direction at the channel outlet or inserted into the channel (Fanguy 2002; Zeng 2002; Xu 2004; Qiu 2007; Martin 2002). In this configuration, the electrode is less expensive and could be replaced more easily.
3. The electrode (wire) is put into the electrode channel through the separate channel transversely perpendicular to the latter (Garcia and Henry 2003; Liu, Vickers, and Henry 2004). This configuration provides greater collection efficiency for the analyte. As far as we know, the lowest detection limit of dopamine (600 pM) was achieved by this configuration (Vickers, Caulum, and Henry 2006).

Electrochemical (EC) detection suffers from the fact that some materials are absorbed on the electrode surface, which would cause a loss of sensitivity (Manica, Mitsumori, and Ewing 2003). So, regenerating the electrodes and regaining a new surface is crucial in electrochemical detection. For integrated microelectrodes, tens of cyclic voltammograms (CVs) were usually carried out to regenerate the surface. It is simple to accomplish; however, the regeneration could not be done simultaneously with electrochemical detection. Another method, the so-called pulsed electrochemical detection (PED), is also developed to maintain surface condition (LaCourse and Modi 2005) (Garcia and Henry 2005). The
PED method employs three distinct potentials. The first potential is for cleaning, the second potential is for regeneration, and the third one is for detection. It has been successfully applied to detection of carbohydrates, thiols, and amino acids; however, this method is much more complicated to manipulate and optimize. Both an expensive instrument and high skill level are therefore needed (Garcia and Henry 2005). The mentioned techniques, CV and PED, are electrochemical treatments, so the regeneration of electrode inevitably disturbs the electrochemical detection and is inherently inefficient.

In the present article, a nonelectrochemical manner to regenerate the electrode surface is described. The unique feature of this configuration is that the solid electrode could change its position in channel. The method seems like a hybrid of a solid electrode and a dropping mercury electrode (DME). There are three advantages to this methodology: it is (1) independent of electrochemical detection, (2) able to regain a brand-new surface, and (3) easy to implement. This method was tested with current–time curve and open circuit potential (OCP). The experiment proved that the surface can be regenerated effectively.

EXPERIMENTAL

Reagents and Chemicals

All reagents used were of analytical grade, and Milli-Q water (18.2 MΩ; Millipore, MA) was used throughout the experiment. Fluorescein isothiocyanate (FITC) (Sigma, USA; 1 mM) was prepared by dissolving 0.038 g FITC in 100 ml water. Potassium ferrocyanide solution (0.5 mM) was prepared in 100 ml KCl (0.1 M) solution. Dopamine (Sigma, USA) solution (2 mM and 200 μM) was prepared in 50 ml KCl (0.1 M) solution. Microwires (100 μm) made of 99.95% platinum were purchased from Alfa Aesar (MA, USA).

Fabrication of Microfluidic Chip

Photolithographic and wet chemical etching techniques were used for fabricating positive relief patterns onto a 1.7-mm-thick 63-× 63-mm glass plate with chromium and photoresist coating (Shaoguang Microelectronics Corp., Changsha, China) (Yao et al. 2004). The glass with positive relief was used as a master. The PDMS prepolymer (five monomers and one curing agent) with excessive Si-H group was cast on this master and cured thermally (75°C). After that, the PDMS plate was peeled from
the master with channels. The reservoirs and cell were punched and trimmed. This channel plate and another blank plate (20 monomers and one curing agent) with excessive vinyl group were bonded together thermally to form an irreversible seal. The channel is 250 μm wide, 20 μm deep, and 2.1 mm long. The chip is 5 mm thick (Fig. 1a). The sampling channel for electrophoresis is simplified and omitted.

**Figure 1.** Schematic diagram of surface regenerable electrode in a PDMS chip. 1 electrochemical station; 2 Inlet; 3 PDMS microchip; 4 Working Electrode (WE); 5 Counter electrode (CE); 6 Reference electrode (RE); 7 Cell; 8 the copper wire (1mm) connected with Pt wire and electrochemical station; 9 stretched glass capillary. (a) The channel is 20 μm deep, 2.1 mm long and 250 μm wide. WE is a 100-μm Pt wire, at the end of channel and piercing it. The distance form WE to WR is about 2 mm. RE is a saturated calomel reference electrode. (b) The Pt wire was inserted in 9 (stretched glass capillary). (c) Fluorescent image of 1 mM FITC as it passes the working electrode. Leakage of FITC was not observed around the Pt wire in experiment. (d) Microscope image of work electrode pierced in the channel. The scale bar represents 100 μm.

Electrode Integration

A piece of glass capillary was pulled by an alcohol blast burner to make tip of glass around 300 μm wide. A platinum wire (100 μm) was placed in
the capillary (Fig. 1b). The capillary was aligned to the channel and was pierced through the chip. Then the capillary was drawn off while the platinum wire was left in the chip (Figs. 1c and d). After the work electrode was ready in place, a copper wire (1 mm) was soldered to the end of work electrode and held in position with epoxy glue. The copper wire was not fixed but curved to certain angle to adjust the position of the working electrode.

The chip was designed to allow the working electrode to regenerate. To determine if fluid leaked from the work electrode, 1 mM FITC was pumped into the channel to visualize it when it passes the work electrode. The fluorescent image was acquired by a color CCD camera (DH-HV1302UC, Daheng Image, Beijing, China) coupled with a television zoom lens (MLM3X-MP, Computar).

**Electrochemical Detection**

All reagents were slightly pumped into the channel. Electrochemical detection of dopamine and K$_4$Fe(CN)$_6$ were conducted by an electrochemical station (CH660A, CH Instruments). The scan range of the cyclic voltammetry was from $-0.2$ to $0.7$ V, and scan rate was variable in certain situations. Initial potential of amperometric was 0.45 V. The saturated calomel reference electrode and one Pt rod (1 mm diameter) as a counterelectrode were put into the cell downstream.

**RESULTS AND DISCUSSION**

The regeneration of electrode surface is essential to electrochemical detection, because the accumulation of absorbed oxide would foul the electrode and lead to the loss of sensitivity. For the integrated electrode, electrochemical treatment to regenerate the surface prevailed. However, tens of CVs hardly get rid of the absorbed oxide and do not retain the surface as brand-new (Manica, Mitsumori, and Ewing 2003); PED needs optimization of the parameters and complicated instrumentation (LaCourse and Modi 2005).

The system described here is a prototypical method, a nonelectrochemical treatment to regenerate the surface of the integrated electrode. The Pt wire as work electrode was pierced into the PDMS channel. The elasticity and hydrophobicity of PDMS prevented leakage from channel. The surface of the electrode passed the channel consecutively, analogous to the dropping mercury electrode (DME) (Fig. 2). The surface of mercury drop is always changing and maintained fresh.
We simply draw the electrode out by 5 mm to achieve the same result as the DME. The schedule to regenerate the electrode is simple and is described here. Before analysis, the surface of electrode is new and fresh (Fig. 2a). By hydrodynamic or electrokinetic driving, the analyte would reach to the electrode. (b) When analyte reach the electrode, the electrochemical reaction occurs on the electrode surface. (c) The accumulation of oxides on the surface fouls the electrode. Pentagram represents the oxide. Arrow indicates the direction of electrode movement. (d) Draw the Pt wire by a little distance and thus the electrode surface is regenerated. (e)–(h) illustrates continues motion of electrode. The curve of copper wire is changed manually to drive the motion of electrode.

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The surfaces of Pt wire and PDMS are hydrophobic, and the contact angle of water is 114° and 98° respectively (Fei, Chiu, and Hong 2008; Huang et al. 2006) (Fig. 3a). (If the inner wall was processed to be hydrophilic, the fluid tended to flow through the channel rather than leak from the gap.) The Young’s module of PDMS is about 750 KPa, so the elastic
force was exerted against the Pt wire when it was inserted (Fig. 3b). Because the elastic force depended on deformation, it was relatively small, as the deformation caused by a Pt wire was small, but it was enough to prevent leakage. Leakage was not observed throughout the experiments. This method seems to be feasible for other elastic material.

To validate this hypothesis, the K₄Fe(CN)₆ solution was pumped gently into the microfluidic chip. The cyclic voltammetric measurement of 0.5 mM K₄Fe(CN)₆ in 0.1 M KCl solution was performed routinely to determine the health of the electrochemical detection system. The CVs were conducted at various scan rates (Fig. 4a). The oxide peak current was linearly proportionally to the square root of scan rate, ranging from 10 to 40 mV/s (Fig. 4a inset). It was in accordance with the Randle–Sevick equation (Bard and Faulkner 2000):

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    i_p = 2.69 \times 10^5 n^{3/2} A D^{1/2} v^{1/2} c
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where \( i_p \) is the peak current; \( n \) is the number of moles of electrons transferred per mole of electroactive species (e.g., ferrocyanide); \( A \) is the area of the electrode in cm²; \( D \) is the diffusion coefficient in cm²/s; \( C \) is the solution concentration in mol/L; and \( v \) is the scan rate of the potential in V/s.

Dopamine is an important neurotransmitter that affects many functions of brain such as sleeping, mood, and learning. Some technology has been developed to analyze dopamine on chip (Garcia and Henry 2003; Liu, Vickers, and Henry 2004) or off chip (Winter, Codognoto, and Rath 2007; Wang et al. 2007; Yang et al. 2006). It is obvious that utilizing a microchip to detect dopamine has many advantages, such as
speed (high efficiency), low consumption, and practical on-site detection. The amperometric i–t curve of 2 mM dopamine solution was performed at 0.45 V (Fig. 4b). The electrode was manually drawn out 5 mm every 50 s. The current rose dramatically as the electrode was pulled but slowly fell and steadied at less than 100 s. The main cause of current jump was that the fresh surface of electrode could supply a more effective area. More substrates would be involved in the electrochemical reaction. The decrement of current is because the diffusion of the dopamine molecular
rather than electrochemical kinetics dominates. When there is a balance between the diffusion and electrochemical kinetics, the current was steady. The same experiment was carried out on much weaker dopamine solution (200 μM), and the results fully met the expectations and confirmed the observations from previous tests.

The OCP (open circuit potential) was used to characterize the condition of the electrode surface. If the electrode is cleaner, the OCP will be smaller, and vice versa. Three i–t curves under 0.45 V detection potential were further performed (Fig. 4c). The OCP measurement and i–t curve were not carried out simultaneously. The OCP was recorded before and after regeneration. Arrows indicate the motion of the electrode. After about 200 s at 0.45 V, the OCP rose from 0.24 to 0.34 V, indicating that the dopamine was oxidized and absorbed on the surface of the electrode; after regeneration, it reduced from 0.34 to 0.24 V, showing that the electrode was regenerated.

CONCLUSIONS

A prototypical method of electrode surface regeneration was developed and demonstrated. The current–time curve of dopamine solution and the OCP of the electrode were employed to testify this prototype system. The current of i–t curve and OCP would come back to initial values by regenerating the electrode, giving evidence that the surface was totally refreshed. It is just like DME to regenerate the surface of a solid electrode rather than liquid one. Manual operation of this system, which may be the major disadvantage, can be sufficiently solved by a stepwise motor. With this promising method, further research into the detection of thiols, carbohydrates, and complex samples can be conducted in the future.

REFERENCES


