Integrating bipolar electrochemistry and electrochemiluminescence imaging with microdroplets for chemical analysis

Suozhu Wu [1], Zhenyu Zhou, Linru Xu, Bin Su*, Qun Fang*

Center for High-Performance and Novel Materials, Institute of Microanalytical Systems, Department of Chemistry, Zhejiang University, Hangzhou 310058, China

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ABSTRACT

Here we develop a microdroplet sensor based on bipolar electrochemistry and electrochemilumines-cence (ECL) imaging. The sensor was constructed with a closed bipolar cell on a hybrid poly(dimet-hylsioxane) (PDMS)-indium tin oxide (ITO) glass microchip. The ITO microband functions as the bipolar electrode and its two poles are placed in two spatially separate micro-reservoirs predrilled on the PDMS cover. After loading microliter-sized liquid droplets of tris(2,2′-bipyridyl) ruthenium (II)/2-(dibutyla-mino) ethanol ($Ru(bpy)_3^{2+}$/DBAE) and the analyte to the micro-reservoirs, an appropriate external voltage imposed on the driving electrodes could induce the oxidation of $Ru(bpy)_3^{2+}$/DBAE and simultaneous reduction of the analyte at the anodic and cathodic poles, respectively. ECL images generated by $Ru(bpy)_3^{2+}$/DBAE oxidation at the anodic pole and the electrical current flowing through the bipolar electrode can be recorded for quantitative analyte detection. Several types of quinones were selected as model analytes to demonstrate the sensor performance. Furthermore, the cathodic pole of bipolar electrode can be modified with (3-aminopropyl)triethoxysilane–gold nanoparticles–horseradish peroxidase composites for hydrogen peroxide detection. This microdroplet sensor with a closed bipolar cell can avoid the interference and cross-contamination between analyte solutions and ECL reporting reagents. It is also well adapted for chemical analysis in the incompatible system, *e.g.*, detection of organic compounds insoluble in water by aqueous ECL generation. Moreover, this microdroplet sensor has advantages of simple structure, high sensitivity, fast response and wide dynamic response, providing great promise for chemical and biological analysis.

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1. Introduction

Over the past decade, microdroplet systems have received considerable attention because they can function as microreactors for high throughput chemical and biological research, such as chemical synthesis, drug discovery, protein crystallization and enzyme assays (Song et al., 2006; Teh et al., 2008; Theberge et al., 2010). Considering the significant size miniaturization of the droplets (typically in femoliter to microliter scale), more sensitive techniques are required for their detection and characterization. Fluorescence technique that can offer an excellent sensitivity has been so far one of the most frequently used detection methods, which however is not very compatible with miniaturized and portable microdevices due to its bulky size and high cost. Given its high sensitivity, low cost and good compatibility with advanced microfabrication technologies (Han et al., 2009; Wang, 2002), electrochemical detection is more adapted for such microsystems. Recently, micro-droplet devices based on the conventional electrochemical detection have been utilized for chemical analysis, including biosensing (Lindsay et al., 2007; Sassa et al., 2010), kinetic studies (Han et al., 2012; Han et al., 2009), cell electroporation (Luo et al., 2006; Zhan et al., 2009) and characterization of droplet flow behavior (Liu et al., 2008). The detection is usually performed by fabricating microelec-trodes at the downstream flow, and thus the aqueous droplets containing analytes can be detected when each of them passes through the microelectrodes (Han et al., 2012; Han et al., 2009; Liu et al., 2008; Luo et al., 2006; Sassa et al., 2010; Zhan et al., 2009). Alternatively, it can be done by inserting the electrode bundle including the working, counter and reference electrodes directly into the droplets (Lindsay et al., 2007).

Recently, bipolar electrochemistry has been demonstrated to be a powerful tool for chemical, biological analysis and high-throughput screening of catalytic nanomaterials (Fosdick et al., 2013; Fosdick and Crooks, 2012; Lin et al., 2012; Loget and Kuhn, 2011; Mavre et al., 2010). In comparison with conventional electrochemistry that pays attention on the redox reactions at

the working electrode, this electrochemical technique focuses on two coupled redox reactions occurring at two opposite poles of a bipolar electrode, namely a reduction reaction at one pole (cathodic pole) and a simultaneous oxidation reaction at the other (anodic pole). Bipolar electrochemistry can be performed in either an open or a closed cell. In the former case (the bipolar electrode is placed inside in a microchannel), the detection relies on electrochemiluminescence (ECL) (Arora et al., 2001; Zhan et al., 2002) or visual metal electro-dissolution (Chow et al., 2010; Duval et al., 2001; Fosdick et al., 2013), because the direct current that flows through the bipolar electrode is not easy to measure (Guerrette et al., 2012; Mavre et al., 2010). Since Manz and coworkers introduced bipolar ECL to the microsystem in 2001 (Arora et al., 2001), many interesting studies have been reported recently, predominantly by the Crooks group, showing broad applications of bipolar electrochemistry in separation (Arora et al., 2001), concentration (Dhopeshwarkar et al., 2008), electrocatalysis (Fosdick et al., 2013; Fosdick and Crooks, 2012), sensing and biosensing (Chang et al., 2012; Chow et al., 2008; Wu et al., 2012; Wu et al., 2011; Wu et al., 2013), cargo transport (Loget and Kuhn, 2012; Sentic et al., 2012), etc. By contrast, when using a closed bipolar cell (two poles of a bipolar electrode are located in two separate compartments), the electrical current could be facilely measured (Cox et al., 2012; Guerrette et al., 2012). For example, it has been employed to study electron transfer at liquid/liquid interfaces (Hotta et al., 2002; Plana et al., 2010) and at microelectrodes and nanoelectrodes (Cox et al., 2012; Guerrette et al., 2012), parallel screening of electrocatalyst (Guerrette et al., 2013; Lin et al., 2012) and sensing with a dual-channel configuration (Zhang et al., 2013). To the best of our knowledge, there is no report regarding the applications of microdroplets-based closed bipolar cell.

Herein we integrate a closed bipolar cell with microdroplets on a hybrid poly(dimethylsioxane) (PDMS)-indium tin oxide (ITO) glass microchip for chemical sensing. The device constitutes an ITO microband functions as the bipolar electrode and two spatially separate micro-holes drilled on the PDMS cover as reservoirs for liquid droplets. After loading microliter-sized liquid droplet of tris(2,2′-bipyridyl) ruthenium (II)/2-(dibutylamino) ethanol ($Ru(bpy)_3^{2+}$/DBAE) and that of analyte, an appropriate external voltage imposed on two driving electrodes can activate the oxidation of $Ru(bpy)_3^{2+}$/DBAE and reduction of the analyte simultaneously at two poles of the bipolar electrode. Using this device, dual ECL imaging/amperometric detection was implemented for hydrogen peroxide (H_2O_2) biosensing. Furthermore, it was utilized for detecting four types of quinone species slightly soluble or insoluble in water by aqueous ECL generation from $Ru(bpy)_3^{2+}$/DBAE oxidation.

2. Experimental

2.1. Chemicals

All chemicals of analytical reagent grade were used without further purification. $Ru(bpy)_3Cl_2$ hexahydrate (98%) was bought from Strem Chemicals (Newburyport, MA, USA). 2-(Dibutylamino) ethanol (DBAE, 99%) and horseradish peroxidase (HRP, 250–330 units mg^{-1}) was obtained from Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA). 2,6-Dichloro-1,4-benzoquinone (DCBQ, > 98%) and 2,3,5,6-tetrachloro-1,4-benzoquine (TCBQ, > 98%) were obtained from TCI (Tokyo, Japan). 7,7′,8,8′-Tetracyanoquinodimethane (TCNQ, 98%) and ferrocene (Fc, 99%) were purchased from Alfa Aesar (Ward Hill, MA, USA). H_2O_2, p-benzoquinone (BQ, > 98%) and sodium citrate were received from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Chloroauric acid ($HAuCl_4$, 47.8% Au) was bought from Aladdin (Los Angele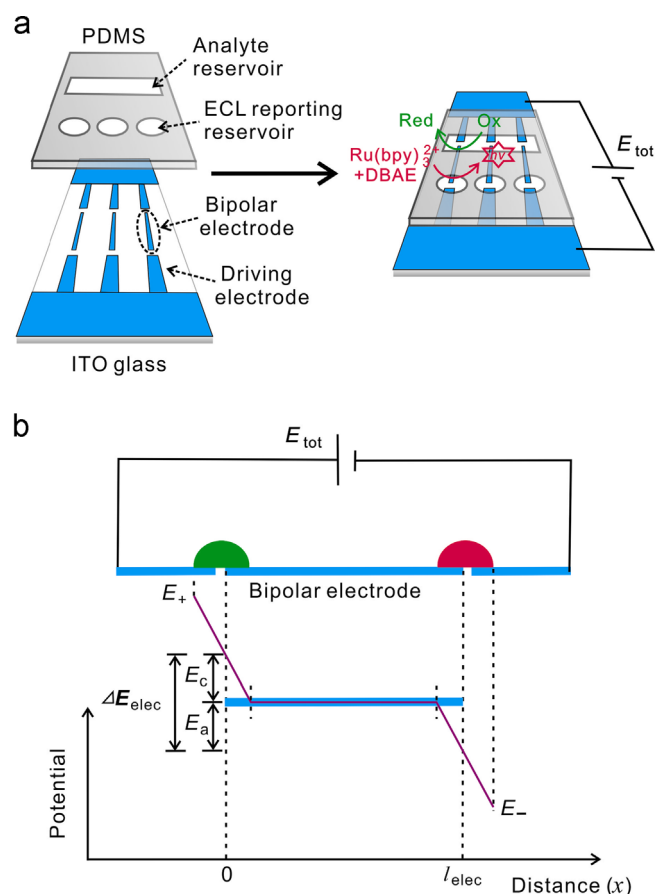s, CA, USA). All aqueous solutions were prepared with ultrapure water (18.2 MΩ cm). Solutions of H_2O_2 and quinones were freshly prepared.

Fig. 1. Schematic illustration of the microdroplet sensor based on bipolar electrochemistry and ECL imaging. (a) The sensor configuration and (b) the sensing principle (the PDMS cover and glass substrate was not shown).

2.2. Chip fabrication

Fig. 1a shows the schematic diagram of the microdroplet sensor based on bipolar electrochemistry and ECL imaging, which was constructed on a hybrid PDMS-glass microchip. The PDMS cover (thickness: ~ 0.1 cm, length: 3.0 cm, width: 2.5 cm) was prepared by a standard replica molding approach according to a previous report (Duffy et al., 1998). Briefly, a mixture of PDMS prepolymer and curing agent (10:1, w/w; Sylgard 184, Dow Corning, MI, USA) was degassed under vacuum for 30 min, and then the mixture was poured onto a silanized glass master (8.0 cm × 7.5 cm) and cured at 75 °C for 1.5 h. After curing, a piece of flat PDMS was formed and peeled from the glass master, followed by drilling micro-reservoirs on it. One rectangular micro-reservoir (length: 1.6 cm, width: 0.5 cm) and desired number of circular micro-reservoirs (diameter: 0.3 cm) for holding analyte and ECL reporting solutions were drilled on the PDMS cover, which are designated analyte and reporter micro-reservoirs, respectively.

Patterned indium tin oxide (ITO) glass substrate (thickness: ~ 100 nm, resistance: < 15 Ω $square^{-1}$; Kaivo Electronic Components Co., Ltd, Zhuhai, China) was fabricated using photolithographic technique, followed by wet chemical etching. Prior to use, ITO glass substrate was sequentially cleaned by sonication in 1 M NaOH dissolved in ethanol, acetone, ethanol and water (15 min each). After cleaning, a positive photoresist layer (thickness: ~ 10 μm, AZ P4620, AZ Electronic Materials USA Corp., Somerville, NJ, USA) was spin-coated on the ITO glass substrate (length: 4.0 cm, width: 2.5 cm)