Biosensors and Bioelectronics 55 (2014) 106–112

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

A droplet-based microfluidic electrochemical sensor using platinum-black microelectrode and its application in high sensitive glucose sensing

Shuqing Gu [a,b,c], Youlan Lu [a], Yaping Ding [a,c,*], Li Li [a], Hongsheng Song [d], Jinhua Wang [d], Qingsheng Wu [e]

[a] Department of chemistry, College of Sciences, Shanghai University, Shanghai 200444, China
[b] Technical Center for Animal Plant and Food Inspection and Quarantine, Shanghai Entry-Exit Inspection and Quarantine Bureau , Shanghai 200135, China
[c] School of Materials Science and Engineering, Shanghai University, Shanghai, China
[d] School of Life Sciences, Shanghai University, Shanghai, China
[e] Department of Chemistry, Tongji University, Shanghai 200092, China

ARTICLE INFO

Article history:
Received 10 September 2013
Received in revised form
20 November 2013
Accepted 1 December 2013
Available online 10 December 2013

Keywords:
Sensor
Microfluidic
Droplet
Pt-black microelectrode
Glucose oxidase

ABSTRACT

We describe a droplet-based microfluidic electrochemical sensor using platinum-black (Pt-black) microelectrode. Pt-black microelectrode was generated by electrodeposition of Pt nanoparticles on bare Pt microelectrode. Scanning electron microscope (SEM) image displays a flower-like microstructure of Pt nanoparticels. Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) indicate that the Pt-black efficiently decreased the charge transfer resistance and improved the electrocatalytic activity towards oxidation of hydrogen peroxide (H_2O_2). Compared with bare Pt microelectrode, the current response on Pt-black microelectrode increased 10.2 folds. The effect of applied potential and electrodeposition time has been investigated in detail. The proposed sensor was validated by performing enzyme activity assay in flowing droplets. For demonstration, glucose oxidase (GOx) is chosen as the model enzyme, which catalyzes the oxidation of β-D-glucose to the product H_2O_2. The enzyme activity of GOx was evaluated by measuring the electrochemical current responding to various glucose concentrations. And the results indicate that this microfluidic sensor holds great potential in fabricating novel glucose sensors with linear response up to 43.5 mM. The analytical applications of the droplet-based microfluidic sensor were tested by using human blood serum samples. Reproducibility, interferences, and long-term stability of the modified electrode were also investigated. The present approach shows the feasibility and great potentials in constructing highly sensitive and low-consumption sensors in the field of droplet microfluidics.

1. Introduction

In recent years, droplet microfluidics has defined a new experimental platform for performing a diverse range of chemical and biological processes with small and portable size, low energy and reagent consumption and rapid manipulation of liquids (Du et al., 2010; Li et al., 2006; Bui et al., 2011; Ali-Cherif et al., 2012). Such systems have found application in biochemical screening (Du et al., 2010), protein crystallization (Li et al., 2006) and enzymatic kinetic assays (Bui et al., 2011; Ali-Cherif et al., 2012), and have a significant impact on other fields such as emulsion based polymerase chain reaction (Zhang et al., 2012), biochemical synthesis (Zhao et al., 2011), and single cell-based analysis (Konry et al., 2011; Debs et al., 2012).

Droplet analytical techniques play a vital role for the development of droplet microfluidics. A wide range of techniques for droplet contents analysis have been established, as well as methods for droplet generation and manipulation. Fluorescence (Bui et al., 2011; Ali-Cherif et al., 2012; Zhang et al., 2012), capillary electrophoresis (Edgar et al., 2006), mass spectrometry (Zhu and Fang, 2010), and Raman spectra (Cristobal et al., 2006) have been successfully applied to measure the chemical compositions of droplets. Many of these techniques require bulky or expensive equipment and there is a need to develop a simple and inexpensive technique to characterize droplets online.

Electrochemical method used the electrode as sensor, directly change the chemical signal of the droplet into an electrical signal. Electrochemical detection possesses a number of attractive attributes for microfluidic systems: (1) it is easier to fabricate microelectrodes

* Corresponding author at: Department of chemistry, College of Sciences, Shanghai University, Shanghai 200444, China. Tel.: +86 21 66 134 734; fax: +86 21 661 32 797.
E-mail address: wdingyp@sina.com (Y. Ding).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.12.002

through microelectromechanical system (MEMS) technology on a microfluidic chip than built an optical devices; (2) different from the optical detection, the sensitivity of the electrochemical sensing the geometry of the channel will not be reduced with channel dimensions; (3) the signal processing system of electrochemical detection and other peripheral equipment is relatively simple and easy to be miniaturized.

Recently, electrochemical technique has emerged as an effective tool for rapid, facile and effective detection of droplets. Up to now, electrochemical method has been implemented in microfluidic systems with continuously flowing droplets for enzymatic reactions (Han et al., 2009, 2012) and droplets flow behavior studies (Liu et al., 2008). These systems employed conventional microelectrodes with relatively low sensitivities. Previously, highly sensitive electrochemical detection of droplets based on modified microelectrode has been implemented for single cell activity assay (Ino et al., 2013), enzymatic glucose detection (Lindsay et al., 2007), and non-enzymatic glucose detection (Wang et al., 2012). However, these systems involve static droplets with rather large volumes. To the best of our knowledge, there has been no report concerning electrochemical microfluidic sensor based on modified microelectrodes in droplet microfluidic systems. Thus, there is a substantially promising development space in this booming field.

Nanomaterials with good biocompatibility and electrocatalytic activities have been widely incorporated in microelectrode modification (Ino et al., 2013; Lindsay et al., 2007; Wang et al., 2012; Chandra et al., 2011; Li et al., 2013; Won et al., 2013; Regiart et al., 2013; Qiang et al., 2010). By physical or chemical modification of the electrode surface, the detection performance has been effectively improved and the application scope has been expanded. Among these nanomaterials, metal nanoparticles are the most commonly used nanomaterials in electrochemical sensor construction. Pt black, a metal layer formed by electro-depositing amorphous clusters of Pt nano-particles, has been used to enhance sensor sensitivity due to its electrocatalytic activities and excellent biocompatibility (Li et al., 2013; Qiang et al., 2010).

Previously, we have reported a droplet-based microfluidic system with concentration gradient for dose-response enzyme inhibition assay of acetylcholinesterase by the electrochemical method (Gu et al., 2013). In this work, we implemented a highly sensitive pt-black microelectrode in the similar system to construct a droplet-based microfluidic electrochemical sensor for enzyme activity assay and glucose sensing in flowing droplets, and demonstrated its utility and potentials in this area in achieving low cost, high throughput and high sensitivity dose-response assay. The analytical applications of the droplet-based microfluidic sensor were validated by using human blood serum samples and the results showed that the proposed sensor has acceptable accuracy and good recoveries.

2. Experimental

2.1. Materials and reagents

All solvents and chemicals used were of reagent grade unless otherwise mentioned. Deionized water was used throughout. Glucose oxidase (GOx, type VII from *Aspergillus niger*; 192.6 KU/g) and β-D-glucose were purchased from Sigma Aldrich (St. Louis, MO). Stock solutions of 1 g/L GOx were prepared in 10 mM phosphate buffered saline (PBS, pH 7.2) and stored at −20 °C. Stock solutions of 1.0 M glucose (freshly prepared, in an acetic acid buffer pH 4.4) were allowed to mutarotate at room temperature overnight before use. $Pb(Ac)_2$ $3H_2O$, H_2PtCl_6 $6H_2O$ and hydrogen peroxide solution (30 wt% aqueous) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Mineral oil obtained from Sigma Aldrich was used as oil phase. Food dyes were purchased from Shanghai Dyestuffs Research Institute (Shanghai, China).

2.2. Preparation of microelectrodes

Pt-black microelectrode: A 1-cm-long Pt wire (100 μm dia., Alfa Aesar) was soldered with a 5 cm-long Cu wire and was used as Pt microelectrode. Electrodeposition of Pt nanoparticles on bare Pt wire was performed using the potentiostatic procedure at −0.1 V for 10 min in a solution of 0.1 M KCl, 2.0 mM H_2PtCl_6 and 1.0 mM $Pb(Ac)_2$ (as a crystal growth promoter) (Wang et al., 2012). The obtained Pt black modified microelectrode was used as working electrode.

Ag/AgCl microelectrode: A 5-cm-long Ag wire (100 μm dia., Alfa Aesar) was polished as described previously. AgCl was electrodeposited on it in 0.15 M NaCl aqueous solution for 1 h with 0.10 mA current and was used as quasi-reference electrode.

2.3. Fabrication of microfluidic device

Microfluidic chip was fabricated based on poly(dimethylsiloxane) (PDMS) using soft lithography (Han et al., 2012; Liu et al., 2008). The microchannels are composed of 150 μm deep fluidic flow channels and 100 μm deep electrode channels. Silicon masters with different thicknesses of SU-8 negative photoresist were fabricated as described in detail elsewhere. Here the thin (e.g., 100 μm) layer was fabricated and developed first and was exposed with a mask which covers the part of the wafer designated to the deeper structures. After fully developing and baking the structures, a second thicker (150 μm) layer of SU-8 was spin-coated onto this developed one-layer master. A degassed 10:1 mixture of a PDMS (Sylgard 184, Dow Corning) precursor with the curing agent (Sylgard 184, Dow Corning) was then poured onto the silicon master and cured at 70 °C overnight. The substrate was then peeled off from the mould and punched with a sharpened flat needle to create inlet and outlet ports. Pt-black microelectrode and Ag/AgCl microelectrode were inserted into the microchannel of the PDMS substrate. And then the substrate was bound to a flat PDMS slab.

2.4. Construction of the microfluidic electrochemical sensor

The structure of the microfluidic electrochemical sensor was similar to that described previously (Gu et al., 2013). Briefly, a fused-silica capillary with a tapered tip was used as sampling probe to inject inhibitor and buffer solutions alternately from a slotted-vials array into a continuously flowing stream of buffer, as depicted in Fig. 1A. Concentration gradient gradually formed at axial direction with the stream flowing. The capillary was connected with a microfluidic channel of a PDMS chip, where the sample stream was combined with enzyme GOx and substrate glucose and then segmented into a serial of droplets by two intersecting streams of mineral oil (Fig. 1B). The flow rate of oil, enzyme, and substrate was controlled by a multichannel syringe pump (Longer Precision Pump Co., Ltd., China) in infusion mode, while the total flow rate was maintained using Harvard Apparatus Pump 11 Elite syringe pump in withdraw mode. Electrochemical experiments including CV and amperometry were all carried out with a CHI 852C electrochemical workstation (Chenhua, China). Electrochemical impedance spectroscopy (EIS) was performed on a CHI 660D electrochemical workstation (Chenhua, China). A stereoscopic microscope (SZM45-T2, Sunny Optical Technology, China) with a CCD camera (MDC 200, Wise Digital Technology, China) was used for direct viewing and recording of the droplet behavior.