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Electrochemical detection of droplet contents in polystyrene microfluidic chip with integrated micro film electrodes

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ABSTRACT

Detection of the droplet contents is a key function for droplet-based microfluidic systems. This paper presents a polystyrene microfluidic device integrated with droplet generator and micro film electrodes for direct amperometric detection of droplet contents without droplet desegmentation and phase separation. The method for fabrication of the full polystyrene micro chip was presented, and the behaviors of the developed polystyrene chip for generation and stabilization of water in *n*-octanol droplets, and detection of H_2O_2 in droplets were examined. The influences of electrode architecture and flow conditions on the electrochemical signals were studied in detail. With the developed chip, a sensitivity of 0.12 A mol^{-1} L cm^{-2} and a detection limit of 10.0 μmol L^{-1} were observed for the determination of droplet contained H_2O_2. The achieved precision for the detection of 21 droplets containing 250 μmol L^{-1} H_2O_2 was 3.8% in relative standard deviation. The way for the aqueous droplets encapsulated by the oil-carrier to contact sensing electrodes was discussed based on the experimental observations, and the potential approaches for further improving the analytical performance were suggested.

1. Introduction

In the recent years, the development of droplet-based microfluidic systems is one of the most attractive research fields in microfluidic community [1]. Compared with two-phase continuous laminar flow, two-phase droplet flow possesses advantages such as rapider mixing of reagents and samples inside the droplets, less communication among droplets, higher sample-throughput, and less sample and reagent consumptions. Hence droplet-based microfluidic systems have become powerful platforms for chemical and biochemical synthesis, drug screening, protein crystallization, enzyme kinetics and so on [1–5].

The detection of the droplet contents is a key function for droplet-based microfluidic systems. The frequently used off-line detection, which is conducted after the droplets are eluted from the microfluidic device, collected and separated from oil phase, was reagent and time consuming [6]. Various on-line detection methods such as fluorescence microscopy, laser induced fluorescence detection, mass spectrometry, electrochemical methods [7–15], have been developed for the analysis of the droplet contents, and recently been reviewed comprehensively [16]. Among these methods, those based on the fluorescence of the analyte or its derivatives were the most commonly used ones. However, either fluorescence microscopy or laser induced fluorescence detector needs quite large and expensive equipments, which greatly reduces the integration degree of the whole system.

Electrochemical detection features easy to be integrated on microfluidic chips, high in sensitivity, quick in response and low in equipment and operation cost [16]. Therefore, electrochemical detection has been widely adopted for on-chip and real-time detection in various microfluidic systems [17,18]. Recently, a few reports on droplet-based microfluidic systems with on-chip electrochemical detection have been published [7,8,19–25]. Some of these reports applied the electrochemical detection to measure the droplet size and velocity, and to count droplets [19–22]. Only a few reports dealt with the electrochemical detection of droplet contents [7,8,23–25]. Two detection modes were adopted for electrochemical detection of droplet contents. One is to directly detect the droplet contents without desegmentation of the droplets. Thus, Han et al. [7] inserted Pt and Ag/AgCl micro wire electrodes into a full polydimethylsiloxane (PDMS) micro channel to form a on-chip amperometric detector for detecting H_2O_2 in aqueous droplets, and applied the developed system to droplet-based enzyme kinetics study. Gu et al. [23] applied the same method to dose–response enzyme inhibition assay with a full PDMS chip. It's quite simple to prepare an on-chip electrochemical detector via inserting wire electrodes into the PDMS channel. However, the electrode-exposing

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area and between-electrode spacing of the inserted electrodes were difficult to be accurately controlled. In addition, the inserted electrode wires were forced to penetrate each droplet, resulting in droplet instability [7]. Due to the discontinuous property of the two-phase droplet flow, direct electrochemical detection of droplet contents suffers problems such as discrete transient signals with relatively poor repeatability. More recently, Liu and Crooks [24] developed a novel PDMS–glass microchip integrated with a micro amperometric detector. In their work, a narrowed channel section was designed as the detection channel, on the bottom of which an array of micro Pt film electrodes was prepared. When entered into the narrowed channel, the droplets were stretched ten times in the length before in contact with the electrodes, resulting in stable and reproducible quasi-steady-state current signals. The other mode of electrochemical detection of droplet contents is to convert the segmented droplets to a continuous flow prior to detection. Thus, Han et al. [8] in their succeeding work reported a microfluidic chip with potentiometric detector for RNA-Mg^{2+} binding kinetics study. In this work, the Mg^{2+} ion-selective microelectrode and Ag/AgCl electrode were inserted to the PDMS channel downstream of a phase separator. Therefore, the concentration of the Mg^{2+} was detected after the aqueous droplets converted to a continuous aqueous flow. Filla et al. [25] reported a PDMS–glass microfluidic device where water droplets were desegmented by leading them into a hydrophilic branch of a T-shaped phase separator. In both reports, a phase separator was used to separate the aqueous droplets from the oil carrier, and the separated continuous aqueous stream was directed to a branch channel for electrochemical detection. This made the channel network complicated. Moreover, any disturbance in flow rate or back pressure would cause incompleteness of the phase separation. In comparison with this detection mode, the first detection mode (direct detection of droplet contents without desegmentation of droplets) is simpler in channel network and chip preparation, easier in flow manipulation, and capable of detect individual droplets. Up to now, no work has been contributed to the systematic study of the factors that influence the electrochemical behavior of the first detection mode.

The previously reported micro chips for on-chip electrochemical detection of droplet contents were all made of either full PDMS or hybrid PDMS/glass substrates. Microchips made of thermoplastic polymers are potentially used as disposable devices because of the advantages such as less expensive, easy to be mass-produced via either hot embossing or injection molding technologies, and a wide range of materials such as polymethyl methacrylate (PMMA), polystyrene (PS), polycarbonate (PC), cyclic olefin copolymer (COC), can be selected for desired applications [26,27]. To the best of our knowledge, no thermoplastic polymer chip with integrated electrochemical detector for on-chip detection of droplet contents has been reported.

In this paper, a full PS microfluidic chip with integrated droplet generator and micro film electrodes for direct amperometric detection of droplet contents was reported. The behaviors of the PS chip for generation and stabilization of water in oil (W/O) droplets, and electrochemical detection of the droplet-contained hydrogen peroxide (H_2O_2) were examined. The influences of electrode architecture and flow conditions on the electrochemical signals were systematically studied. The analytical performance of the developed chip for detection of droplet contained H_2O_2 was evaluated.

2. Experimental

2.1. Materials

PS sheets were purchased from Hangzhou Baiersi Plastic Co. (Hangzhou, China); *n*-octanol and hydrogen peroxide (30%, v/v) were from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China); Cetyltrimethylammonium bromide (CTAB) was from Guangzhou Chemical Reagent Factory (Guangzhou, China); A silver electroplating bath (containing 0.29 mol L^{-1} sodium silver sulfite ($NaAgSO_3$), saturated sodium sulfite, 0.19 mol L^{-1} sodium carbonate and 0.51 mol L^{-1} monopotassium phosphate) and a platinum electroplating bath (5 mmol L^{-1} hexachloroplatinic acid and 0.5 mmol L^{-1} lead acetate prepared with pH = 4.0 Britton–Robinson buffer) were prepared in-home.

All chemicals were of analytical grade or better, and double-distilled water was used to prepare solutions throughout the work.

2.2. Preparation of the microchip

The microchips (Fig. 1), consisting of a PS substrate with microchannel network (Fig. 1a) and a PS cover sheet with integrated micro film electrodes (Fig. 1b), were prepared as before [28]. Briefly, gold electrode-bases with a desired configuration were deposited onto the PS cover sheet via UV-assisted region-selective electroless plating. Then the gold electrode-bases for working and the counter electrodes were modified to Pt electrodes via platinum electroplating. It was conducted by scanning the gold electrode-

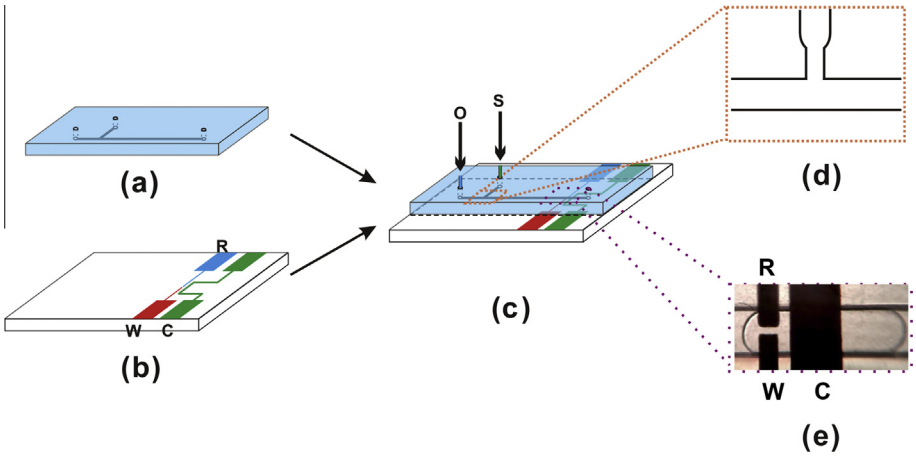

Fig. 1. Schematic diagrams of the PS microfluidic chip for on-chip electrochemical detection of droplet contents. (a) the PS substrate with the micro-channels; (b) the PS cover sheet with the integrated micro metal film electrodes; (c) the bonded microchip, O: inlet port for oil carrier, S: inlet port for aqueous sample solutions; (d) enlarged view of the droplet generator tip; (e) a CCD image of the sensing parts of the three-electrode system, W, working electrode, 100 μm in width; R, reference electrode, 100 μm in width; C, counter electrode, 250 μm in width; the between-electrode spacing was 50 μm.