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Electrochemical microfluidic chip based on molecular imprinting technique applied for therapeutic drug monitoring

Jiang Liu[a], Yu Zhang[b], Min Jiang[b], Liping Tian[b], Shiguo Sun[b], Na Zhao[b], Feilang Zhao[c], Yingchun Li[a,b,*]

[a] College of Science, Harbin Institute of Technology (Shenzhen), Shenzhen, China
[b] Key Laboratory of Xinjiang Endemic Phytomedicine Resources, Ministry of Education, School of Pharmacy, Shihezi University, Shihezi, China
[c] Jiangsu Devote Instrumental Science & Technology Co., Ltd., Huai'an, China

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ABSTRACT

In this work, a novel electrochemical detection platform was established by integrating molecularly imprinting technique with microfluidic chip and applied for trace measurement of three therapeutic drugs. The chip foundation is acrylic panel with designed grooves. In the detection cell of the chip, a Pt wire is used as the counter electrode and reference electrode, and a Au–Ag alloy microwire (NPAMW) with 3D nanoporous surface modified with electro-polymerized molecularly imprinted polymer (MIP) film as the working electrode. Detailed characterization of the chip and the working electrode was performed, and the properties were explored by cyclic voltammetry and electrochemical impedance spectroscopy. Two methods, respectively based on electrochemical catalysis and MIP/gate effect were employed for detecting warfarin sodium by using the prepared chip. The linearity of electrochemical catalysis method was in the range of 5×10^{-6}–4×10^{-4} M, which fails to meet clinical testing demand. By contrast, the linearity of gate effect was 2×10^{-11}–4×10^{-9} M with remarkably low detection limit of 8×10^{-12} M (S/N=3), which is able to satisfy clinical assay. Then the system was applied for 24-h monitoring of drug concentration in plasma after administration of warfarin sodium in rabbit, and the corresponding pharmacokinetic parameters were obtained. In addition, the microfluidic chip was successfully adopted to analyze cyclophosphamide and carbamazepine, implying its good versatile ability. It is expected that this novel electrochemical microfluidic chip can act as a promising format for point-of-care testing via monitoring different analytes sensitively and conveniently.

1. Introduction

Therapeutic drug monitoring (TDM) is essential in clinical drug therapy, which aims to guarantee effectiveness of drug and meanwhile avoid its adverse effect. At present, several approaches including spectrum analysis (Sachse et al., 2006; Stobiecka, 2014; Stobiecka and Chalupa, 2015), chromatography (Malfará et al., 2007), mass spectrometry (Ansermot et al., 2013), immunization (Fan et al., 2014) and capillary electrophoresis (Haselberg et al., 2013) have been reported for TDM work. However, these methods suffer from several disadvantages, such as serious interference from sample matrix, time-consuming process, costly instruments and complicated operation. Moreover, most of the methods require several milliliter volume of sample, which brings large sufferance to patients.

Microfluidic detection technology (MDT) is recognized as one of the most promising analytical tools, due to its admirable merits, such as miniaturized components, microliter level of demanded sample volume, easy operation, adaptability to realize automation, low cost, etc. (Yamada et al., 2015), which is especially suitable for TDM and also plays an important part in an emerging technology, point-of-care testing (POCT) (Vashist et al., 2015). There are some applications in TDM based on electrochemistry in pharmaceutical analysis (Cao et al., 2014; Du et al., 2011; Kovachev et al., 2010). However, some obvious problems exist in the present microfluidic chip. i) The majority of electrodes in the chip are planar, whose effective area is limited and only nano-ampere level current signal can be generated (Chikkaveeraiah et al., 2011). Low signal is unfavorable for sensitivity enhancement and easy to be influenced by environment and/or other factors. ii) Most quantification approaches depend on the electrical signals produced by redox reactions of analyte molecules, which is called "electrochemical catalysis" determination. However, analyte owning sufficient electrical activity is the prerequisite for this method, which shuts the detection door towards many compounds with weak or without electrochemical activity. iii) Small sample volume in micro-

* Corresponding author at: College of Science, Harbin Institute of Technology (Shenzhen), Shenzhen, China.
E-mail address: liyingchun@hit.edu.cn (Y. Li).

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fluidic chip detection (at microliter-level) leads to weak signals and consequently low sensitivity (usually below tens of nA/mM) and unsatisfying detection limit (usually at μM level) (Gu et al., 2014; Li et al., 2012), which can barely meet the practical demand for trace measurement. iv) The use of three-electrode system in microfluidic chip more or less makes it difficult to simplify sensor setup and minify size of chip.

To avoid the above mentioned limitations of microfluidic chip, its preparation was improved in this work. Firstly, nanoporous-metallic material with large specific surface area is adopted as working electrode to remedy the defect of planar electrode. This is conducive to enhancement of electrical signal, raising the detection performance of the chip, especially sensitivity. Secondly, molecular imprinting technique (MIT) is introduced to decorate working electrode, and then the measurement strategy based on the so-called "gate-controlled effect" is adopted (Li et al., 2015a, 2015b). "Gate-controlled effect" owns some evident advantages like anti-disturbance capability and versatility. The former is attributed to the specificity of molecularly imprinted polymer (MIP) film for analyte, just like that of antibody for receptor (Schirhagl, 2014), which ensures more accurate and reliable analysis. The latter feature is related to the fact that this strategy is independent of electrochemical activity of analyte, which expands the range of detectable drugs in using microfluidic chip for TDM. Previously, we have developed a series of ultrasensitive and selective electrochemical sensors by using MIT on the basis of gate effect, where MIP membrane serves as promising sensing agent because of its superb recognition ability towards target molecules, along with its reusability and physicochemical stability (Li et al., 2016a; Stobiecka et al., 2009; Zhang et al., 2016). However, to our best knowledge, there is no concerned report about MIP membrane acting as functional material and used in fabrication of microfluidic chip for TDM. Furthermore, compared with traditional three-electrode system, two-electrode electrochemical sensor is more beneficial for microminiaturization of sensing platform (Gu et al., 2014; Horny et al., 2016).

Herein, a novel electrochemical microfluidic chip was established based on MIT, and two-electrode system was adopted in the chip for electrochemical determination. Specifically, a Pt wire acts as both counter and reference electrode, and a 3D nanoporous Au–Ag alloy microwire (NPAMW) modified with MIP works as working electrode. Two strategies, i.e. electrochemical catalysis and gate effect were adopted for detecting trace therapeutic drug warfarin sodium (WFS), which is a widely used oral anticoagulant drug with narrow therapeutic window and its activity has to be monitored by blood testing. At last, this system was successfully applied to determine WFS in plasma of rabbit, and related pharmacokinetic parameters were obtained. In order to verify its versatility, the similar chips were fabricated and utilized for assay of cyclophosphamide and carbamazepine.

2. Experimental

2.1. Materials and chemicals

Au/Ag alloy microwire (AMW) (40:60 wt%; 400 μm in diameter) was obtained from Suzhou Cold Stones Tech. Co. Ltd. (Suzhou, China), and annealed at 800 ℃ for 8 h in a muffle furnace before use. Platinum wire with a diameter of 400 μm was obtained from Innochem Technology Co. Ltd. (Beijing, China). Warfarin sodium (WFS), cyclophosphamide (CPA), carbamazepine (CBZ), aspirin (ASP), hydrochlorothiazide (HCT), and vitamin K4 (VK4) were purchased from Adamas Reagent Co., Ltd. (Shanghai, China). Resorcin was bought from Alfa Aesar (Shanghai, China). Other chemicals, such as $[Fe(CN)_6]^{3-/4-}$, NaOH, HNO_3, methanol and phosphate buffer solution (PBS, KH_2PO_4 and K_2HPO_4) were obtained from Guangfu Tech. Co. Ltd. (Tianjin, China). All of the chemicals are analytical reagent grade or better.

2.2. Instruments

All electrochemical measurements were carried out with a computer-controlled CHI 760E Electrochemical Workstation (Chenhua Instruments Co., Shanghai, China). A bare or a modified NPAMW in fabricated microfluidics chip served as working electrode, and a platinum wire acted as counter electrode and reference electrode. The surface morphology of NPAMW was characterized by scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDS) (Scanning Electron Microscope, Zeiss Supra 55VP). Working solutions and sample solutions were pumped into the chip by a BT100L peristaltic pump with independent multi-channel (Baoding Lead Fluid Tech. Co. Ltd., Hebei, China). For comparison, high-performance liquid chromatography (HPLC) was used for detecting WFS, and the details can be found in supporting information.

2.3. Fabrication of microfluidic chip

Poly-methylmethacrylate (PMMA) plates were used as the chip substrates, where the channels and the chamber were prepared via laser etching. NPAMW was obtained by dealloying method and then electrodes and substrates were assembled. Details for the preparation procedure were elaborated in supporting information. Subsequently, NPAMW electrode was modified with MIP film through electrochemical polymerization. Functional monomer and polymerization conditions were optimized previously (Li et al., 2016b) and used in this work. Briefly, pre-polymerization solution containing functional monomers (resorcin) and templates (WFS) was pumped into the microfluidic chip at 0.5 mL/min. At the same time, cyclic voltammetry (CV) was carried out between 0 V and 1.0 V (vs. Pt wire) for 70 cycles at a scan rate of 50 mV/s. Afterwards, 0.1 M NaOH was pumped into the chip at 0.5 mL/min, and WFS embedded in polymer film was extracted by CV scanning from −0.5 to 0.5 V for two cycles at 100 mV/s. As for CBZ-imprinted polymer preparation, the process was nearly identical to that for WFS-MIP film, except that the template was CBZ instead of WFS. As for CPA-MIP preparation, the functional monomer was *o*-aminophenol according to pre-optimization work (results not shown) and polymerization was carried out by using CV between 0 and 0.8 V for 60 cycles. Afterwards, template molecules were washed from the imprinted film by CV scanning between −1 and 1 V in 0.1 M NaOH. For comparison, non-imprinted polymer (NIP)-modified electrodes were prepared in the same way without adding templates during electropolymerization.

2.4. Assay procedure with the microfluidic chip

In this work, two strategies, electrochemical catalysis and gate effect, were used for measurement of WFS. The former detecting method is based on catalysis of working electrode (NPAMW without decoration of MIP) towards WFS, which is a direct detection manner. Its process was as follows: sample solution containing WFS was pumped into microfluidic chip and then differential pulse voltammetry (DPV) measurement was performed. The gate effect measurement is an indirect strategy, which is based on the peak current change of working electrode (MIP modified NPAMW) towards probe ions before and after adsorbing WFS. The process includes five steps: i) Probe solution containing $[Fe(CN)_6]^{3-/4-}$ was pumped into microfluidic chip, and CV was operated to get the reduction peak current (I_1) of $[Fe(CN)_6]^{3-}$. ii) Sample solution was pumped and allowed WFS to adsorb on the MIP-NPAMW. iii) Distilled water was pumped to wash nonspecifically sorbed WFS. iv) The first step was repeated and the reduction peak current (I_2) was recorded. Then the current shift (ΔI) was calculated from the change of I_1 and I_2. v) The last step was eluting the embedded WFS from the MIP film by CV scanning in NaOH solution.