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Hand-drawn&written pen-on-paper electrochemiluminescence immunodevice powered by rechargeable battery for low-cost point-of-care testing

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In this paper, a pen-on-paper electrochemiluminescence (PoP-ECL) device was entirely hand drawn and written in commercially available crayon and pencil in turn for the first time, and a constant potential-triggered sandwich-type immunosensor was introduced into the PoP-ECL device to form a low-cost ECL immunodevice proof. Each PoP-ECL device contained a hydrophilic paper channel and two PoP electrodes, and the PoP-ECL device was produced as follows: crayon was firstly used to draw hydrophobic regions on pure cellulose paper to create the hydrophilic paper channels followed with a baking treatment, and then a 6B-type black pencil with low resistivity was applied for precision writing, as the PoP electrodes, across the hydrophilic paper channel. For further point-of-care testing, a portable, low-cost rechargeable battery was employed as the power source to provide constant potential to the PoP electrodes to trigger the ECL. Using Carbohydrate antigen 199 as model analyte, this PoP-ECL immunodevice showed a good linear response range from 0.01–200 U mL^{-1} with a detection limit of 0.0055 U mL^{-1}, a high sensitivity and stability. The proposed PoP-ECL immunodevice could be used in point-of-care testing of other tumor markers for remote regions and developing countries.

1. Introduction

Since the first microfluidic paper-based analytical device (μ-PAD) was constructed by Martinez et al., 2007, the μ-PADs have showed great and robust prospect in the molecular analysis of biological fluids, such as urine, serum and blood, environmental detection, and health monitoring in developing countries, resource-limited and remote regions (Martinez et al., 2010; Mukhopadhyay, 2009; Sia and Kricka, 2008; Whitesides, 2011). Especially with the rapid developments and sophisticated applications of μ-PADs in colorimetry (Abe et al., 2008; Bruzewicz et al., 2008), electrochemistry (Lu et al., 2012; Nie et al., 2010), chemiluminescence (Yu et al., 2011; Wang et al., 2012), electrochemiluminescence (ECL) (Delaney et al., 2011; Wang et al., 2013), absorbance and fluorescence methods Carrilho et al., 2009b, μ-PADs have emerged as privileged devices in integrated analytical systems, and have gained considerable attention and interest (Martinez et al., 2010; Mukhopadhyay, 2009; Sia and Kricka, 2008; Whitesides, 2011). Therefore, the design and fabrication of μ-PADs, particularly for those who have no professional skill or vocational training, have become a hot spot in this flourishing field.

Presently, many means have been used to fabricate μ-PADs, including inkjet etching (Abe et al., 2008), polydimethylsiloxane plotting (Bruzewicz et al., 2008), photolithography (Apilux et al., 2010), plasma etching (Li et al., 2008), and wax-printing (Carrilho et al., 2009a). In these methods, wax-printing is the most easiest means of mass production available, and has been considered the most effective in the production of μ-PADs for laboratory research and industrialization application. When encountering unpredictable chemical testing items for which there were no relevant optimized μ-PADs, an easy-to-use method may be critical to prepare new μ-PADs for rapid point-of-care determination, and the proposed method should be adjusted and improved according to the test applications in situ as quickly as possible. In this paper, we proposed a hand-drawn&written method in which the hydrophobic treatments and electrodes developments on paper were entirely realized by hand-drawing&writing using crayon and pencil. In this way, the shape and width of the paper channel and PoP electrodes on μ-PADs can be quickly optimized for

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comprehensive and accurate results on the spot without any electronic instruments.

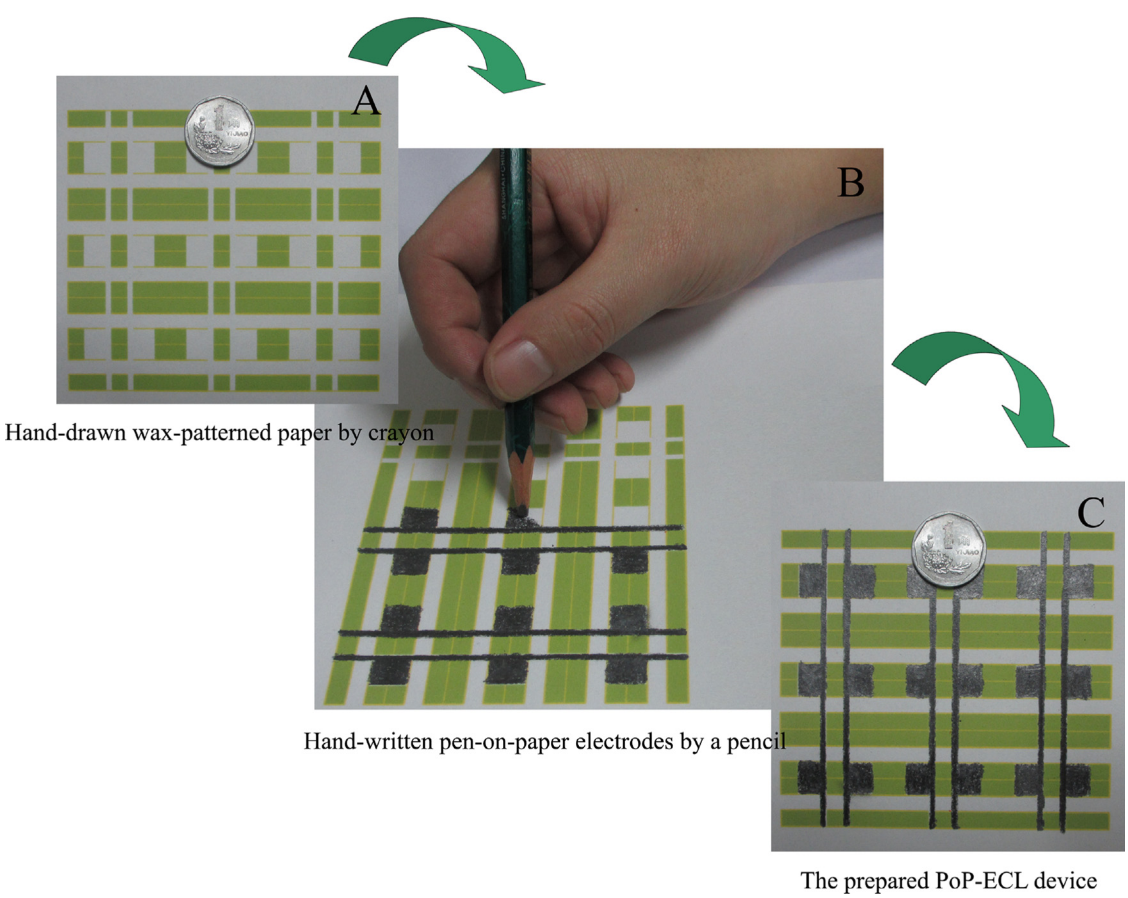
ECL integrates the merits of chemiluminescence and electrochemistry, and shows excellent sensitivity, wide dynamic concentration response range and high selectivity (Jie et al., 2007; Liu et al., 2008; Richter, 2004; Zou et al., 2005). Especially when established on μ-PADs (Delaney et al., 2011) based on conductive electrodes, ECL not only continues to impact diverse areas ranging from chemical analysis to the molecular-level understanding of biological processes, but also increases the scope of options for detections on μ-PADs. To date, the conductive electrodes have been deposited by sputter coating (Siegel et al., 2009), inkjet printing (Gans et al., 2004), airbrush spraying (Siegel et al., 2010) and screen printing. However, most deposition methods are either costly or employ dilute inks that readily permeate the paper substrate. Consequently, a new precept for making conductive electrodes on μ-PADs would be helpful. Using a black pencil, we directly write conductive carbon electrodes on paper to construct pen-on-paper ECL device. Another key problem that retards the application of ECL in remote regions and developing countries is the power supply – electrochemical workstation, which is costly and bulky. It makes sense to practice new excitation mode for ECL.

As one of the most important biological analytic methods, immunoassay based on the specific recognition between antigen and antibody is widely used in clinical cancer screening, early diagnostic application and biological researches. Carbohydrate antigen 199 (CA 199) is a well-known and useful tumor marker used in diagnosing cancer of the colon, pancreas, and other organs (Henlyn et al., 1982; Metzgar et al., 1982). Herein, we fabricated a pen-on-paper electrochemiluminescence (PoP-ECL) device, and a constant potential-triggered sandwich-type ECL immunosensor was established on the μ-PADs to detect CA 199. The PoP-ECL device consisted of a hydrophilic paper channel and two PoP electrodes, which were made using commercially available crayon and pencil. Firstly, crayon was used to draw hydrophobic regions on pure cellulose paper to create the hydrophilic paper channels, and the wax-covered paper was baked soon afterwards. Then, we employed a 6B-type black pencil to write carbon electrodes (named PoP electrodes) across the hydrophilic paper channel. To construct the immunosensor, chitosan was used to modify the paper working regions between two PoP electrodes to covalently immobilize antibodies on the PoP-ECL device, and $Ru(bpy)_3^{2+}$ – gold nanoparticles (Ru@AuNPs) were used as the ECL luminophore. A rechargeable battery was used as a constant-potential mode power supplier to trigger the ECL for detection. The aim of this study is to explore simple, sensitive, low-cost, disposable and portable point-of-care testing devices for remote regions and developing countries.

2. Materials and methods

2.1. Materials and reagents

All other reagents were of analytical grade and used as received, and all solutions were prepared using Millipore (model Milli-Q) purified water. Whatman pure cellulose chromatography paper #1 was obtained from GE Healthcare Worldwide (110 Pudong, Shanghai, China) and used with further adjustment of size. Crayon, 6B-type black pencil and a rechargeable battery were obtained from a local Wal-Mart store. CA 199, capture anti-CA199 antibody ($McAb_1$) and signal anti-CA199 antibody



Scheme 1. Schematic representation of the fabrication of PoP-ECL device..