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Fully-drawn origami paper analytical device for electrochemical detection of glucose

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ABSTRACT

In this work, an origami paper-based analytical device for glucose biosensor by employing fully-drawn pencil electrodes has been reported. The three-electrode system was prepared on paper directly by drawing with nothing more than pencils. By simple printing, two separated zones on paper were designed for the immobilization of the mediator and glucose oxidase (GOx), respectively. The used paper provides a favorable and biocompatible support for maintaining the bioactivities of GOx. With a sandwich-type scheme, the origami biosensor exhibited great analytical performance for glucose sensing including acceptable reproducibility and favorable selectivity against common interferents in physiological fluids. The limit of detection and linear range achieved with the approach was 0.05 mM and 1–12 mM, respectively. Its analytical performance was also demonstrated in the analysis of human blood samples. Such fully-drawn paper-based device is cheap, flexible, portable, disposable, and environmentally friendly, affording great convenience for practical use under resource-limited conditions. We therefore envision that this approach can be extended to generate other functional paper-based devices.

1. Introduction

Advancements in miniaturization and lowering of costs of electrochemical biosensors have led to selective and sensitive sensors capable for rapid on-site analysis [1–6]. Paper, a commonly material seen in laboratories, has been extensively exploited as an attractive substrate for the development of cost-effective and portable electrochemical paper-based analytical devices (ePADs) [7–9]. Paper substrates offer many advantages for ePADs. Not only is paper widely available and inexpensive, it is lightweight, biodegradable, flexibility and can be rolled or folded into 3D origami configurations. Therefore, paper-based sensors are meeting many public requirements and becoming more and more welcome. Commonly, the electrode integrated with ePADs are fabricated via inkjet printing, sputter coating or air brush spraying techniques, using inks or pastes prepared by mixing suitably conductive materials with polymeric binders [10–12]. However, these procedures are either costly or employ dilute inks that readily permeate the paper substrate.

Graphite pencil, a day-to-day tool, is essentially a nanocomposite of fine graphite powders with intercalated clay particles. It can easily make graphite traces on paper by dry drawing using a gentle force. Since the obtained pencil trace exhibits relatively high conductivity, pencil drawing is the simplest approach for obtaining conducting tracks, and can be utilized in fabricating paper-based functional devices [13,14]. To date, the pencil tracks with desired geometry have been applied as electrodes in Li-air battery and zinc oxide ultraviolet sensor [15,16]. Compared to other popular methods of electrode integration with paper, pencil-drawing has no critical requirement for the ink and is easily operated. Fresh paper chips can be rapidly fabricated without supporting equipments, affording great convenience for practical use in resource-limited areas.

Glucose is an important medical analyte because it is closely related to the diseases such as glucose metabolism disorders and islet cell carcinoma [17]. At present, most of the current glucose biosensors are based on glucose oxidase catalytic oxidation reaction on glucose [18,19]. Since glucose oxidase does not have long term stability, the effective immobilization of enzymes is a key step to fabricate the sensitive and stable biosensors. An ideal immobilization method should be cheap, simple, enzyme friendly, and can retain well the bioactivities of the enzyme. Cellulose paper contains fine fiber matrix and highly porous microstructure with reasonable mechanical strength, which can store reagents in active form within the fiber [20,21]. This makes it a favorable support for enzyme immobilization through simple adsorption mechanism. Therefore,

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paper is expected to serve both as a suitable substrate for electrode integration and as an effective microstructured reactor for enzyme.

Here, we design and report an origami paper-based analytical device for glucose biosensor by employing fully-drawn pencil electrodes. It comprises two zones separated by a central crease. One is the detection zone where electrodes are fully-drawn with a pencil and ferrocenecarboxylic acid was introduced as the electron transfer mediator for the catalytic oxidation of glucose. Another is the enzyme immobilization zone defined by a circle of hydrophobic barrier on paper. The two zones are brought into contact by folding the device along the central crease. With a sandwich-type scheme, the origami paper-based device could be used as an amperometric detector for glucose detection with good reliability and suitable reproducibility. This fully-drawn pencil-on-paper approach proved to be favorable for rapidly fabricating inexpensive paper-based sensors. We envision that this strategy may become a driving force for rapid, cheap, flexible, and reliable devices to be developed for clinical emergency as well as large-scale use in developing regions where reliability, affordability, and user friendliness are major challenges.

2. Experimental

2.1. Materials and instrumentation

Whatman grade 1 chromatographic paper was purchased from GE Healthcare Worldwide (Shanghai, China) and used with further adjustment of paper size. Commercial 6B grade pencils (Staedtler Mars, Germany) were obtained from a local store. All reagents used were of analytical grade. Glucose oxidase (GOx) from Aspergillus niger (Type V, 100000 U g^{-1}) was purchased from Sigma-Aldrich (St. Louis, MO, USA). D-(+)-glucose and ferrocenecarboxylic acid (FCA, >98%) were purchased from TCI (Shanghai, China). Dopamine (DA), ascorbic acid (AA) and uric acid (UA) at the analytical grade were obtained from Aladdin (Shanghai, China). Phosphate buffer solutions (PBS, 0.05 M) were made from Na_2HPO_4 and NaH_2PO_4 with its pH value adjusted using 0.05 M H_3PO_4 or NaOH. The stock solution of D-(+)-glucose was prepared in pH 7.0 PBS and allowed to equilibrate overnight before use. Other aqueous solutions were prepared with ultrapure water (18.1 mΩ) obtained from a Millipore water purification system. Commercial glucometer was purchased from Sinocare Inc (Changsha, China). All electrochemical experiments were performed at room temperature (except in experiments on temperature effects) using a CHI1230B electrochemical working station (CH Instrumentation, Shanghai, China).

2.2. Design and fabrication of the device

The schematic diagram of the fabrication of the origami paper-based analytical device was shown in Scheme 1. Firstly, Whatman grade 1 chromatographic paper was cut into A4 size (210 mm × 297 mm). The shape of the paper-based sensor, which contains a detection zone and an enzyme zone, was designed using Adobe illustrator CS4. In addition, to ensure the reproducibility of the electrodes written by graphite pencils, the patterns of working electrode (WE), reference electrode (RE) and counter electrode (CE) were pre-designed on the detection zone. Then the patterns were printed on the paper with a laser printer (Hewlett–Packard, Model HP 1010 LaserJet) (Scheme 1A). The printed paper was baked in an oven at 150 °C for 120 min to form hydrophilic areas. After the heat treatment, the paper was allowed to cool at room temperature and cut into pieces (Scheme 1B). Each individual PAD contains an enzyme zone and a detection zone, both of them are consists of a circular region with the diameter of 7 mm. Subsequently, pencil-drawn electrodes with certain size were written uniformly on the detection zone by a common 6B pencil (Scheme 1C). The WE is 3 mm × 2 mm, the RE and the CE are 2 mm × 2 mm, respectively. The graphitic layers were deposited repeatedly until a certain range of sheet resistance was obtained.

2.3. Functionalization of the device and electrochemical measurements

To functionalize the paper device, GOx (5 μL) and FCA solution (5 μL) with different concentrations were added to the enzyme zone and detection zone respectively and allowed to dry at room temperature (25 °C), as shown in Scheme 2A. After which, the two zones are brought into contact by folding the device along the central crease (Scheme 2B). We refer to this as FCA/GOx/PAD. The PAD immobilized with only mediator or enzyme is defined as FCA/PAD and GOx/PAD, respectively. For the electrochemical experiments, sample solution in pH 7.0 PBS with the volume of 10 μL was dropped on the detection zone for amperometric analysis. The effect of applied potential was investigated with potential shifting from 0.1 to 0.6 V using amperometric experiments. PBS with different pH values from 4.0 to 9.0 was used to study the effect of solution pH on the response of the biosensor. To optimize the mediator and GOx loading, different concentrations of FCA (5, 10, 15, 20 mM) and different amount of GOx (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 U/disc) were tested using the FCA/GOx/PADs. Unless otherwise indicated, all measurements were performed in triplicates on paper discs immobilized with 20 mM FCA and 2 U/disc GOx. In voltammetric measurement, potential scanning was performed according to the need of experiment. In experiments on temperature effect, the fabricated sensor was placed in a controlled thermostatic incubator for 30 min and then taken out for measurements at once under room temperature. In the amperometirc experiment, ΔI is defined to be the decreased current after 20 s.

3. Results and discussion

3.1. Design of the ePAD and morphology characteration of the pencil electrode

We have chosen paper as a substrate for the fabrication of the electroanalytical device because it is widely available, flexible, easily creased and written by pencils. It also does not break into sharps and can be disposed of by burning. The roughness of the paper endows large surface areas for physical adsorption of biomolecules and other regents. We took advantage of these properties to immobilize the redox mediator and GOx on the surface of the paper. To minimize the fouling of the electrode and maintain the activity of the immobilized enzyme, we have separated the detection zone and the enzyme zone spatially (Scheme 2A).

In addition, the flexibility and foldability of the paper substrate allows us to bring the electrodes into contact with the reaction mixture only when the electrochemical detection step was ready to be performed. After completing the immobilization of the mediator and enzyme, the device was folded along a central crease to bring the electrodes in contact with the solution of the electroactive reagent of the enzymatic reaction (Scheme 2B).

Pencil drawing on paper allows for an extremely facile, green and rapid method to fabricate electrode prototypes, as designs and geometries can be easily altered. While drawing, friction between the pencil and the paper rubs off graphite particles on the paper, which are essentially conducting tracks. The changes during this manufacturing process can be elucidated by SEM. Paper contains large amount of cellulose fibers and exhibits a rough and porous surface morphology (Fig. 1A), which is favorable for the adhesion of the graphitic particles. While drawing, graphitic deposits adhere