All-inkjet-printed "lab-on-paper" with Electrodes and Microchannels

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Keywords
printed electronics, micro-PADs, point-of-care, green chemistry

Abstract
A facile and novel fabrication method for paper-based micro-chips, usually called µPADs (microfluidic paper-based analytical devices) or "lab-on-paper", has been developed. Through inkjet-printing and green chemical integration technology, lab-on-paper was successfully fabricated. Conductive electrodes were printed on paper substrates prepared from highly-beaten cotton linter pulps. For patterning of microchannels, volatile organic compounds (VOC)-free UV-curable ink was printed as surrounding microchannel patterns followed by UV curing. For enzymatic electrochemical detection, poly(dopamine) (PDA) were used as an immobilization support of enzyme. By applying this method to determination of glucose concentrations, electrochemical response was successfully enhanced.

Introduction

"Lab-on-Paper"

"Lab-on-Paper", often called µPADs (microfluidic paper-based analytical devices) [1], is a new type of paper-based microchips mounting microchannels, and has recently attracted soaring attention for new point-of-care devices. Since paper is a biocompatible, low-cost and disposable material, it is suitable for home healthcare use and for use in developing countries.

As shown in Figure1, the microchannels used in the devices represent a unique concept, which allow realizing systems requiring only a small volume of sample without any external equipment for sample delivery because sample flow in the microchannels is derived from capillary force. The possibility to perform simultaneous multiple assays is also one of the features of the microchannel layout.

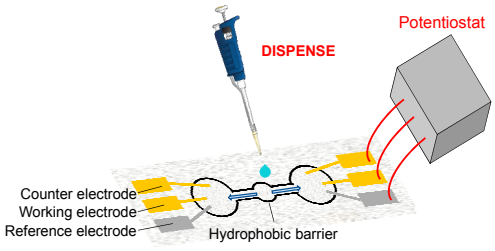

Figure 1. *Schematic of lab-on-paper*

Electrochemical sensing

Electrochemical detection in micro-chip is a well-studied method. However, lab-on-paper has been dominantly used as a colorimetric sensor. Although it has some advantages such as use of "cell phone camera" for detection and fairly inexpensive method, the background color from analyte samples like blood impairs the detection accuracy. On the other hand, since electrochemical detection isn't affected by colored sample, it is more suitable for blood test than colorimetric one. By using electrodes, electrochemical sensing can be conducted in lab-on-paper.

Fabrication methods for electrodes on filter paper have been reported too [5, 6]. In the reported works, electrodes were printed by screen-printing, but sensing reagents were dispensed by pipetting, which is unsuitable for mass-production. Inkjet-printed electrodes have been previously reported, however, there is a drawback that they require high temperature heating process which may cause damage to paper substrate.

The sensing systems of these methods are mainly based on enzymatic electrochemical method. Enzymatic electrochemical method has several useful features including selectivity and the variation of detection targets, though, in conventional methods, the immobilization of enzyme on electrodes was not found.

Enzyme immobilization

Enzyme has been used in electroanaylses conventionally. Enzymes are usually immobilized by some way in order to keep them from being detached from electrodes, and achieve stabilized electronic signals. There are various methods for enzyme immobilization such as covalent bonding, physical absorption, cross-linking, and incorporation. However, depending upon the kind of these immobilization methods, the activity of enzyme intensively fluctuates. Considering these methods comprehensively, another method to immobilize enzyme to electrodes is demanded.

Superior functions of poly(dopamine) (PDA) have been frequently reported since 2007 [7]. PDA has a great ability to immobilize biomolecules like enzyme [8]. In addition, it has also other useful functions including adherent ability to arbitrary substrates [9]. Therefore, it can be used as an immobilization support of enzyme.The facile preparation method of PDA nanoparticles was previously reported [10]. In conventional method, only pH and temperature control of dopamine solution are required for preparation of PDA nanoparticles. By using this method, enzyme supporting materials can be easily obtained.

Advantages of using inkjet technology

Inkjet technology is applicable to various types of materials, including biomaterials and low viscosity materials, as "ink" (ink means fluid materials for printing). This technology is not only reproducible but also offers low-cost production. It is suitable for the fabrication of entire lab-on-paper. Our previous work demonstrated a microchannel fabrication method by inkjet-printing technology [2, 3]. This method can not only fabricate microchannel-patterning, but also dispense sensing reagents.

The novelties of this work

The use of volatile organic compound (VOC) solvents and the required pre-treatment for the paper substrate are still hurdles for mass-production. In order to realize the commercialization for lab-on-paper, our group previously developed the "UV-curable fabrication method", which requires neither VOC solvents nor pretreatment process [4].

In this work, we have developed the fabrication method for lab-on-paper as an electrochemical sensor through inkjet-printing and green chemical integration technology. Not only microchannel patterning, but also electrodes can be printed without VOC solvents or heating process. For enzyme immobilization, PDA is used as an enzyme support. Therefore, this work has viability to establish a practicable method to fabricate lab-on-paper.

Experimental

Preparation of test paper

In order to improve the printability of paper substrate, test paper exclusively for this purpose was originally prepared from a cotton linter pulp under the optimized condition. An Ash-less filter pulp (Advantec Tokyo. Co., Japan), beaten 30,000 revolutions to 96 mL Canadian Standard Freeness in a PFI mill, was used in preparation of filter paper substrate without any chemical additives. Handsheets with a density of 0.67 g/cm^3 were prepared from the pulp slurries with filtered tap water. In the dehydration process, the handsheets were pressed at a pressure of 0.34 MPa for 5 min. For thorough drying, the resulted handsheets were kept to be attached to metal plates at 23°C and 50% relative humidity for one day. The resulted paper was used as printing substrates.

Printing of electrodes

We used a DMP-2831 (Fujifilm Dimatix Inc., Santa Clara, USA), a printer for research applications. As working and counter electrodes, PEDOT:PSS electrodes were fabricated. Gold electrodes were also fabricated for the same purpose. In addition, Silver electrodes were fabricated as reference electrodes. Unless otherwise stated, all chemicals were obtained from Wako Pure Chemical Industries (Osaka, Japan), Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), or Sigma-Aldrich (St-Louis, USA).

PEDOT:PSS electrodes were fabricated in the following process. PEDOT:PSS (Clevios P Jet HC, H.C. Starck GmbH, Frankfurt, Germany) was diluted to 0.3-fold concentration with a 10 %(v/v) ethylene glycol aqueous solution (PEDOT:PSS ink). Ultra-pure water (Millipore, Billerica, USA) was used for dilution. The PEDOT:PSS ink was printed on the paper substrates.

For fabrication of gold electrodes, an Au ink was printed on the paper substrates.

Silver electrodes were fabricated in the following process. To avoid the process of heating at a high temperature, the *in situ* reduction method of Ag ion previously reported [11] was applied. A 340 g/L sodium ascorbate aqueous solution prepared as a reducing ink was printed first on the paper substrates. Then, a 500 g/L silver nitrate – 10 %(v/v) ethylene glycol aqueous solution prepared as an Ag ink was printed exactly on the same location with the reducing ink.

Patterning of microchannels

We used a PX-101 model (EPSON, Suwa, Japan) office printer. UV-curable inks were prepared as reported previously. Briefly, monomers, purified by washing with 5 % NaOH solution several times following dissolution in hexane, were octadecyl acrylate and decanediol diacrylate mixed in a 70:30 weight ratio. The ratio of monomers to the initiator (2,2-dimethoxy-2-phenyl acetophenone or Irgacure 651) was 85:15 (w/w). Microchannel patterns were printed on the topside of substrates after the prepared UV-curable ink was loaded in clean cartridges. Next, the printed patterns were exposed for 60 seconds to UV-light (600 mW cm-2 at 365 nm) under LC-6 UV spot light source (Hamamatsu Photonics, Hamamatsu, Japan). In order to prevent a liquid sample from leaking from the channels three-dimensionally, rectangular patterns were also printed from the bottom side to cover the microchannel patterns. The rectangular patterns were also exposed to UV light under the same condition. (Figure 2)

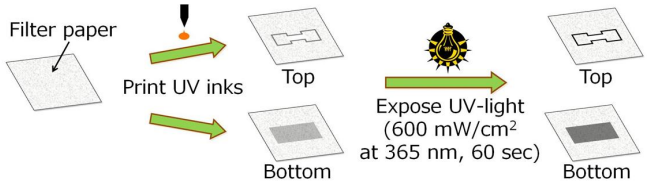

Figure 2. *Fabrication process of the microchannels with UV-curable inks*

Printing of PDA nanoparticles

PDA nanoparticles were synthesized according to previously reported process by Lee and co-workers [10]. Briefly, 180 mg of dopamine hydrochloride was dissolved in 90 mL of Ultra-pure water. Then, 0.76 mL of 1 M NaOH aqueous solution was added to the solution at 50 °C under vigorous stirring. After 5 hours, the dark brown solution was obtained. Approximately 74 mg of PDA nanoparticles powder was retrieved in the resultant solution by several times of centrifugation (20 kG) followed by freeze dry. One mg/mL PDA nanoparticles colloid prepared as a PDA ink was printed at the foot of both working and counter electrodes.

Electrochemical analysis for glucose solution

We used a three-electrode electrochemical analyzer (ALS/CHI Model 660A, CH Instruments, Inc., Austin, USA) for electrochemical analysis.

The mechanism of enzymatic electrochemical detection is shown in Figure 3. Briefly, when glucose is oxidized by Glucose oxidase (GOx), mediator is reduced, and then current can be obtained. Therefore, a good linear relationship between glucose concentration and current value can be seen.

For electrochemical detection of glucose, cyclic voltammetry (CV) and chronoamperometry (CA) (data not shown here) were used as electrochemical technics. By using CV, not only the information of redox peaks, and but also calibration curve of glucose concentration vs. current value can be obtained. As well as CV, calibration curve can be achieved from CA.

A mixture solution of 2.5 µL of 2 g/L GOx - 1 g/L peroxidase – 0.1 M KCl – 0.1M PBS buffer (pH 7.0) was applied to the inlet area of the fabricated lab-on-paper. After 20 min drying *in vacuo*, 5µL of the glucose standard solution (in 0.1 M KCl – 0.1 M PBS buffer (pH 7.0)) was applied to the inlet area. Electrochemical analysis was conducted after 2 min incubation for sample solution.

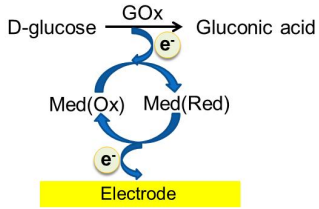

Figure 3. *Schematic of the reaction mechanism for glucose detection*

Results and Discussion

Fabricated lab-on-paper

Through these processes detailed above, lab-on-paper was successfully fabricated. Their appearance in the case of printing PEDOT:PSS ink is shown in Figure 4.

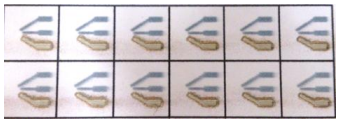

Figure 4. *Photograph of lab-on-paper*

Electrochemical performance of printed electrodes

A quantitative electrochemical analysis of glucose was conducted by using fabricated gold electrodes (data not shown). Although a good linear relationship between the observed current and glucose concentration (R2=0.979), it seemed to be difficult to achieve a reproducible performance of fabricated gold electrodes.

On the other hand, fabricated PEDOT:PSS electrodes showed more stable electrochemical performance than gold ones. Thus, we chose PEDOT:PSS as both working and counter electrodes.

A cyclic voltammetry of the fabricated lab-on-paper was conducted and the result is shown in Figure 5. Both of oxidation and reducing peak can be successfully observed.

Conclusions

We established a novel fabrication method for lab-on-paper. Due to the integration of several unique technologies, the realistic fabrication method applicable to industrial manufacturing process was demonstrated: (i) in order to improve printability of substrate,

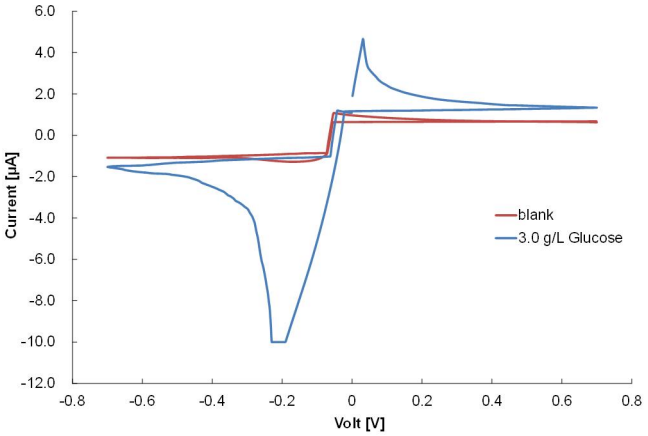

Figure 5. *CVs measured with fabricated lab-on-chip in the absence (red) and presence of glucose at 50 mV/s scan rate*

high density test paper was successfully prepared from a highly-beaten cotton linter pulp, (ii) three kinds of electrodes were fabricated respectively by printing on the paper substrate, (iii) microfluidic patterns were fabricated on the paper substrate surface by exposure to UV light after inkjet printing of hydrophobic UV-curable inks, and (iv) PDA nanoparticles were successfully prepared and printed at the foot of the electrodes as a support for enzyme immobilization. By using lab-on-paper fabricated as described above to detect concentrations of glucose solutions, electrochemical response could be successfully achieved.

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Author Biography

Kento Maejima received the B.S. degree in applied chemistry from Keio University, Kanagawa, Japan (2011) and he is pursuing his master of agriculture in biomaterial science at University of Tokyo (since 2011). His work has focused on the development of paper-based diagnostic microchips.