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Paper-based chemiluminescence immunodevice with temporal controls of reagent transport technique

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ABSTRACT

A paper-based chemiluminescence (CL) immunodevice for detection of three biomarkers with the temporal controls of reagent transport technique was developed in this work. Sugar barriers were built on the paper-based chip for the controlled reagent transport technique to create programmable flow delays for the later CL detection. Different amount of sucrose which can manipulate the reagent migration rate was drawn precisely on the microchannels by a craft-cutting instrument. The paper-based immunodevice with three channels was fabricated by the cutting method. Also the localized incision protocol with one side adhesive tape and paper-folding method were adopted here to isolate the detection zone from flow channels. This simple origami step of the detection zone can eliminate possible reagent diffusion and flowing during antibody immobilization steps and numerous washings. By using the gold nanoparticles (AuNPs) as the carrier for loading multi horseradish peroxidase (HRP) labeled signal antibody, greatly enhanced sensitivity was achieved by HRP catalyzed luminol CL system. Under a sandwich immunoassay, carcinoembryonic antigen (CEA), carcinoma antigen 125 (CA125) and carbohydrate antigen 199 (CA199) were detected on the paper-based chip. A limit of detection (LOD) of 0.03 ng/mL, 0.2 U/mL, 0.2 U/mL was demonstrated, respectively. We believe that this paper-based CL immunodevice with controlled reagent transport technique can provide a new strategy of sensitive detection of multi-biomarkers in point-of-care (POC) diagnostics field.

1. Introduction

Recently, microfluidic paper-based analytical devices have become a very promising platform for point-of-care (POC) diagnosis field [1]. Immunoassay has been performed on the paper-based device in POC [2–5] and different detection method including colorimetric [6], electrochemistry [7], electrochemiluminescence (ECL) [8] and fluorescence [9] has been used on the paper-based immunodevice. Besides, with the advantage of high sensitivity, wide calibration range and simple instrumentation, chemiluminescence (CL) [10–12] has also been a common detection method on the paper-based device. In combination with the microchannel on the paper-based device, the CL signal of luminol system was always recorded for tens of seconds [13] to get the maximum signal for the quantitative analysis. If the paper-based device was designed with multiple microchannels, different migration time in the microchannel should be obtained for capturing the CL maximum signal of the analyte.

As there was no supporting pump or pressure source on the paper-based device [14,15], the reagent migration on paper was entirely depended on the capillary force. However, if we want to get different migration time on paper, the common way was to design different length or width of microchannel on paper [16–18]. What is more, the reagent retention effect increased with length of the hydrophilic channel and the reagent migration would finally cease in a very long microchannel. Different reagent migration time cannot be achieved with the same length and width microchannel. To solve this problem, dissolvable sugar barrier technique was used in a few papers [19,20]. Different migration time in the same length microchannel was obtained in Fu's work [19]. Different amount of sugar was added in the channel to cause different time delay effect [19] in the microchannel. In addition, we noticed that the barrier in Fu's [19] work was established just by a pipette. The sugar barrier may not be added accurately with the same origin of the microchannel and this would influence the repeatability of the delay time. What is more, different time delay can also be obtained in Lutz's

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work [20] by using the dissolvable sugar on paper. Programmable flow delays were created with every microchannel wicking into the sucrose of different concentration. This mode, however, was not suitable for washing procedure on the paper-based immunodevice [21]. For, on the one hand, the sucrose would be washed off from the microchannel with the common washing, and on the other hand, cross contamination can be produced in the microchannel.

Therefore, a new and simple approach to constructing the dissolvable sugar barriers in the paper-based immunodevice was employed in this work. Different amount of sucrose was drawn on the microchannels by a craft-cutting instrument. The cutter of the cutting plotter [22] was changed to an assembly pen which can hold the sugar solution inside. With the setting origin, the sucrose can be precisely drawn on the same position on the microchannel. Thus, the reagents applied to each channel can arrive at the test zone at different time. The delay time from one channel to another was about 20 s and the maximum CL signal can be recorded in every detection zone. To prevent the washing solution from diffusing and spreading into the flow channels, an improved origami step [23–25] with an incision was used in this work. Single-sided adhesive tape was affixed to the microchannel to help making the incision. The origami strategy was much simpler and cannot influence the sugar barrier in the microchannel.

As CL detection was adopted in this work, enzymes such as horseradish peroxidase (HRP) [26,27] and alkaline phosphatase [28–30]) were often be used as the labels for CL immunoassay. Nowadays, more attention has been paid to various signal amplification strategies in the field of CL immunoassay to achieve higher sensitivity. Therefore, enzyme-functionalized nanostructured materials [31–37] have been adopted as the label for signal amplification function, because the increased enzyme loading toward nanoparticle can subsequently enhance the signal for target detection. Gold nanoparticles (AuNPs) [31,32], which possessed high surface-to-volume ratio and unique size-dependent optical properties, can be used as the enzyme-functionalized nanoparticle [34–37] or as the carrier [38–41] to load more signaling molecules for the signal amplification strategy. As a result, the signal amplification strategy was adopted for CL detection on the paper-based chip by using AuNPs as the carrier for loading multi HRP labeled signal antibody.

In this work, we described a paper-based CL immunodevice with the reagent controlled technique for the determination of three biomarkers. Temporal controls of reagent transport were achieved by drawing the sugar barrier precisely on the microchannel. As the drawing origin can be set by the cutting plotter, good reproducibility obtained with certain delayed time in the same length microchannel. What is more, multi HRP loaded AuNPs bioconjugates were used as the signal amplification strategy with luminol CL system. With the sandwich immunoassay mode, a limit of detection (LOD) of 0.03 ng/mL for CEA, 0.2 U/mL for CA125 and 0.2 U/mL for CA199 was performed in human serum on this paper-based immunodevice. What's more, multiplexed detection of cancer biomarkers by CL or electrochemiluminescence (ECL) method on paper-based device have been reported by other groups [24,42,43]. Luminol CL system was performed on the paper microzone plate in Wang's work [42] with LOD of 0.05 ng/mL for CEA and 0.33 U/mL for CA125. CEA and CA199 have been detected in Ge's paper [24] by CL method with LOD of 0.02 ng/mL for CEA and 0.06 U/mL for CA199. While, with ECL method [43], three biomarkers were detected with LOD of 0.6 U/mL (CA125), 0.17 U/mL (CA199) and 0.5 ng/mL (CEA). In comparison to the above CL or ECL methods, the sensitivity in our work was high and can be fulfilled in the real samples. The proposed paper-based CL immunodevice represented a new useful approach to the manipulation of fluid transport and established a transferable strategy for sensitive detection of biomarkers.

2. Experimental

2.1. Materials and reagents

Anti-CEA, anti-CA125, anti-CA199 (monoclone, Ab1); HRP labeled detection antibodies (Ab2); CEA, CA125 and CA199 (antigen) were purchased from Biocell Co. (Zhengzhou, China). Bovine serum albumin (BSA), NaH_2PO_4, Na_2HPO_4, Chitosan (90% deacetylation and a relative molecular mass of 400,000 g/mol), chloroauric acid ($HAuCl_4$) and glutaraldehyde were purchased from Sinopharm Group Chemical Reagent Company (Shanghai, China). Sodium citrate, sucrose and hydrogen peroxide were purchased from Xi'an Chemical Reagent Company (Xi'an, China). Luminol (3-aminophthalhydrazide) was obtained from Kangpei Technology Company (Xi'an, China).

Phosphate-buffered saline (PBS; 0.01 M, pH 7.4) which was prepared by mixing the stock solutions of NaH_2PO_4 and Na_2HPO_4 was used as washing buffer. Blocking buffer for the residual reactive sites was PBS containing 0.1% BSA. The 0.01 M stock solution of luminol was prepared by dissolving luminol (1.77 g) in 0.1 M sodium hydroxide solution and then diluted to 1 L with water. The luminol solution was stored in the dark for one week prior to use to ensure that the reagent had stabilized. Working solutions of H_2O_2 were freshly prepared by dilution of a 30% reagent solution (Shanghai, China). Sucrose was prepared every day from 50% (w/v) reagent solution. All chemicals and reagents were of analytical grade and used without further purification. Whatman chromatography paper #1 (WCP#1) (200.0 mm × 200.0 mm, 0.18 mm thick) was purchased from Sigma (U.S.A.). The clinical serum samples were provided by healthy adult volunteers from Shaanxi Normal University Hospital. Millipore Milli-Q water (18 MΩ/cm) was used in all experiments.

2.2. Apparatus

The CL intensity was detected and recorded with a model IFFS-A CL Analyzer (Xi'an Remex Electronic Sci. Tech. Co. Ltd., Xi'an, China). CL data acquisition and treatment were performed using RFL-1 software (Xi'an Remax, Xi'an, China). The paper was cut by the cutting plotter which was made by Graphtec Craft Robo-S (Graphtec Corporation, Japan). UV absorption spectra were measured on a UV-1800 spectrophotometer (Shimadzu, Japan). The transmission electron microscopy (TEM) images of AuNPs were taken by a Hitachi H-600 TEM (Tokyo, Japan). Dynamic Light Scattering (DLS) was used to see the size distributions of the particles on a photon correlation spectrometer (Brookhaven BI-90Plus, USA). The environmental scanning electron microscopy (SEM) images of bundle fiber were taken with Quanta 200 environmental scanning electron microscopy (Hitachi, Japan).

2.3. Fabrication of sugar barrier on paper-based chip

The fabrication of the paper-based chip and the sugar barrier on the chip was shown below. As illustrated in Scheme 1, the pattern of the device which consisted of three channels was firstly designed using CorelDraw X6. The design was then exported as an AutoCAD file into the controller software of a craft cutter, ROBO Master-Pro (Graphtec Corporation). Using a carrier sheet (Graphtec Corporation, Japan), the paper was cut by the cutting plotter (Graphtec Craft Robo-S, Graphtec Corporation). The cutting blade was 1 mm for cutting. The cutting pressure was 230 g and the cutting speed was 10 mm/s. The details of the dimension also can be seen from Scheme 1. Then the chip was prepared for later use.