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A novel microchip electrophoresis-based chemiluminescence immunoassay for the detection of alpha-fetoprotein in human serum

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ABSTRACT

A sensitive immunoassay method based on microchip electrophoresis chemiluminescence (MCE-CL) detection technology was developed for the detection of tumor marker alpha-fetoprotein (AFP). This method adopts the non-competitive immunoassay mode, and was conducted after AFP reacted with excessive horseradish peroxidase (HRP) labeled monoclonal antibody. The extreme pH value was adopted in the electrophoresis buffer solution. The use of brij 35 as an additive of electrophoresis buffer increased dramatically the resolution (Rs) and the reproducibility of the analysis. Under the optimized experimental conditions, effective separation of the immune complex Ag-Ab* and free Ab* was achieved within 60 s. The peak height of the immune complex Ag-Ab* was taken as quantification of AFP. Good linearity was observed within AFP concentrations ranging from 10 ng/mL to 150 ng/mL, and the detection limit was found to be 7.2 ng/mL (1.0×10^{-10} M). The present method was successfully applied for the detection of AFP in human serum from both healthy and cancer patients, and the AFP levels in the both were found be in the range of 16.5–23.4 ng/mL and 416.2–825.4 ng/mL, respectively.

1. Introduction

Alpha-fetoprotein (AFP), an oncofetal glycoprotein, is commonly used as a marker for hepatocellular carcinoma (HCC). Under healthy conditions, AFP comes from embryonic hepatocytes and its concentration begins to decline in fetal blood approximately two weeks after birth. In adult serum, the normal content of AFP is low than 25 ng/mL [1]. An elevated AFP concentration in serum may be an early indication of HCC, hepatoblastoma, and germ cell tumors [2,3]. Therefore, AFP is considered to be a specific clinical marker for the diagnosis of primary liver cancer. Similarly, AFP concentrations increase with disease progression in patients with pancreatic cancer, lung cancer, and hepatic cirrhosis [4,5]. Periodic detection of AFP concentration in human bodily fluids for the clinical analysis of treatment outcomes, prognosis assessment, prediction of recurrence and metastasis is very important.

Currently, the commonly used AFP detection methods include radioimmuno-assay [6], enzyme-linked immunoassay (ELISA) [7], chemiluminescence immune- assay (CL-IA) [8], capillary electrophoresis immunoassay (CE-IA) [9–11] and immunosensors [12,13]. These methods usually require long analysis times and complex liquid handling procedures. Additionally, these methods utilize expensive antibody reagents, which results in high costs, ultimately restricting their widespread application. In order to improve the sensitivity of AFP detection methods, a variety of nanomaterials including grapheme [14], carbon nanotubes [15], gold nanoparticles [16], PdNi nanoparticles [17] and mesoporous silica [18] have been utilized for signal amplification in the methods. In above mentioned methods, highly sensitivity was obtained, however, most of these methods require tedious surface modification of electrode or liquid handling procedures, which make the assays time-consuming. Therefore, it is necessary to develop a new immunoassay method with fast detection and high accuracy for clinical AFP detection.

The immunoassay, which is a detection method based on specific responses that arise from the binding of an antibody with an antigen, has high selectivity and sensitivity, and is therefore extensively used in clinical diagnoses and biochemical analyses [19]. However, routine immunoassay requires extensive amounts of expensive reagents, long analysis time and complex operation procedures.

Microchip electrophoresis (MCE) is a new separation assay technique developed after capillary electrophoresis (CE). MCE has the advantages of high efficiency, fast analysis, less sample and reagent consumption, and high automation and integration degree; therefore, it has been widely adopted in chemical analyses [20].

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Chemiluminescence (CL) detection is a highly sensitive detection system for MCE. When MCE-CL is used in conjunction with immunoassay, the resulting analyses have high selectivity of immunoassay and high sensitivity of CL detection. Therefore, it has been shown that the combination of microfluidic systems and immunoassay is a highly effective separation assay technique [21]. However, no study has reported the use of MCE-CL for AFP detection.

In the present study, a new non-competitive immunoassay method based on the MCE-CL technology was established for AFP detection. Following the reaction of AFP antigen (Ag) with an excess of HRP labeled anti-AFP antibody (Ab*), MCE-CL method was used to conduct AFP detection. Free Ab* and the immune complex (Ag-Ab*) were separated within 60 s. The method has been successfully used for the detection of trace AFP in human serum, confirming the utility of the assay.

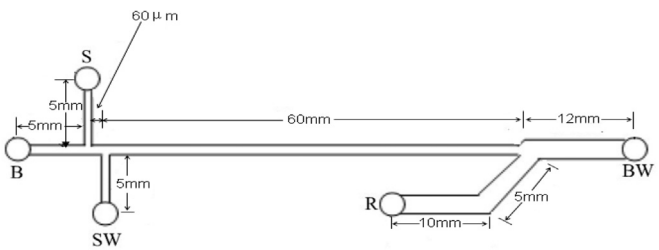
2. Experimental

2.1. Reagents and solutions

The AFP immunoassay kit and the horseradish peroxidase (HRP) labeled mouse anti-AFP monoclonal antibody were purchased from Zhengzhou Bioassay Biotechnical Co., Ltd. (Zhengzhou, China). Luminol was purchased from Fluka (Buchs, Switzerland). Brij 35, iodophenol (PIP) and H_2O_2 were purchased from Shanghai Chemical Company (Shanghai, China). All other chemicals were of analytical reagent grade and used without further purification. Water was purified by employing a Milli-Q plus 185 equip from Millipore (Bedford, MA), and used throughout the work. AFP and HRP-mouse anti-AFP monoclonal antibody were diluted using 20 mM phosphate buffer (pH 7.2). The electrophoresis buffer solution was 10 mM Na_3PO_4 (pH 10.2) and contained 0.004% (w/w) Brij 35 and 1.0 mM luminol. The oxidizer solution was 45 mM $NaHCO_3$ (pH 9.0) and contained 90 mM H_2O_2 and 1.2 mM PIP.

2.2. Experimental devices

The MCE-CL detection system was self-constructed in the lab [22]. The glass/polydimethylsiloxane (PDMS) microchip with an expanded Y-shape CL detection pool was designed according to the literature [23] (Scheme 1). The chip had an area of 9.5 cm by 2 cm. The top width of the channel measured 65 μm (except at R and BW, where the distance was 250 μm) and the depth measured 25 μm. The effective length of the separation channel was 60 mm. The distance from the buffer reservoir (B) to the T-intersection was 5 mm. The distance between the separation channel and the sample reservoir (S) or the sample waste reservoir (SW) was 5 mm. The two T-intersections were 60 μm. The sampling volume was calculated to be about 190 pL. The distance from the oxidizing agent reservoir (R) to the Y-intersection was 1.5 cm, and the Y intersection was 1.2 cm from the buffer waste reservoir (BW).



Scheme 1. Schematic diagram of the layout of the glass/PDMS microchip. S: sample reservoir; B: buffer reservoir; SW: sample waste reservoir; BW: buffer waste reservoir; R: oxidizing agent reservoir.

2.3. Immunoreaction procedure

The non-competitive mode was adopted for the immunoassay. The principle binding process was as follows:

Ag + Ab*(excess)—Ag − Ab* + Ab*

First, 10 μL of AFP antigens with different concentrations or the serum sample were mixed with 10 μL of 0.25 μg/mL HRP labeled mouse anti-AFP monoclonal antibody solution in a micro-centrifuge tube. The mixture was then diluted to 50 μL using the phosphate buffer (pH 7.4) and incubated at 37 °C for 40 min. The solution was then used for MCE-CL analysis.

2.4. MCE procedure

Prior to and between any two test of electrophoreses, all chip channels were rinsed thoroughly with 0.1 M NaOH, water and the buffer solution for 10 min each. All the channels were filled with the buffer solution under a vacuum negative pressure of ~50 mm Hg, and then, each corresponding reservoir was filled with different solutions for separation. Samples were injected in pinched mode. A voltage of 650 V was applied to S, while SW was connected to the ground. Meanwhile, B and BW were applied with 250 V and 400 V, respectively. The injection time was 15 s. During separation, 2600 V was applied to B, and BW was connected to the ground. Meanwhile, 1550 V was applied to S and SW to avoid samples leaking into the channels during separation. R was applied with 500 V. The sample components migrated to the Y-intersection, where they mixed with oxidizer solution to produce CL signal, which was then collected with an object lens (placed at the Y-intersection of the channels) and transferred to a photomultiplier tube (PMT). Finally, the signal was recorded via a HW-2000 chromatography work station (Zhejiang University Star Information Technology, Hangzhou, China).

3. Results and discussion

3.1. Optimization of the CL conditions

The pH of the oxidizer solution is a major factor affecting CL intensity. In this experiment, 45 mM $NaHCO_3$ was adopted as the buffer to study the influence of varying pH values (pH 8.0–10.0) on the CL intensity. The results are shown in Fig. 1a. It can be seen that the CL intensity first increased and then decreased with continuously increasing pH. When the pH was 9.0, the highest CL intensity was observed. Therefore, 9.0 was selected as optimal pH value of $NaHCO_3$ solution.

H_2O_2 was used as the oxidizing agent in the experiment; its concentration also plays a major role in CL intensity. The influence of the H_2O_2 concentration on the CL intensity was studied within the range of 50–120 mM. The results indicate that the CL intensity increased with the increase of H_2O_2 concentration between 50 and 90 mM. When the concentration was above 90 mM, CL intensity showed a downward trend as the H_2O_2 concentration increased. Therefore, 90 mM was taken as the ideal H_2O_2 concentration.

The PIP is a sensitizing agent that is extensively used in the luminol-H_2O_2-HRP CL system. PIP greatly increases the CL intensity of the system; therefore, its concentration has notable influence on the luminescence intensity. The influence of the PIP concentration on the CL intensity was investigated between 0.6 and 1.4 mM; the results are shown in Fig. 1b. As can be seen, the CL intensity increased gradually with increasing PIP concentration from 0 to 1.2 mM. After the concentrations are above 1.2 mM, the CL intensity decreased with increasing PIP concentration. Hence, 1.2 mM was selected as the optimal PIP concentration.