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Real-time immunoassay with a PDMS–glass hybrid microfilter electro-immunosensing chip using nanogold particles and silver enhancement

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Abstract

This paper presents the development of a PDMS–glass hybrid electro-immunosensing chip for real-time measurement of an antigen–antibody reaction through an electrical signal. Using a microfilter and microbeads, the antigen was easily immobilized in the detection zone where the microelectrodes were located. The immuno-reaction was detected by measuring the electrical resistance between microelectrodes using gold nanoparticles with silver enhancement. An immunoassay test with the developed chip was performed for the antigen of protein A, the specific first antibody of anti-protein A, the nonspecific first antibody of HBsAg monoclonal IgG, and the second antibody anti-rabbit IgG. The electro-immunosensing chip reduced the antigen–antibody reaction time to 10 min, thus reducing the overall assay time to about 1 h. The electrical resistance varied according to the concentration of the specific first antibody, the detection limit of which was 10 ng/ml. Compared to conventional enzyme-linked immunosorbent assays (ELISAs), the process of performing an immunoassay using the electro-immunosensing chip was relatively simple and required less time to complete. In addition, the electro-immunosensing chip required less sample volume.

Keywords: Immunoassay; Electro-immunosensing; Microfilter; Microbead; Nanogold particle; Silver enhancement; PDMS–glass microbiochip

1. Introduction

Immunoassays are among of the most important analytical methods for clinical diagnoses because of their extremely high sensitivity and specificity. The most commonly used immunoassay method is the enzyme-linked immunosorbent assay (ELISA), which is a very useful and powerful method for estimating antigen–antibody reactions [1–3]. The signal of a conventional ELISA is produced by an enzyme conjugated to a secondary antibody, and is often detected using fluorescence. Despite its usefulness, ELISA has several disadvantages, in that it requires a relatively long reaction time, a large number of routine processing steps, large sample volume consumption, and a complicated detection system. In order to overcome these drawbacks, current studies have focused on the miniaturization of immunoassay devices using microfluidic and microelectromechanical system (MEMS) technology [4–8]. Especially, many studies of miniaturized immunoassay devices have been based on microbiochips, as they allow for a short reaction time, small sample volume consumption, can be developed using an easy procedure, and can be mass-produced [8–23].

For immunoassays using a microbiochip, various techniques such as fluorescence [9–15], absorbance [16–20] and electrochemical [21–25] methods can be applied to detect the immuno-reaction. Among these methods, most immunoassay chips apply an optical detection method using fluorescence or absorbance [9–20]. However, this method requires large and expensive instruments such as a confocal laser scanning

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microscope, and such systems can be difficult to adapt to a portable microbiochip. Methods detailing the electrochemical detection of an immunoassay using a microchip have recently been reported [21–25]. These detection methods utilize an electrical signal resulting from a redox reaction of an enzyme tagged with an antibody, thereby demonstrating the potential of the miniaturization of detection systems. However, some enzymes are difficult to immobilize on a substrate or do not retain their enzymatic activity because of the instability of the protein. In addition, this immobilization method has low reproducibility due to denaturation resulting from the changes in the *in vitro* environments such as temperature, moisture, and pH [26]. Another electrochemical technique has been to use nanoparticles in the electrochemical detection of an immunoreaction because they have good physical properties such as a high surface-to-volume ratio, a unique absorption spectrum, and high electrical conductivity [27]. In addition, silver enhancement is often employed when using gold nanoparticles for immunoassays in order to amplify the electrochemical signal [28]. The usefulness of nanoparticles and silver enhancement has been demonstrated in an immunoassay utilizing a colorimetric microarray detection system [29]. Recently, gold nanoparticles and silver enhancement combined with gold microelectrodes have been used for determining DNA concentrations [30], suggesting a potential basis for an immunoassay that uses fast electrical detection by measuring the resistance between microelectrodes that have undergone silver enhancement.

Magnetic beads coupled with antibodies have been used previously for the electrochemical detection of immunoassays [22,31]. The use of microbeads has a number of advantages for microchips based on microfluidics. First, the surface-to-volume ratio is greatly increased, which results in increased sensitivity due to the higher efficiency of interactions between the samples and reagents. Secondly, the analytes attached onto the beads can be easily transported in a microfluidic system. Finally, various surface modifications are available for microbeads, thereby allowing for multiple functions in a single microfludic chip [32,33]. Indeed, the magnetic bead method is useful for the miniaturization of a bio-analytical system. However, a delicate procedure for the microfabrication of a planar electromagnet surface is needed for the separation and fixing of the microbeads. In addition, external devices such as a power supply are required to generate the magnetic field.

In this paper, we discuss the development of an inexpensive glass–PDMS (polydimethylsiloxane)-based electro-immunosensing chip that offers both a simple and accurate detection method. In order to immobilize bio-samples on the detection zone of the chip, we used microbeads and a pillar-type microfilter. The proposed immobilization method utilized the high mobility of microbeads, which provided a high efficiency of interaction between samples and reagents. By using the microfilter, external devices to fix microbeads were not necessary and any antibody that did not react with the antigen was easily filtered. Thus, we were able to immobilize the antigen on the detection zone wherever microelectrodes were located. The immuno-reaction was recognized by measuring the electrical resistance between the microelectrodes in the detection zone of the chip. Gold nanoparticles and silver enhancement were used to further improve the efficiency of the immunoassay. To minimize the time needed to complete the chip immunoassay, processing times needed for the immuno-reaction and the silver enhancement were optimized. The performance of the chip was also optimized for various concentrations of the first antibody.

2. Methods and materials

2.1. Detection principle

The detection principle of the immuno-reaction using an electrical signal is shown in Fig. 1. Microbeads covered with antigens are injected into the chip and are gathered over the electrode by the microfilter (Fig. 1(a)). The primary antibodies, which are specific to the antigen, are injected and the antigens bound on the surface of the microbeads capture the specific primary antibodies (Fig. 1(b)). Next, washing of the chip is performed. When the first antibody is nonspecific, the antigen–antibody reaction does not occur and the first antibodies are washed out. Second antibodies conjugated with nanogold particles are then injected. The second antibodies react with the specific first antibodies, and the resulting antigen–antibody complexes tagged with the

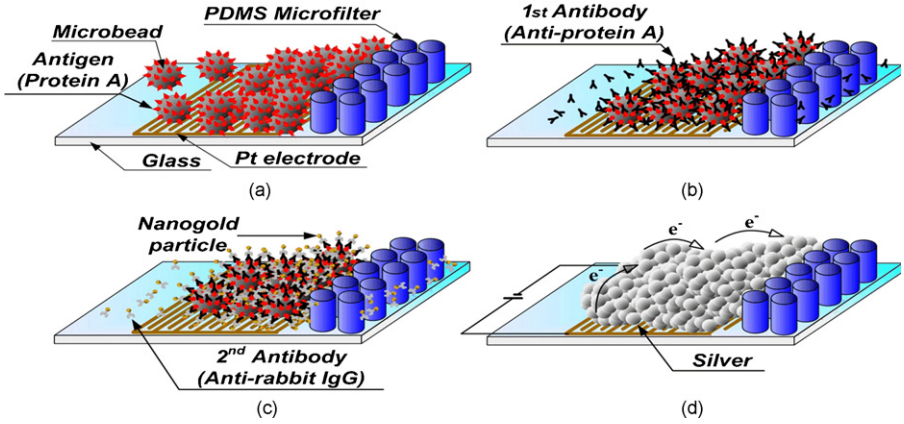

Fig. 1. Illustration of electrical detection immunoassay: (a) gathering of the microbeads covered with antigens, (b) antigen–antibody reaction, (c) reaction with gold-conjugated antibody, (d) signal enhancement with silver enhancement.