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Paper-based microfluidic sensing device for label-free immunoassay demonstrated by biotin–avidin binding interaction

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ABSTRACT

Efficient diagnosis is very important for the prevention and treatment of diseases. Rapid disease screening in ambulatory environment is one of the most pressing needs for disease control. Despite there are many methods to detect the results of immunoassays, quantitative measurement for rapid disease screening is still a great challenge for point-of-care applications. In this study, a fabrication method for depositing carbon nanotube bundles has been successfully developed for realization of functional paper-based microfluidic sensing device. Quantitative detection of label-free immunoassay, i.e., biotin–avidin binding interaction, was demonstrated by direct measurement of the current change of the biosensor after single application of the target analyte. Sensitivity of 0.33 μA/ng mL^{-1} and minimal detectable analyte concentration of 25 ng/mL were achieved. The time necessary for the detection was 500 s which is a large reduction compared with the conventional immunoassay. Such paper-based biosensor has the benefits of portability, fast response, simple operation, and low cost and has the potential for the development of rapid disease screening devices.

1. Introduction

Immunoassay is one of the widely used and standard diagnostic techniques nowadays [1]. This bio-analytical technique is based on the specific interaction between an antibody and its antigen. Hence, antigen of a particular disease can be detected by the known antibody. Conventionally, immunoassay is performed on a microplate and involves a series of washing, mixing, and incubation steps between each application of reagents. The entire process requires operating in a well-equipped laboratory and handling by well-trained personnel. That makes diagnostic service in hospital expensive and time-consuming. However, efficient diagnosis is very important for the prevention and treatment of diseases. The need of rapid disease screening devices for quantitative measurement that can operate in clinics or home has been emphasized recently [2–4]. Rapid disease screening for point-of-care (POC) applications often refers to devices which are portable, have fast response, are simple to operate, and are inexpensive [2]. Such technologies hold great impact on improving global health [3,4].

Microfluidic systems built by silicon, glass, or polymer substrates have been extensively developed for POC applications and a lot of excellent bio-analytical demonstrations have been reported recently [5–7]. Although these systems are much more simplified than conventional analytical instruments, they are still not readily accessible to untrained personnel. There are few commercialized microfluidic POC products in the market [8]. In recent years, paper-based microfluidics, or paperfluidics, has been proposed for a new class of POC diagnostic device because paper is inexpensive, thin, lightweight, and disposable [9]. Paperfluidics is realized by patterning sheets of paper into hydrophilic channels bounded by hydrophobic barriers based on the technologies such as photolithography [10,11], wax printing [12,13], polydimethylsiloxane (PDMS) printing [14], and plasma treatment [15]. The barriers define the dimension, i.e., width and length, of the channels and the thickness of the paper defines height of the channels. Therefore, aqueous solution can be passively transported by wicking through the paper fibers. Based on this development, biological analyses, e.g., glucose detection, on paperfluidics were demonstrated by determining the color intensity of the reacted sites visually [16]. Although the colorimetric detection is straightforward, changes of color intensity are difficult to distinguish for quantitative detection because of the influence of color perceptions of people and environmental illumination. To pursue quantitative analysis, digital devices, such as cell phone camera and photosensor, have been used for image capturing and analysis by computer software [17,18]. Moreover, electrochemical detection was also applied using paperfluidics for quantitative biological

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assays for detection of glucose, uric acid, and lactate [10,19,20]. Conductive carbon and silver pastes were screen-printed on paper to fabricate the detection electrodes. The aforementioned techniques could achieve an important feature of POC devices, i.e., the result was analyzed from a single application of the sample, but they could only detect an enzymatic reaction. Moreover, many nanoparticles were used for the paper-based immunosensor, such as gold nanoparticles, quantum dots, and graphene [21]. Gold nanoparticles and quantum dots were demonstrated to be used in lateral-flow test-strip devices [22–25]. They worked as a label conjugated to secondary antibody for generating optical or electrochemical signal. In addition, graphene was used as a working electrode for the electrochemical detection of immunoassay [26,27]. In this demonstration, horseradish peroxidase (HRP) was used as a label to generate detectable signal detected by graphene electrode. The above excellent demonstrations still rely on a label to generate detectable signal of immune-reaction. Using a label can generate detectable signal; but, it might induce extra operations and time cost.

In this work, we propose a method to fabricate carbon nanotube (CNT)-based biosensor on paper for implementation of a quantitative label-free immunoassay and demonstration of the detection of a biotin–avidin binding interaction. Result can be directly measured by the current change of the biosensor after single application of a sample. The biotin–avidin binding interaction has high affinity and specificity and a lot of standard reagents utilize this binding interaction for diverse detection schemes [28,29]. Because the biotin–avidin interaction is well known and stable, we used it to develop our paper-based microfluidic sensing device. CNT was used as the biological transducer because the conductivity of CNT is highly related to the protein binding on its sidewall. A number of papers in literature have reported that a functionalized single CNT across a pair of metal nano-electrodes can work as label-free protein biosensor [30–33]. However, the difficulty of processing a single CNT is a hurdle for its practical realization. Therefore, use of CNT bundles across the electrodes has been proposed to realize individual functional device. Temperature sensing and pH monitoring of analytes have been respectively demonstrated using the CNT-deposition techniques of ac electrophoresis [34] and spray method [35]. Here, we have adopted a vacuum filtration method to deposit CNT bundles with well-defined dimension to fabricate the biosensor on paper. This method was originally developed for the fabrication of conductive CNT films for use in light-emitting diode (LED) [36–38]. It reported that the method provides deposition of CNT with homogeneity and controlled thickness [36]. We have re-examined it and successfully fabricated a CNT-based sensor on paper to monitor the pH value of the analyte solutions [39,40]. Based on these developments, we have functionalized the CNTs and fabricated a biosensor on paper for label-free immunoassay. Biotin was immobilized on CNT's sidewall and CNTs conjugated to biotin were then deposited on paper by vacuum filtration method to form the biosensor. Avidin suspended solution was applied to the biosensor and their binding interaction was measured by the current change of the biosensor. The CNT-based biosensor showed a sensitivity of 0.33 μA/ng mL^{-1} and a minimal detectable analyte concentration of 25 ng/mL. Such detection is a label-free method and the result of immunoassay can be quantitatively analyzed from single application of sample, which is suitable for POC applications. By demonstrating label-free biotin–avidin detection, label-free immunoassay of specific disease can be extended by replacing the proven antigen–antibody complex.

2. Materials and methods

2.1. Chemicals and reagents

Commercially available single-wall CNTs were used in this study and purchased from Centron Bio-chemistry Technology Co., Taiwan. Filter paper of 800 nm pore size was utilized and purchased from Whatman, USA. Biotin, avidin, bovine serum albumin (BSA), N-Hydroxysuccinimide (NHS), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), hydrochloric acid (HCl), and sodium hydroxide (NaOH) were obtained from Sigma, Taiwan. PDMS material (Sylgard®184) was purchased from Dow Corning, USA. Buffer used throughout this study was phosphate-buffered saline (PBS; 0.2 M Na_2HPO_4, 0.2 M NaH_2PO_4, pH 6.7).

2.2. Fabrication of the CNT-based biosensor

The fabrication process of the CNT-based biosensor on paper is illustrated in Fig. 1. The CNTs were functionalized based on the reported protocol [41]. The CNTs were bathed in HCl under sonication for 0.5 h to create acid functionality through carboxyl groups (–COOH) on CNT's sidewall. Acid treated CNTs were washed in deionized water, collected by filtration, and dried. The treated CNTs (0.6 mg) were dispersed in PBS buffer (15.5 mL) under sonication for 2 h. NHS solution (2.3 mL of 50 mg/mL) and EDC solution (1.2 mL of 36 mg/mL) were then added to the dispersion and stirred for 0.5 h. Thereafter, biotin solution (1 mL of 20 mg/mL) was added and the solution was then stirred for 24 h at 4 °C followed by the centrifugation and repeated washing with deionized water. Therefore, biotin functionalized CNT suspended solution was prepared for the deposition process. On the other hand, the filter paper was treated by the blocking process (0.1% BSA in PBS) in order to eliminate the non-specific protein binding. Vacuum filtration process was then conducted using a metal mask covering on the filter paper for defining the geometry of the biosensor. The metal mask contained openings of the patterns of the biosensors. It was designed by computer software and

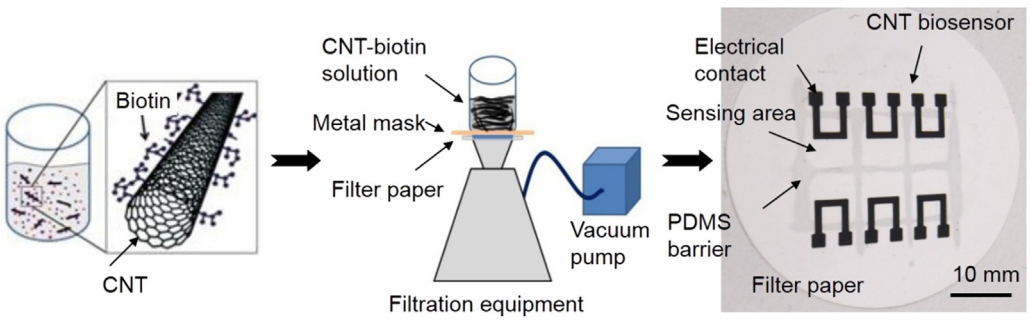

Fig. 1. Fabrication process of the CNT-based biosensor on paper based on vacuum filtration process and photograph of the biosensors with PDMS barrier.