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Development of a Paper-based Carbon Nanotube Sensing Microfluidic Device for Biological Detection

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Abstract—**Carbon nanotube (CNT) has been utilized for the biological detection due to its extremely sensitive to biological molecules. A paper-based CNT sensing microfluidic device has been developed for the detection of protein, i.e., biotin-avidin, binding. We have developed a fabrication method that allows controlled deposition of bundled CNTs with well-defined dimensions to form sensors on paper. Then, polydimethyl siloxane (PDMS) was used to pattern the hydrophobic boundary on paper to form the reaction sites. The proposed fabrication method is based on vacuum filtration process with a metal mask covering on a filter paper for the definition of the dimension of sensor. The length, width, and thickness of the CNT-based sensors are readily controlled by the metal mask and the weight of the CNT powder used during the filtration process, respectively. Homogeneous deposition of CNTs with well-defined dimensions can be achieved. The CNT-based sensor on paper has been demonstrated on the detection of the protein binding. Biotin was first immobilized on the CNT's sidewall and avidin suspended solution was applied to the site. The result of the biotin-avidin binding was measured by the resistance change of the sensor, which is a label-free detection method. It showed the CNT is sensitive to the biological molecules and the proposed paper-based CNT sensing device is a possible candidate for point-of-care biosensors. Thus, electrical bio-assays on paper-based microfluidics can be realized to develop low cost, sensitive, and specific diagnostic devices.**

I. Introduction

In the past decade, carbon nanotubes (CNTs) have been reported to have excellent electrical, mechanical, chemical, and structural properties. However, difficulties of processing a single CNT to be a functional device have hampered the practical realization of their properties. CNT bundles have been proposed to work as an individual functional device. Thermal sensing has been demonstrated and the CNT bundled device showed very low power consumption [1]. CNT-based devices are suggested to have some advantages: small size with larger surface; fast response and good reversibility [2] and easy functionalization [3]. Paper-based microfluidics, or microfluidic paper-based analytical devices (μPADs), has been attracted attention recently for a new class of diagnostic devices [4]. Many functions in conventional analysis system including sample manipulation and detection can be performed on a sheet of paper. Paper-based microfluidics is a promising technology for the development of diagnostic assays in developing countries and harsh environments because of their low cost, simplicity, flexibility, and disposability. Paper has a number of advantages compared with the conventional substrates, i.e., silicon, glass and polymer, using in microfluidic devices. Paper is inexpensive, thin, lightweight, available in a wide range of thickness, and disposable. Paper can transport aqueous fluids by wicking and passive pumping is realized. Well-defined pore sizes in paper can be manufactured that can separate suspended solids from samples before the bio-assays. Paper is biocompatible with various biological samples and can be modified by a wide range of functional groups that can be covalently bound to proteins. Fabrication of the paper-based microfluidic devices can be realized by patterning sheets of paper into hydrophilic channels bounded by hydrophobic barriers. The hydrophobic barriers can be fabricated by different methods such as photolithography [5, 6], wax printing [7, 8]. The patterned barriers define the shape, width and length, of the paper-based microfluidic channels and the thickness of the paper defines the height of the channels. The aqueous fluids can be transported along the channels by wicking in the hydrophilic fibers of paper. Because of their advantages, paper-based microfluidic devices have the potential to be good alternative for diagnostics over traditional microfluidic systems.

Based on our previously proposed fabrication method of CNT-based sensor on paper substrate [9], a paper-based CNT sensing microfluidic device is proposed and demonstrated on the detection of biotin-avidin binding activity. CNT-based sensor was fabricated directly on a sheet of paper to form the device. Biotin was first immobilized on the CNT's sidewall and avidin suspended solution was applied to the reaction site, which is defined by the PDMS boundary. The biotin-avidin binding activity was measured by the resistance change of the sensor. Such detection is a label-free method and does not rely on bulk equipment. The proposed device has a high potential to be develop to a low cost, simple, and specific point-of-care diagnostic device.

II. Materials and Methods

A. Materials

Paper used in this study was 800nm pore size filter paper from Whatman, USA. Multi-wall CNTs was purchased

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commercially from Centron Bio- chemistry Technology Co., Taiwan and prepared by chemical vapor deposition (CVD). Biotin, avidin, N-Hydroxysuccinimide (NHS), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydro-chloride (EDC), bovine serum albumin (BSA), sodium dodecyl sulfate (SDS), and Bio-Rad Protein Assay (Dye Reagent Concentrate) were purchased from Sigma, USA. Polydimethylsiloxane (PDMS) (Sylgard® 184) was purchased from Dow Corning, USA. Buffer used in this study was phosphate-buffered saline (PBS; 0.2M phosphate (Ha_2HPO_4), 0.2M phosphate (NaH_2PO_4), pH6.7).

B. Fabrication method of protein-immobilized CNT-based sensor

The paper-based CNT sensing microfluidic device mainly consists of CNT-based sensors and their corresponding reaction sites bounded by PDMS polymer. Multi-wall CNTs were used as received without any further purification or intentional doping. The axial dimension and the diameter of the CNTs provided from the manufacturer were 5-15μm and 10-20nm, respectively. To prepare the protein-immobilized CNT-based sensors on paper, the CNT power was first bathed in hydrochloric acid (HCl) under sonication for 2 hours to create acid functionality mainly through carboxyl groups (-COOH) on the sidewalls of the CNTs. Then, acid treated CNTs were washed in running DI water and then dried. The treated CNTs (1.2mg) are dispersed in PBS buffer (5.5ml) and then bath-sonicated using a 150W sonicator for 1 hour. After bath-sonicated, 2.3ml NHS solution (50mg/ml) and 1.2ml EDC solution (10mg/ml) were then added to the dispersion and stirred for 30 min at room temperature. Hence, 1ml biotin solution (10mg/ml) was added and then stirred for 24 hours at 4°C. Hence, the solution was performed by centrifugation and repeated washing with DI water. Therefore, biotin functionalized CNTs were prepared for the fabrication of the CNT-based sensor on paper. Vacuum filtration process was then performed using a metal mask covering on a filter paper for the definition of the dimension of the sensors, as shown in Figure 1. The metal mask was design by computer software and fabricated by the laser micro-machining. The filter paper was the substrate where the CNT-based sensors were located. During the process, the biotin-immobilized CNT suspended solution was poured into the container of the vacuum filtration equipment and sucked by the vacuum force. As the solution went through the opening of the metal mask and the pores of filter paper, the CNTs were trapped on the paper surface, forming a homogeneous layer. The filter paper was then removed from the vacuum filtration equipment gently and dried in a low temperature dehumidifier. Hence, the CNT-based sensors could be fabricated on the paper. In this study, a fixed widths and length of the sensors were fabricated on a sheet of paper. There were two $2\times2mm^2$ square blocks at the ends of the sensors for the electrical contacts for further characterization experiments. For the control of the thickness of the sensors, weight of the CNT powder used can determine

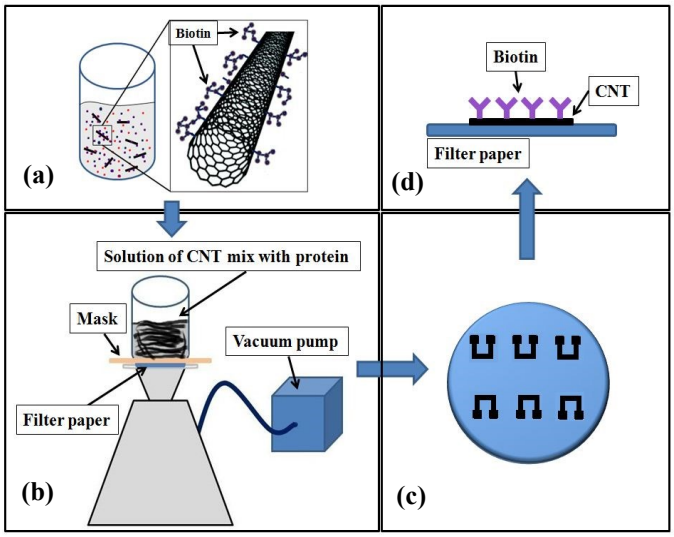

Figure 1. Fabrication process of the paper-based CNT sensing microfluidic device. (a) Immobilization of biotin on CNT's sidewall. (b) Deposition of CNT on paper using vacuum filtration process. (c, d) CNT-based sensors on a paper.

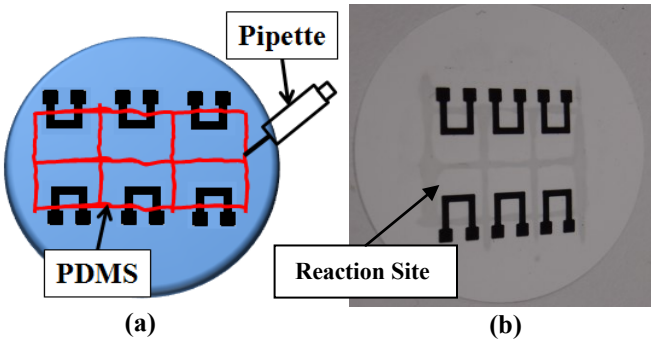

Figure 2. (a) Schematic representation of the application of PDMS mixture by pipetting. The red lines represent the trajectory of the PDMS application. (b) Photograph of the PDMS boundary on the paper-based CNT sensing microfluidic device.

the deposition thickness during the vacuum filtration process. More CNT powder used can fabricate thicker sensors. In our experiment, 1.2mg CNT powder was dispersed in 10ml buffer as the master solution. During the vacuum filtration process, a certain volume of CNT suspended solution was used to fabricate the sensors on paper. The volume was calculated by the proportional weight of the CNT in the master solution. For example, 1ml master solution contained 0.12mg CNTs. Therefore, the length, width, and thickness of the CNT-based sensors on paper can be well-controlled using this fabrication method. The paper was still flexible and the CNT-based sensors did not show cracks on the surface under microscopic observation.

C. Confirmation of the biotin immobilization on CNT's sidewall

Protein assay was used for the investigation of the biotin immobilization on CNT's sidewalls. BSA formulated solution

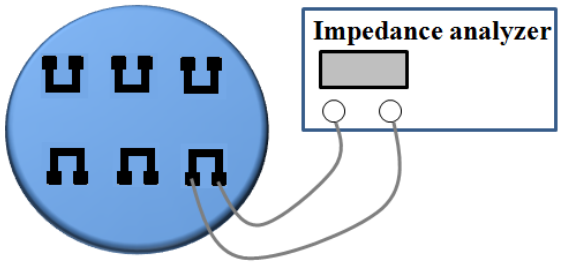

Figure 3. Experimental setup of the measurement of the CNT-based sensor.

was utilized to generate the standard curve. During vacuum filtration process, the remnant solution was collected to analysis the biotin concentration. Enzyme-linked immunosorbent assay (ELISA) is used to measure the optical density (OD) values of the BSA formulated solution and the remnant solution. The biotin concentration can be estimated by the comparison with the BSA formulated solution. Therefore, with the known concentration of the original biotin solution, the concentration of the biotin on CNT can be estimated.

D. Fabrication of hydrophilic reaction sites on paper

PDMS is widely used to fabricate the microfluidic systems for biological and chemical applications because of its advantages of optical transparency, non-toxicity, chemical resist, and biocompatibility. Patterning paper using PDMS material for paper-based microfluidic device can receive the above benefits. Moreover, because PDMS is an elastomer, the patterned paper could be bent and folded without destroying the integrity of the channels. Also, since organic solvents are not involved in the fabrication process, hydrophilic reaction sites could retain the original purity. PDMS patterned paper-based microfluidic device has been successfully fabricated, as shown in Figure 2. The PDMS mixture was prepared by thoroughly mixing the PDMS pre-polymer and curing agent in a weight ratio of 10:1. The PDMS mixture was then degassed under a vacuum chamber until the air bubbles escaped from the mixture. Then, it was applied by a pipette on paper along the pre-designed trajectory and cured at 4°C for 2 hours. Finally, the hydrophilic reaction sites can be formed across the CNT-based sensors, as shown in Figure 2(b).

III. RESULTS

A. Characterization of the CNT-based sensor

The CNT-based sensors were loaded onto a probe station for the I-V measurement. Potentials from -5 to 5V with 0.1V step were applied respectively across the sensors by an impedance analyzer (VersaSTAT 4, Ametek, USA). The sensors were in dry and atmospheric condition during the measurement, and the process is shown in Figure 3. I-V characteristic of the CNT-based sensors is shown in Figure 4.

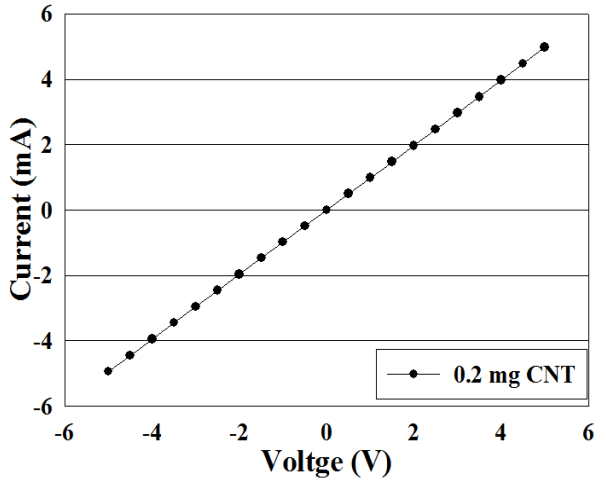

Figure 4. The I-V characteristics of the CNT-based sensors.

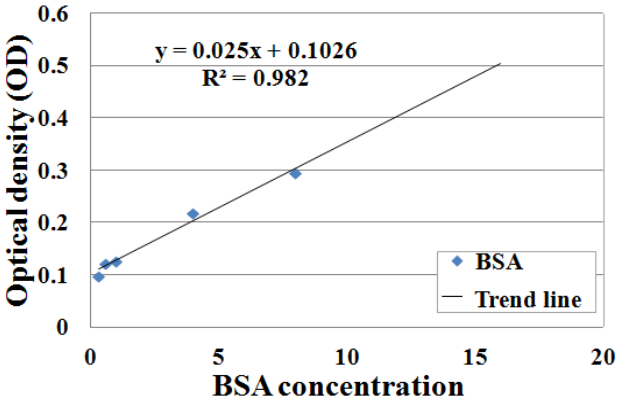

Figure 5. Standard curve of the BSA formulated solution..

It showed a linear relationship between voltage and current, which is a stable resistance characteristic. Vacuum filtration provides a repeatable and well-controlled method for depositing the bundled CNTs for fabricating a functional CNT-based sensor.

B. Estimation of the biotin concentration on CNT

The standard curve of the BSA formulated solution is shown in Figure 5. The protein concentration was represented by OD value. The BSA concentration was in 0.3-8μg/ml. The OD value of the remnant solution was then measured. Because biotin in 1mg/ml was utilized for the immobilization, the determination of biotin concentration in remnant solution can estimated the biotin concentration on CNT. The OD values of the BSA formulated solution and the remnant solution were summarized in Table I. Therefore, the biotin concentration on CNT can be estimated to be 0.994mg/ml. The protein assay provided an evidence for the biotin immobilization on CNT's sidewall.

C. Biological detection

The avdin suspended analyte solution (10μl) was applied to

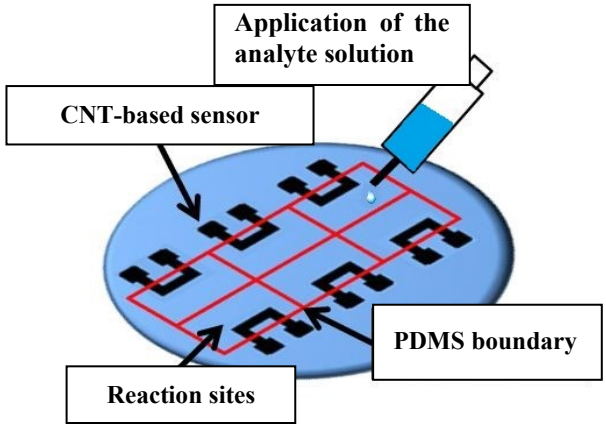

Figure 6. Illustration of the experimental process for the protein detection using the proposed paper-based device.

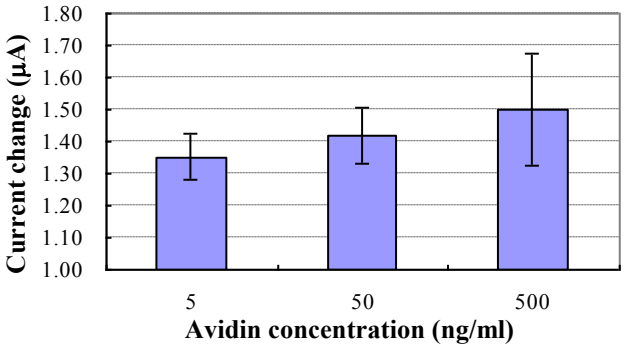

Figure 7. The relationship of the avidin concentration and the current change of the sensor.

the hydrophilic reaction sites to study the biotin-avidin binding response using the CNT-based sensor, as shown in Figure 6. When the avdin solution is applied to the site, the sensor was wetted. The biotin molecules on the CNT's sidewall caught the avdin molecules and the conductivity of the sensor was affected. Potential of 0.1V was applied across the sensor and its current change was measured before and after the application of analyte solution. It was observed that the current change of the sensor was proportional to the concentration of the avidin, as shown in Figure 7. This preliminary result showed the CNT is sensitive to the biological molecules and the proposed paper-based CNT sensing device is a possible candidate for point-of-care biosensors.

IV. Conclusion

In this study, a paper-based CNT sensing microfluidic device has been developed and biotin-avidin binding activity has been detected by the CNT-based sensor. It shown the CNT-based sensor is a possible candidate for biological biosensors. It is expected that the CNT-based sensor can detect more and more biological molecules. According to this preliminary result, it will have more applications on paper-based CNT sensing microfluidic device.

TABLE I. The OD values and corresponding concentrations of the BSA formulated solution and the remnant solution

BSA formulated solution					
BSA Concentration (μg/ml)	0.3	0.6	1	4	8
OD (a.u.)	0.098	0.122	0.127	0.219	0.295
Remnant solution					
OD (a.u.)	0.254				
Biotin Concentration (μg/ml)	6.056				
Biotin immobilized CNT's sidewall					
Biotin Concentration (μg/ml)	994				

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