

A HIGH SENSITIVITY CARBON NANOTUBES ENHANCED PZT DIAPHRAGM-BASED IMMUNOSENSOR ARRAY

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ABSTRACT

In this paper, a high sensitivity carbon nanotubes (CNTs) enhanced piezoelectric diaphragm-based immunosensor array for detecting anti-goat IgGs is reported. Anti-goat IgGs with 6 different concentrations was successfully detected at the same time. The average diameter of CNTs was characterized by AFM, changing from original 58 nm to 82 nm after absorbing goat IgGs. A nearly linear relationship between the frequency depression and concentration of anti-goat IgGs was obtained. The sensitivity (~ 227.68 Hz/ng) of the biosensor with CNTs is almost 6 times higher than that of a same type sensor without CNTs enhancement.

KEYWORDS

Carbon nanotube, PZT, Diaphragm, Immunosensor.

INTRODUCTION

Among the numerous reports on piezoelectric biosensors available in the literatures, micro-machined cantilever, quartz-crystal microbalance system (QCMS) and membrane biosensors are the three main players. Although, micro cantilever based piezoelectric biosensors are still the most popular device because of its high sensitivity and label-free detection [1-3], some problems, such as low quality merit factor, the fragility of the devices, are hard to be solved due to the physical structure limitation of cantilevers.

Therefore, QCMS has been developed to overcome these problems in piezoelectric cantilever biosensors [4-6]. The reported QCMS did take over cantilever biosensors in some applications due to its good frequency stability and reproducibility. As the new diseases continuously emerge, a low cost biosensor array with rapid response is strongly demanded. Unfortunately, QCMS is unable to full fill the requirements as the solid quartz crystal lacks of integration.

In order to address this issue, micro-diaphragm based piezoelectric biosensors have been recently investigated [7-8]. Our previously work reported a mass sensitive piezoelectric diaphragm biosensor array for multiple immunoassays. The sensor was developed by MEMS fabrication techniques and two different bio-entities were successfully detected simultaneously. The mass sensitivity of the device was estimated to be about 6.25 Hz/ng [9]. Nevertheless, this sensitivity still doesn't yet match the practical requirements.

Sensitivity and limit of detection are the two crucial

measures to judge the performance of biosensors. At the same concentration of target analyte, a sensor with higher sensitivity can generate a stronger output signal. This is very important in the clinic applications because it can provide clearer and more accurate health information on the patients. On the other hand, the limit of detection refers to the capability of detecting the minimum concentration of the analyte. It is useful for the doctors to provide effective treatments or monitoring for the patients in their early stage of illness. Hence, it is necessary to develop a piezoelectric diaphragm-type biosensor array with high sensitivity and high limit of detection.

To improve the sensitivity of the above-mentioned biosensor, the sensing surface of a biosensor needs to be modified. Among various reported methods and materials, gold nanoparticles are the mostly used material because of its unique physical property that provides a three dimensional platform to enhance the sensitivity of the biosensors [10-12]. However, this method is not suitable for micro-machined piezoelectric diaphragm-based biosensors. Because of the high density of the gold, the deposited gold particles may cause the micro mechanical deformation of the thin piezoelectric diaphragm due to the relatively heavy weight of gold. In addition, the gold particle modified platform also has reliability problems during the immobilization process due to the point contact between the particles and the plan surface. As such, CNTs will be used as an alternative material to gold particles in this study. One of the extraordinary features of CNTs would be its extremely high surface area, which ranks the highest among all nanomaterials. Theoretical calculations have predicted that the specific area for multi walled CNTs can be as high as 400 m²/g [13-15]. At the same concentration, a larger number of probe molecules could be adsorbed on the surfaces or inside the tubes for interacting with target molecules. Therefore, CNTs serve as an amplifier to enhance the output signals. As the results, the sensitivity and the limit of detection are dramatically increased [16]. Recently, some researchers demonstrated that CNTs are able to enhance the electrochemical reactivity of some important biomolecules [17-19] and useful for label-free electrochemical detection of DNA hybridization [20]. However, in the reported methods, CNTs were very often deposited on the electrodes with applied voltage, which may cause certain damage to the proteins and make the testing process unstable and inaccurate. Hence, in this paper, CNTs were deposited on the piezoelectric

diaphragm. The high specific area of CNTs was used to absorb more bio-molecules to enhance the sensitivity and the limit of detection the mass sensitive biosensor array.

FABRICATION OF PIEZOELECTRIC DIAPHRAGM-BASED BIOSENSOR ARRAY

Fabrication Processes

The piezoelectric sensor array was fabricated by standard MEMS fabrication technology with five main steps. Firstly, a thin TiO_2/Pt (15/200 nm) layer was deposited on the both side of a silicon-on-insulator (SOI) wafer as the bottom electrode. Afterwards, a thin $\text{Pb}(\text{Zr}_{0.52}\text{Ti}_{0.48})\text{O}_3$ (PZT) film ($\sim 0.7 \mu\text{m}$) was deposited by the sol-gel deposition technique [21]. One spin coating process offered a thin layer with thickness of 50 nm, and the process was repeated 15 times to achieve a desired thickness. In the third step, the PZT film was patterned and etched in the specific areas to open the access to the bottom electrode. In order to minimize parasitic capacitance induced by the patterned electrode wiring, a Si_3N_4 layer ($\sim 350 \text{ nm}$) was deposited to serve as an insulation layer. A Ti/Pt (10/200 nm) film was sputtered and patterned as the top electrode. Finally, the bulk silicon in the backside was etched by holes deep reactive ion etching (DRIE).

Fabrication Results

Figure 1 (a) shows the optical images of the fabricated sensor array which consists of eight individual sensors. The two large rectangular pads and the eight square pads with number are the bottom electrode and top electrode respectively. Despite the independent top electrodes, all the sensors share the same bottom electrode.

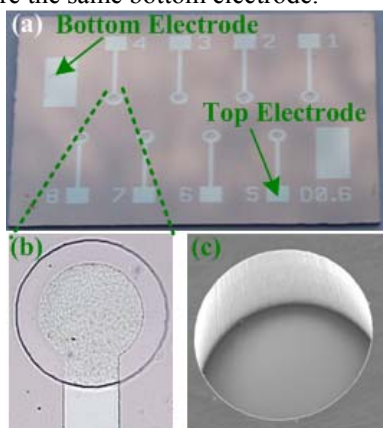


Figure 1. Images of the fabricated biosensor array. (a) Top view of an optical image of the device. (b) Enlarged optical image of the active PZT diaphragm. (c) SEM image of the reaction chamber on the backside of the diaphragm.

The enlarged image of an active PZT diaphragm is shown in Figure 1 (b), in which the silver color top electrode is smaller than the circular diaphragm. On the backside of the sensor array, there are eight DRIE-etched holes that serve as reaction chambers during the

bio-immobilization process and they are located exactly underneath the PZT diaphragm. The SEM image of one chamber with a diameter of 0.6 mm is presented in Figure 1(c).

To increase the sensing surface area, pre-synthesized CNTs by thermal vapor deposition were coated onto the PZT diaphragm. Due to the hydrophobic nature of CNTs, they need some chemical and physical treatments before dispersing into solution. The CNTs were firstly mixed into a dilute sodium dodecyl sulfate (SDS) solution which was centrifuged for an hour at 50°C followed by a sonication for 4 hours at 40°C . Afterwards, the CNTs become hydrophilic and are uniformly distributed in the solution, and ready for the subsequent bio-immobilizations.

Bio-immobilization

To investigate the effect of the CNTs on the detection sensitivity, two identical sensor arrays were used during the experiment. Before the bio-immobilization, one sensor array was firstly deposited with CNTs. The sketched immobilization processes are depicted in Figure 2. Firstly, the silane (5%) functionalized CNT solution and silane (5%) solution were dropped into the reaction chambers in the first and second sensor array, respectively. After 45 min deposition at room temperature, the reaction chambers were washed by ethanol. Secondly, goat IgG (100 $\mu\text{g}/\text{ml}$) was added in both sensor arrays. The two sensor array was loaded into the culture chamber ($\sim 37^\circ\text{C}$) for 30 min, and then washed by tris-buffered-saline (TBS washing buffer, pH8.0) and de-ionized (DI) water and dried under nitrogen (N_2) air flow. Afterwards, blocker caseins (1 $\text{mg}/\mu\text{l}$) were added to block the unoccupied space to avoid any unexpected physical absorption. The excess blockers were washed away by TBS and DI water. Lastly, anti-goat IgGs with concentration of 1, 10, 100, 1000, 10000 and 100000 ng/ml were dropped into 6 sensors in each array for the hybridization between the antigens and antibodies. The two remaining sensors without adding any antibodies were used as references.

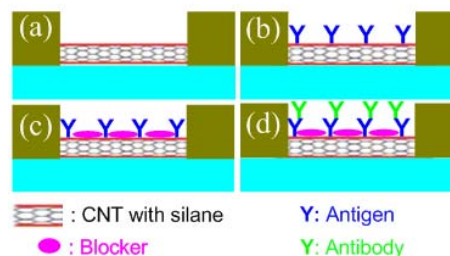


Figure 2. Sketched immobilization processes for the CNT enhanced PZT biosensor.

CHARACTERIZATION

Physical structure of CNTs

Figure 3 shows the FSEM and AFM images of the CNTs. Figure 3(a)-(b) shows the overall view of the CNT forest before and after bonding with goat IgGs. Both images were taken with the same scale. It is obvious that CNTs in figure 3(b) have larger diameter than that in figure 3(a). To have a further study of the diameter change of CNTs, AFM was conducted and the images were shown in figure 3(c)-(d).

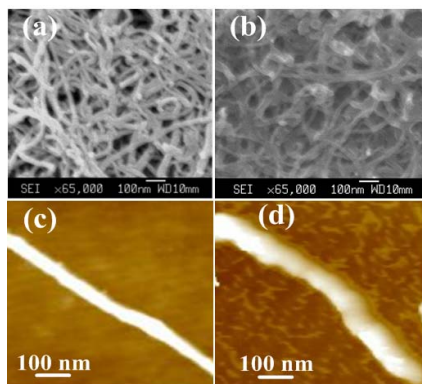


Figure 3. FESEM (a, b) and AFM (c, d) micrographs of CNTs. (a) & (c) CNTs were pretreated by SDS. (b) & (d) CNTs after absorbing goat IgGs.

One purified CNT possesses an average diameter of 58–66 nm (Fig.3 (c)) before the immobilization. However, after absorbing the goat IgGs, the diameter increased to 82–105 nm (Fig.3 (d)). The CNT diameters were changed significantly after the functionalization, attributed to the bonded IgGs through the functional group from silane.

Resonant frequency measurement

Due to the mass sensitivity nature of the PZT biosensor, the resonant frequency decreased after the external bio-entities were captured because the extra weight was added on the surface.

In order to have a detailed study on this relationship, the resonant frequencies of the sensors were measured after each immobilization or reaction process. For the accuracy consideration, all the measurements were constantly conducted in gas phase with a temperature of 30 °C. The frequency was measured by an Agilent impedance analyzer together with a probe station. After each immobilization, frequency depression among the sensor array varied from 2500 to 3500 Hz and 950 to 1500 Hz for the sensor array with and without CNTs, respectively. Detailed frequency shifts after immobilizing goat IgGs, blocker and anti-goat IgGs are presented in Figure 4. It is clear that the sensor with CNTs has much larger frequency shift for the same immobilization process. In Figure 4 (a), the frequency depression after adding high concentration Blocker is smaller than that of after capturing anti-goat IgGs. This

indicates that the amount of successfully bond blocker was relatively small, in another word, most of the surfaces were occupied by the goat IgGs.

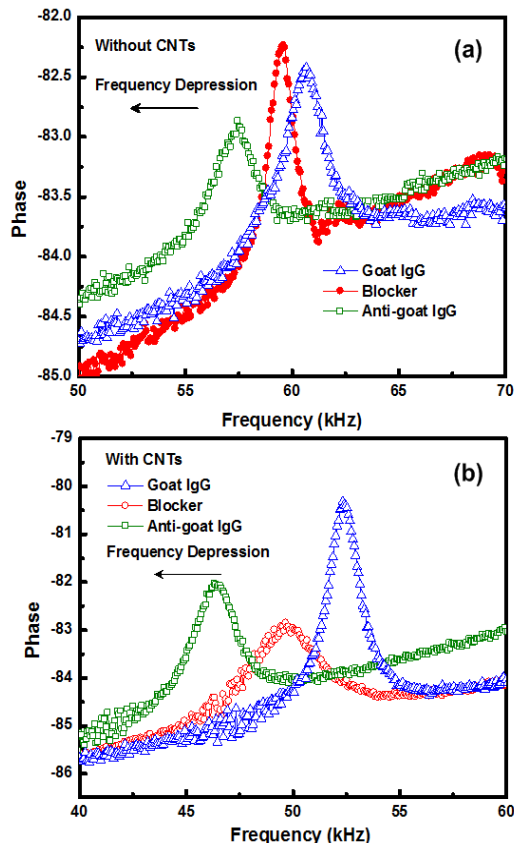


Figure 4. Detailed frequency shift of the two-sensor array (a) without CNTs, (b) with CNTs after each immobilization processes

However, in Figure 4 (b), a comparable frequency depression is clearly observed for the 3 immobilization processes. This can be explained by this fact: due to the hollow tube structure of CNTs, certain amounts of blockers were dispersed into the tubes which implying that CNT surfaces were not fully utilized by the applied goat IgGs and extra surfaces were still available for absorbing blockers. This may attribute to the low concentration of the goat IgGs in which there are no sufficient amount of goat IgGs to occupy the large surface area of the deposited CNTs.

The relationship between the frequency depression and concentration of the anti-goat IgG is shown in Figure 5. The data was plotted with the average values of five measurements. The curves have a nearly linear relationship in the low concentration range (< 10 µg/ml) and a saturation phenomenon in the high concentration range. At the same concentration level, the frequency changes of the CNT-enhanced sensors are much higher than those of the sensors without CNTs. This is because of more molecules being absorbed or captured by the CNTs during the immobilizing processes.

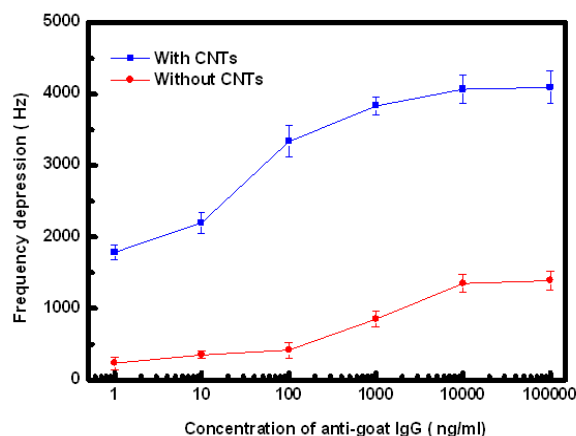


Figure 5. Relationship between the frequency depression and concentration of the added anti-goat IgG.

At the concentration of 1 ng/ml, the frequency depressions for biosensor with and without CNTs are 310 Hz and 1920 Hz, respectively. Based on this significant difference, it can be creditably predicted that if the lower concentration anti-goat IgGs were used in the last process, the biosensor with CNTs will have higher limit of detection. The sensitivity of the biosensor array with and without CNT enhancement was calculated to be 227.68 and 39.2 Hz/ng, respectively.

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