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A new biosensor based on PVDF film for detection of nucleic acids

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Abstract

Nucleic acid testing (NAT) promises stable, safe, selective and specific detection of infectious and inherited diseases. However, conventional technologies, such as gel electrophoresis, ultraviolet spectrometry and fluorescent PCR, are labor-intensive and time-consuming, and require specialized instruments and professional staff. In this study, we developed a new diaphragm mass biosensor based on polyvinylidene fluoride (PVDF) piezoelectric film for detection of nucleic acids. A capture probe immobilized on the gold film of the biosensor via Au–S reaction has been designed. To improve the specificity of the biosensors, 6-mercapto-1-hexanol (MCH) as a blocking reagent after capture probe immobilization was employed. The nucleic acid hybridization between capture probe and target analyte was used in the experiment. The real mass load added on the diaphragm was proportional to the amount of target nucleic acids. The fluorescent measurement was used to validate the experiment and served as a control. The biosensor developed in the study can provide a cost-effective platform for NAT, thus holding potential for disease screening.

Keywords: PVDF piezoelectric film; Diaphragm mass biosensor; Nucleic acid testing

1. Introduction

Mass sensors have been massively investigated and applied into biomedicine for molecule detection, drug discovery due to several significant advantages like real time, free labeling, good response, and high sensitivity [1,2].Two parameters are used to describe the mass sensor: S_m (the mass sensitivity) and Q value (mechanical merit factor). S_m means the shift of resonance frequency caused by a unit mass on the surface of diaphragm. Q value describes the sharpness of the resonance peaks and reflects the minimum frequency change which could be detected. The working principle of the device is that the resonance frequency will be changed by the mass load introduced by reaction between the target species and the sensing element immobilized onto the surface of the sensor platform [3,4]. The gold film is often used for electric conduction and biological immobilization layer in fabricating piezoelectric diaphragm biosensors [5–7].

The fabrication and application of piezoelectric diaphragm biosensors are being widely investigated due to many advantages, such as compact size, high sensitivity, easy integration with an analysis circuit, and rapid response. Micro-cantilever [8,9], quartz crystal microbalance (QCM) [10] and micro–electromechanical system (MEMS) [6,11] are among the most studied mass sensors. Even though we can obtain high mass sensitivity, however, the great bulk (QCM), fragility (micro-cantilever) and the complicated fabrication process hinder the applications of such biosensors.

Polyvinylidene fluoride (PVDF) piezoelectric film has advantages of low cost, light weight, easy of fabrication, good stretch and flexile for working in liquid. In this study, we have designed and fabricated a new kind of biosensor based on PVDF thin film used as a diaphragm platform. Mass sensitivity

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was obtained though immobilized quantitative nucleic acids on the diaphragm surface. Immobilized nucleic acids as the probes to combine targets, and this process was confirmed by the fluorescent microscope.

2. Experiments

2.1. Fabrication processes

Commercial polyvinylidene fluoride (PVDF) thin film with a thickness of 28 μm (Precision Acoustics, UK), PMMA plate with a thickness of 3 mm (Evonik, Germany), UV-light adhesive (XSSS, China) and nylon screws were used to fabricate the piezoelectric biosensor. The PVDF film was cut into a piece of 10 mm × 80 mm along the poling direction, and then stretched and fixed on a stainless steel frame. 1-mm-thick patterned PMMA mask plates were fixed on both sides of the PVDF film. Gold electrodes were sputtered on the both sides of PVDF film via above PMMA masks by a DC sputtering apparatus. The PVDF film was clamped and glued by UV curing adhesive between two pieces of 3-mm-thick PMMA plates with the patterned holes in them, as shown in Fig. 1. The setup was tightened by four nylon screws at four corners and was exposed under an ultraviolet light with the light intensity of 13 mW/cm^2 for 90 s to cure the adhesive. The steel frame was then removed from the setup and silver wires with a diameter of 25 μm were adhered on the gold electrodes by the silver epoxy for electrical measurements.

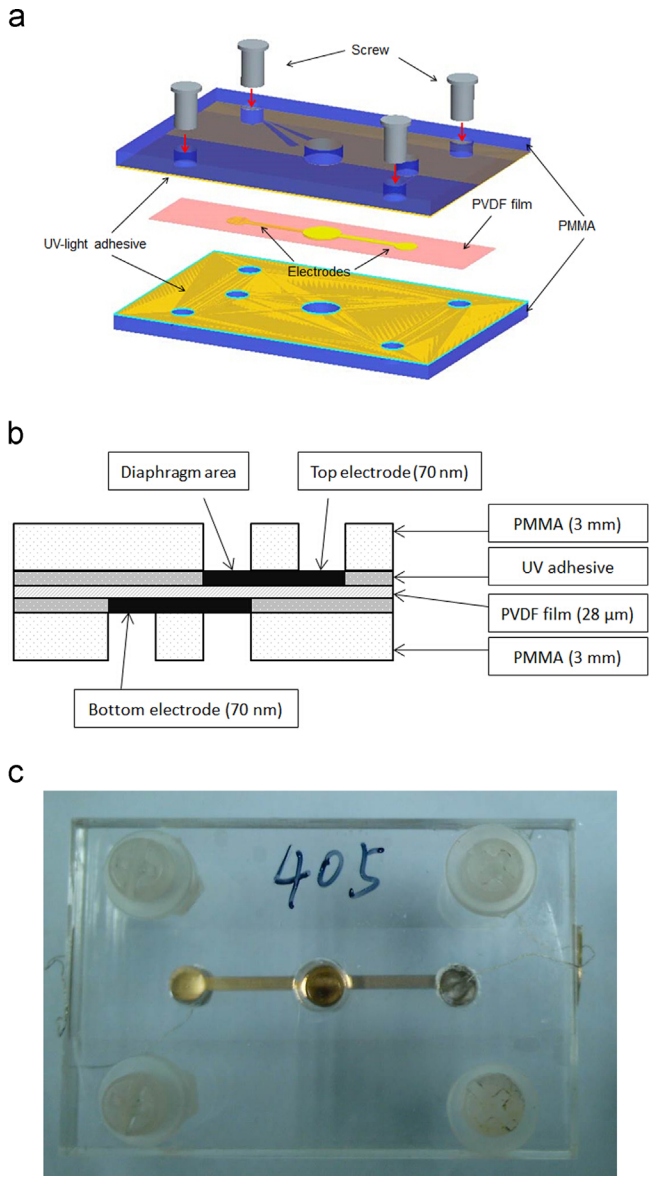

Fig. 1. (a) Schematic drawing of the fabricated biosensor using PVDF film as piezoelectric layer; (b) cross-sectional view of the biosensor shown in (a); (c) photo of the biosensor shown in (a).

2.2. Immobilization and hybridization

Piranha solution (70% H_2SO_4:30% H_2O_2) was used to clean and modify the gold film surface of the diaphragm. Then, the single-stranded DNA (ssDNA), 5′-

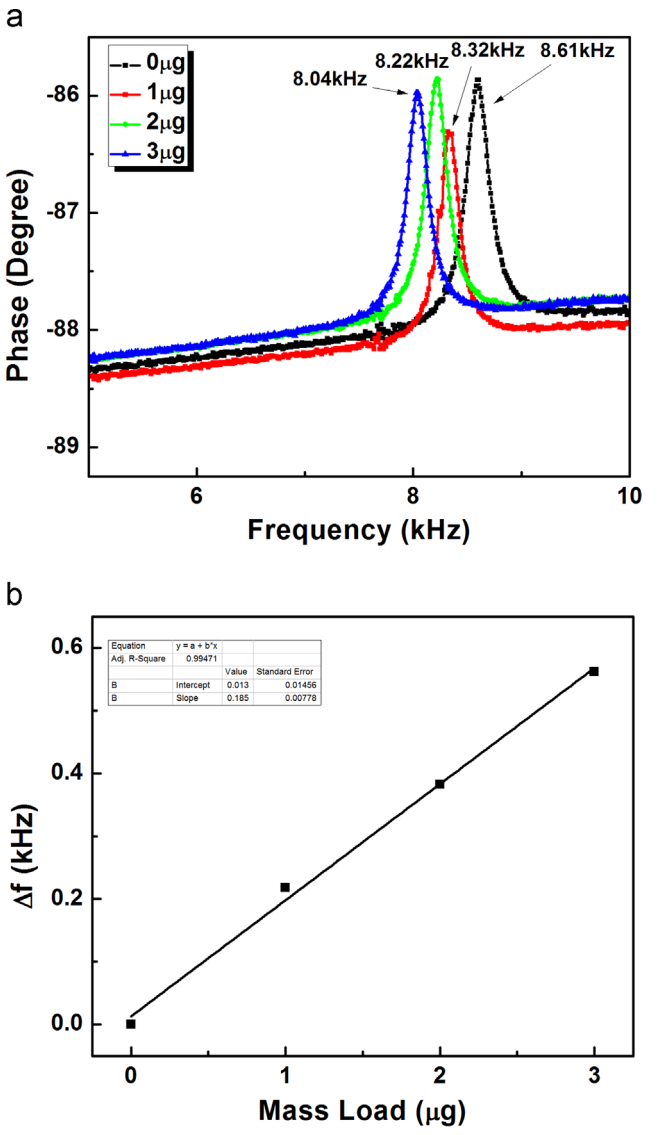

Fig. 2. (a) Resonance spectra for nucleic acid immobilization; (b) relationship of frequency shift (Δ*f*) and mass load (*m*).

CACAACAGACGGGCACACACTACT-3′, was used in the test of mass sensitivity[12,13]. The ssDNA was added on the surface of diaphragm. 1 μL solution, containing 1 μg of the ssDNA, was added on the gold surface of diaphragm and then dried at 37 °C for 30 min. An impedance analyzer (Agilent 4294A) was used to measure the impendence spectrum of the biosensor. The resonance spectra of the biosensor were measured from 8.61 kHz to 8.32 kHz (Fig. 2). Above steps were repeated until total 3 μL solution was added to the biosensor. The dependence of the resonance frequencies as a function of mass load was obtained.

During the hybridization process as shown in Fig. 3, the piranha solution (70% H_2SO_4:30% H_2O_2) treatment was performed at the first step after the sensor was fabricated. At the second step, the capture probe, 5′-6-FAM/CACAACAGACGGGCACACACTACT/C6-SH-3′, was used to immobilize on the diaphragm surface, while the sequence 5′-6-FAM/CACAACAGACGGGCACACACTACT-3′ was used as a control. 60 μL 1.0 M KH_2PO_4 solution of the ssDNA was dropped into the chamber and kept at room temperature for more than 6 h. Third, the chamber was washed by ultrapure water thoroughly. Then the sensor was dried at 37 °C for 30 min. A fluorescence microscope was used to check whether the capture probe was immobilized on the gold film. If not, the above step was repeated once again. If so, the experiment moved to the fourth step for 6-mercapto-1-hexanol (MCH) treatment. 30 μL blocking reagent of MCH was dropped into the chamber and deposited at room temperature for 2 h. Fifth, the fluorescence of the diaphragm surface was observed and recorded. Sixth, 20 μL solutions contain complementary ssDNA (HEX-modified) with the concentrations of 10 nM and 1000 nM were added into the chamber, respectively, as the target analytes. Seventh, the fluorescence of the resulting chamber was further recorded. It should be noted that the frequency spectrum of the sensor was measured after each fluorescent observation as well. The biological immobilization is similar to the dip-and-dry process [14].

3. Results and discussion

Resonance frequency of the sensor reduced when DNA was immobilized on the surface of the diaphragm. The frequency spectrum is shown in Fig. 2(a). Resonance frequency shift (Δf) is defined as $f_0 - f$, where f_0 is the frequency without any mass load, f is the frequency with mass load. If f_1 represents the resonance frequency with one microgram mass load, $\Delta f_1 = f_0 - f_1$, the rest can be done in the same manner. From the equation [11]: $S_m = -\text{frequency shift}/\text{mass load} = -\Delta f/m$, the mass sensitivity of the sensor is calculated as shown in Fig. 2(b). The slope of the line is 0.185, which means the mass sensitivity of 0.185 kHz/μg is obtained for the diaphragm with a diameter of 5 mm. The sensitivity value obtained is higher than previous biosensor based on PVDF thin film [15].

When the nucleic acid was immobilized or hybridized on the diaphragm, the resonance frequency of the sensor shifted. In this work, we can observe macroscopic image by modifying the nucleic acid by FAM and HEX. Fluorescent observation results are shown in Fig. 4. A sequence of nucleic acid probe (FAM-modified) was immobilized on the surface of PVDF-based

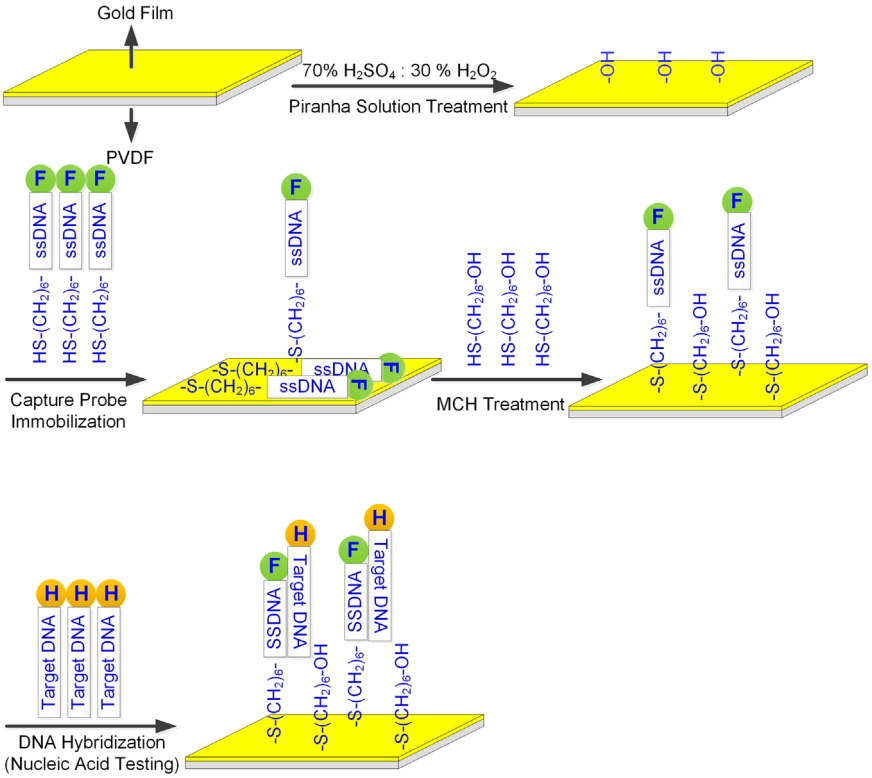

Fig. 3. Process of hybridization.

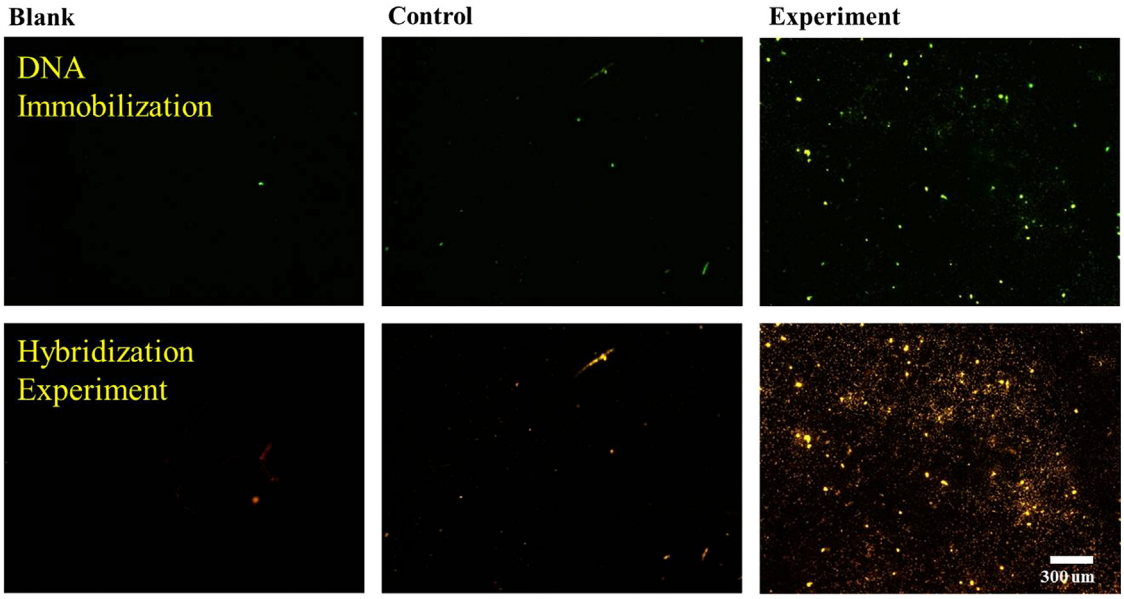

Fig. 4. Fluorescent observation results.

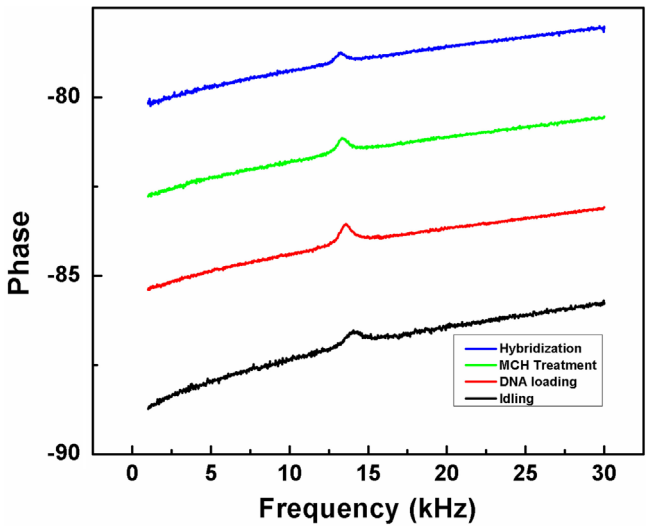

Fig. 5. Frequency spectra for hybridization.

biosensors via Au–S reaction [16]. The hybridization experiment was achieved by the addition of the complementary HEX-DNA.

The frequency spectrum change after addition of the HEX-modified nucleic acid with the concentration of 10 nM is shown in Fig. 5. The resonance frequency decreases from idling (black line) after addition of nucleic acid (red line). The resonance frequency increases since the nonspecific adsorption was washed through MCH treatment (green line), and the mass load was reduced. Via the nucleic acid hybridization between the capture probe and the target analyte, the real mass load added on the diaphragm was proportional to the amount of the target nucleic acids, and the resonance frequency decreases (blue line). When the concentrations of target are 10 nM and 1000 nM, the resonance frequency shifts are 0.19 kHz and 1.6 kHz, respectively. Larger resonance frequency shift appears for higher concentration.

4. Conclusions

In this paper, nucleic acid hybridization on diaphragm of biosensor was investigated. Fluorescent molecule modified nucleic acid was used to observe the biological immobilization and combination from macroscopic view. The sensitivity of the sensor with diaphragm diameter of 5 mm was calculated to be 0.185 kHz/μg. The value of frequency shift is directly proportional to the concentration of target solution. Real-time, low-cost, environmental-friendly and the simple fabrication process are the characteristics of the biosensor.

Conflict of interest

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

Acknowledgments

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