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Detection of a carcinoembryonic antigen using aptamer-modified film bulk acoustic resonators

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ABSTRACT

An AlN-based based film bulk acoustic resonator has been fabricated using a magnetron sputtering method, which was employed as a biosensor for detection of a carcinoembryonic antigen. The resonator consisted of an AlN piezoelectric layer and a Mo/Ti Bragg reflector which had a working resonance near 2.0 GHz. The carcinoembryonic antigen binding aptamers were self-assembled on the top gold electrode as the sensitive layer to capture the carcinoembryonic antigen molecules. When the sensitive layer absorbs the target molecules, the resonance frequency of the sensor decreases proportionally in response to the increase of the mass loading. The resonant frequency shifts of various carcinoembryonic antigen concentrations in the range of 0.2–1.0 mg/mL were measured. The sensor modified with the carcinoembryonic antigen binding aptamer exhibits a high sensitivity of 2284 Hz cm^2/ng and Q value around 520, exhibiting a high performance. These positive results suggest that the aptamer-immobilized film bulk acoustic resonator is a promising candidate for detection of the carcinoembryonic antigen.

1. Introduction

Biosensors have been widely employed as important tools for a variety of biology and biochemical applications including biomolecular interactions and clinical diagnosis. High sensitivity, label-free, real-time response, and miniaturization are the critical drivers for common usage. Acoustic sensors such as surface acoustic wave (SAW) and film bulk acoustic resonator (FBAR) have been developed for biosensing applications in the last decade due to their higher resonant frequency, greater sensitivity, lower cost, and smaller size than comparable quartz crystal microbalance (QCM) sensors [1–3]. Furthermore, AlN-based FBAR sensors offer promise in other applications because of their compatibility with monolithic microwave integrated circuits [4].

Over the past few years, remarkable results have been reported in the applications of FBAR for liquid sensing, gas sensing, and biological detection [5–7]. But there are few reports concerning label-free detection of carcinoembryonic antigen (CEA) with an aptamer modified FBAR biosensor. CEA is one of the most widely used tumor markers in the clinical diagnosis of breast, colorectal, and lung cancer making monitoring of CEA very important for clinical tumor diagnoses [8–10].

Aptamers are types of single-stranded DNA or RNA sequences that can be synthesized in vitro guaranteeing a specific binding affinity for corresponding targets [11]. The aptamer immobilized immunoreaction is a more attractive than the comparable method of antigen binding where the antibody is adsorbed onto a gold electrode surface. Aptamer modified films are widely used to fabricate biosensors, because of their significant advantages including simplicity, lower cost, high binding affinity, and specificity. In addition, the unique characteristics of the aptamer modified films have helped to improve the sensitivity of biosensors [12]. In practice, aptamers are normally functionalized with a thiol group at one end which helps to anchor them onto the gold electrode surface resulting in an aptasensors. The other end of the aptamer probe is labeled with redox moieties to bind CEA which promotes high binding affinity and specificity with corresponding target biomolecules [13].

In this paper, we report on a label-free film bulk acoustic resonator sensor with an immobilized aptamer and its application for the detection of CEA. The dependence of the resonant frequency shifts of various CEA concentrations was investigated, and the sensitivity of the aptamer-immobilized FBAR sensor for CEA

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detection was measured. Furthermore, the selectivity of the sensor was evaluated by comparing its detection for CEA in comparison to cystadenocarcinoma antigen(CA125), beta-Actin (Actin), and bovine serum albumin (BSA). The experimental results demonstrate that the aptamer-immobilized FBAR sensor is a promising candidate for sensitive detection of CEA.

2. Experimental

2.1. Reagents

CEA (≥95%, SDS-PAGE), 6-mercapto-1-hexanol (MCH), and tris (2-carboxyethy) phosphine hydrochloride (TCEP) were purchased from Sigma–Aldrich (USA). All other reagents were analytical grade. CEA binding aptamer sequences (3′-SH-ATACCAGCTTATTCAATT-5′) were artificially synthesized and purified by Sangon (Shanghai, China).

2.2. Fabrication of FBAR

A detailed description of the fabrication process is given in our previous work [14]. The metal films were deposited using the JGP450 sputtering system. The AlN film was deposited onto a *p*-type monocrystal Si substrate using RF reactive magnetron sputtering. The target was a 60-mm-diameter aluminum foil of 99.995% purity. The alternating layers of Ti and Mo were applied to the substrate using DC magnetron sputtering to create a three-period Bragg acoustic reflector. The thickness of each layer of the Bragg reflector was one quarter wavelength of the resonant wave which achieved an acoustic mirror response. The deposition parameters were optimized in order to improve the crystal quality and reduce the surface roughness. The optimized deposition conditions, which ensured a highly *c*-axis oriented AlN film, are shown in Table 1.

A Ti buffer layer with 10 nm thickness was deposited by DC magnetron sputtering to enhance adhesion characteristics between AlN piezoelectric film and top Au electrode. The top Au electrode was patterned as one port ground-single-ground using sputtering and a lift-off photolithography process.

To support manual dispensing of biomolecules on the device, polybenzoxazole (PBO) barriers were produced using a second photolithographic process to separate the sensor cells on the planar device.

2.3. Self-assembly CEA aptamer sensitive bio-layer

As shown in Fig. 1, CEA aptamers were self-assembled on the top Au electrode of the AlN-based FBAR for the detection of CEA biomolecules. The CEA aptamer sensitive layer was coated on the top Au electrode surface by manual dispensing employing a precision pipette. Prior to modification, the Au electrode surface was pretreated and dried in flowing nitrogen gas. This was accomplished as follows: the resonant zone of the Au electrode was first dipped in 50 μL of 10 μmol/L CEA binding aptamer solution which included 10 μL of 5 μM TCEP. In order to assemble the sensitive layer of the aptamer through the Au—S bond with the Au electrode, the sensor was covered with 50 μL of 10 mM MCH and preserved in a sealing assembly for 12 h at refrigerated temperature. Then the sensor was thoroughly washed with Tris–HCl buffer (25 mM Tris–HCl, 150 mM NaCl, pH 8.0) and deionized water to remove the nonspecific bonded CEA aptamer to obtain a well-aligned CEA binding aptamer layer.

Table 1
The optimized deposition conditions for each layer.

	Deposition time (s)	Power (W)	Pressure (Pa)	Ar flow (Sccm)	N_2 flow (Sccm)	Temperature (°C)
AlN	3600	RF 200	0.2	12	18	300
Ti	405	DC 80	0.5	12	0	300
Mo	400	DC 80	0.5	12	0	300
Au	100	DC 80	0.5	12	0	25

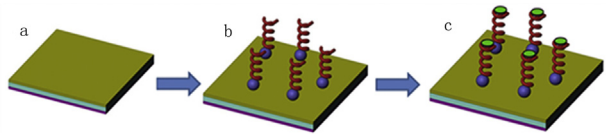

Fig. 1. Schematic illustration of AlN-based FBAR modified with aptamers to detect CEA. (a) AlN-based FBAR, (b) CEA aptamers modified Au electrode, and (c) using the sensor to capture CEA.

2.4. Detection of CEA

The sensor with immobilized sensing interface was immersed in various concentrations of CEA and reacted for 2 h at a temperature of 37 °C, followed by washing with the Tris–HCl buffer and deionized water to remove the unbound CEA. The AlN-based FBAR sensor was used to measure the frequency shift with various CEA concentrations. The selectivity of the modified sensor was also determined. To determine the selectivity of the modified sensor, the frequency properties of CA125, beta-Actin, and BSA were also monitored.

2.5. Test equipment

The crystal orientation, microstructure, and the thickness of the sensor layers were determined by X-ray diffraction (XRD, Bruker Advanced D8) and field-emission scanning electron microscopy (SEM, JEOL 7100F). The frequency characteristics of the AlN-based FBARs were measured using the vector network analyzer (Agilent E5071C) and probe station (PE-4, EverBeing, Taiwan) after a standard short-open-load (SOL) calibration.

3. Results and discussion

Fig. 2 illustrates the SEM image of an AlN thin film on Mo/Ti Bragg layer with Si substrate, including the Au electrode and PBO barrier layer. As shown in Fig. 2a, the AlN thin film has conformed, dense grains with a truncated hexagon morphology in the top view, which is consistent with a hexagonal wurtzite structure. The cross-section view morphology of the FBAR is shown in Fig. 2b, the FBAR consists of a piezoelectric stack (Au/AlN/Mo) and a Bragg reflector with three-fold acoustic Mo/Ti mirror deposited on Si substrate. The highly *c*-axis oriented AlN film with 1.7 μm thickness has a dense columnar structure. Mo/Ti Bragg layers have uniform thickness, and the interfaces between the layers are clearly visible and smooth ensuring the minimization of loss of the acoustic energy in the FBAR. The bottom Mo electrode serves as electrical ground plane, and the top is a one port ground-single-ground patterned Au electrode (G–S–G pattern) for measurement. PBO barrier layer has been processed laterally the sensor area to support manual dispensing. The inset in Fig. 2b is the micrograph of the patterned Au electrode with an active area of $300 \times 300\ \mu m^2$.

Fig. 3 shows the X-ray pattern which was employed to analyze the orientation of the AlN film grown on the Mo/Ti Bragg reflector with Si substrate and Au top electrode. In the XRD patterns, (0 0 2) AlN, (1 1 1) Au, (1 1 0) Mo, and (1 1 0) Ti are observed. The (0 0 2) peak of the AlN film is located at 35.9° and exhibited a preferred *c*-axis orientation corresponding with the wurtzite hexagonal