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ALPHA-FETOPROTEIN GENOSENSOR BASED ON QUARTZ CRYSTAL MICROBALANCE

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ABSTRACT

In this study, a genosensor based on quartz crystal microbalance (QCM) has been developed for the detection of single-stranded oligonucleotide specific to alpha-fetoprotein (AFP) gene. The quartz crystals were coated with polyethyleneimine (PEI) and then crosslinked with glutaraldehyde (GA). The oligonucleotide probe was immobilized onto the PEI-GA film. The coatings were characterized by using atomic force microscopy. Hybridization reaction between probe and the complementary strand was monitored by the QCM system in real-time. This work demonstrated that QCM is a suitable tool for the real-time monitoring of hybridization on the crystal surface without using any label.

KEYWORDS: Piezoelectric Sensor, Alpha-fetoprotein, Quartz Crystal Microbalance, Resonance Frequency, Atomic Force Microscopy, Hybridization, Gene Sensor.

1. INTRODUCTION

Biosensors are powerful analytical tools that take advantage of the high selectivity and sensitivity of a biologically-active material. They are among the most frequently employed devices in clinical chemistry laboratories, as well as in the medical, environmental monitoring, pharmaceutical, diagnostic, and food industries. Biosensors are integrated with a physicochemical transducer, such as optical, electrochemical, thermometric, magnetic or piezoelectric (PZ) [1]. Among these, PZ biosensors are mass sensing devices

well known to be quite sensitive and selective in detecting biological elements such as proteins [2], antibodies [3], DNA [4] and microorganisms [5].

A typical quartz crystal microbalance (QCM) sensor consists of a thin plate of quartz sandwiched between two metal electrodes [6]. Electrodes are prepared by depositing metal vapor on both surfaces of the quartz crystal. The resonance frequency of crystal is adjusted by the amount of metal deposited on the surface. The metals used as electrodes are gold, silver, platinum, aluminum and nickel etc. Gold electrodes are usually preferred in sensor applications due to their inertness [7].

With its piezoelectric property, quartz crystal is an active element of QCM device, used as transducers in chemical and biochemical sensors. The quartz is a SiO_2 monocrystal with a zinc-blend structure type and each elementary cell consists of three SiO_2 molecules. Quartz is present in nature in two different forms: α-quartz and β-quartz. α-quartz form is the most used in PZ applications since it is resistant to high temperatures and insoluble in water [8].

The basic principle of QCM is based on the relationship between the oscillation frequency of PZ quartz crystal and the total deposited mass on the electrode. As it was first described by Sauerbrey in 1959 [9], any increase in mass on the surface of the PZ quartz crystal causes a decrease in the vibrational frequency of the piezoelectric crystal. QCM biosensors have various applications such as pathogen [10, 11], DNA or gene sequence [12, 13], protein [14] and tumor antigen detection [15]. PZ quartz crystal detectors are selective, simple, ease of use, low cost and can provide a real-time and label-free response [16].

Alpha-fetoprotein (AFP) is a reliable and commonly preferred indicator for determining hepatocellular carcinoma (HCC). AFP mRNA can be a potential marker for the detection of HCC cells in the circulation or in tumor tissue. Determination of this protein in nanogram levels is critical for the early diagnosis of HCC [17, 18]. The aim of this study was to develop an AFP gene sensor based on QCM enabling real-time measuring of deposited mass on the crystal surface in nanogram levels without using any labels.

2. MATERIALS AND METHODS

Chemicals

The 20-mer single-stranded synthetic oligonucleotides used as probe and complementary strand were purchased from Exiqon. PEI, glutaraldehyde (GA), and all other chemicals were obtained from Sigma. The reagents were

of either HPLC or analytical-grade. Highly purified double-distilled water (≥20MΩcm at 25ºC) was used in all experiments. All experiments were performed at room temperature.

QCM system

QCM experiments were performed by using an electrochemical quartz crystal microbalance system (EQCM, 400B, CH Instrument, USA) at 25ºC. The quartz crystals used were polished and AT-cut with a fundamental frequency of 7.995MHz, with 5.1mm diameter gold electrodes.

Crystal coating

The crystals were coated by using the poly(ethyleneimine)/glutaraldehyde (PEI/GA) method as previously described [19]. At first, the crystals were cleaned with Piranha solution (30% H_2O_2, 70% H_2SO_4) for 1 minute. Later, the crystals were washed with ultrapure water and ethanol, and dried in air to obtain a clean gold crystal surface. The initial frequencies of the quartz crystals were recorded as F_0 before coating. PEI solution (40g/L) of 5μL was dropped onto freshly cleaned crystals and then dried at room temperature. Later, the crystals were washed with ultrapure water and ethanol. Further, incubated with 10 μL of Glutaraldahyde (GA) (0.3M) for 40 min, and dried in air at room temperature. Then, the crystals were washed again with ultrapure water and dried in air. The frequency of the quartz crystal was recorded as F_1. The frequency shifts of coated crystals were calculated by using the following formula:

$$\Delta F=F_0-F_1$$

Atomic force microscopy

AFM technique provides valuable information on surface morphology and topography, which is not easily obtainable by other imaging systems. In this study, the sensor surface was characterized using atomic force microscopy. AFM experiments were performed by a NI-AFM model atomic force microscope (Nanomagnetics Instruments, Ankara, Turkey). All measurements were performed in air using a Tap300A1 model cantilever in dynamic mode (Budget Sensors, Innovative Solutions, Sofia, Bulgaria) with a resonance frequency of 320kHz. The quartz crystal surface regions were randomly scanned at 40μm x 40μm with 40N/m force constant.

Immobilization of the AFP probe

For probe immobilization, 50μL of 2μM oligonucleotide probe was dropped on the PEI/GA-coated quartz crystal. After 40 minutes incubation at room temperature, the crystal surface was gently rinsed with phosphate buffer (pH 7.4) to remove free probe molecules and dried in air at room temperature. After drying, the resonance frequency of the crystal was measured again at room temperature.

Determination of hybridization

PEI/GA coated PZ crystal was located into the Teflon cell (CH Instrument), only one side of the crystal was in contact with phosphate buffer (pH 7.4). Following the one-hour incubation to reach the steady-state frequency of crystal, 100μL of 100μM complementary oligonucleotide solution was injected into the buffer and the frequency changes were monitored. The sequences of the 20-mer probe and target oligonucleotides are presented in **Table 1**.

Table 1. The sequences of the probe and complementary strands.

Oligonucleotides	**Sequence**
Probe	5'-AACAGCAGAGTGCTGCAAAC-3'
Complementary	5'-GTTTGCAGCACTCTGCTGTT-3'

3. RESULTS AND DISCUSSION

Coating of the PZ Crystal and probe immobilization

The crystal frequency was recorded before and after coating and following probe immobilization. The polymer coating and probe immobilization were confirmed by the decrease in crystal frequency given in **Table 2**.

Table 2. Frequency shifts of crystals in response to coating and immobilization

Step	**ΔF (Hz)**
PEI-GA Coating	460 ± 22
Probe Immobilization	157 ± 17

The morphology and topography of coated quartz surfaces were investigated by AFM analysis. PEI coating has been mostly used in many successful biosensor applications to provide a thin and uniform coating with complete spreading [20]. Figures 1 and 2 show the high-resolution surface topography of a 40μm x 40μm area of the quartz crystal surface coated with PEI/GA film. The AFM images of the coated crystal surfaces revealed a homogeneous distribution of nanometer-sized (100-900 nm) clusters on the crystal surface.

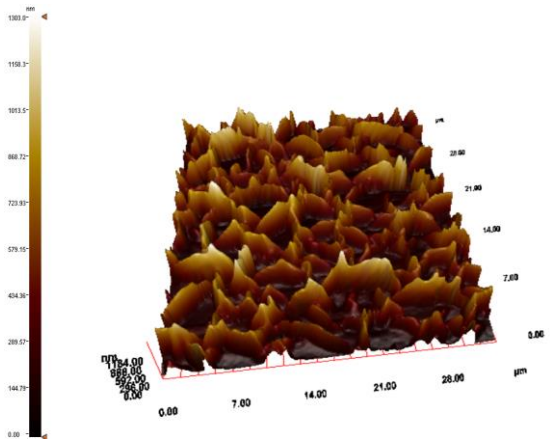

Figure 1. Three-dimensional surface topography of PEI/GA coated quartz crystal.

Determination of hybridization

In QCM gene sensors, determination of hybridization reaction is carried out following the change in frequency of crystal causing the interaction between a specific DNA sequences (oligonucleotide probes) immobilized on the electrode surface of the quartz and the complementary oligonucleotide in solution [21]. In this study, the interaction between the probe on the crystal surface and the complementary sequence was monitored by QCM system in real-time. Fig. 3 shows frequency shifts in response to interaction of the probe on the quartz crystal with complementary oligonucleotide. Figure shows decrease in the crystal frequency proportional to the mass increase on the crystal (Fig. 3).

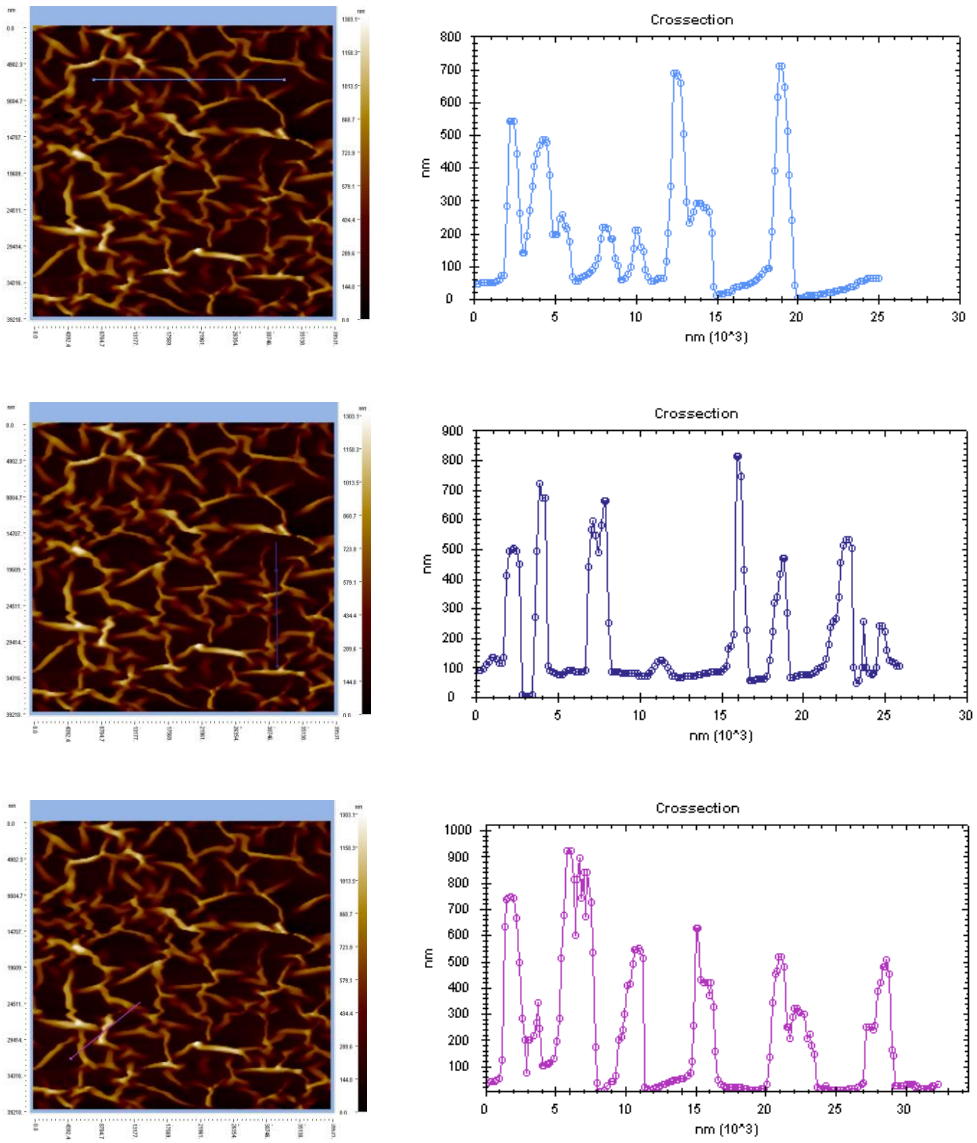

Figure 2. Cross-sectional analysis of PEI/GA coated crystal.

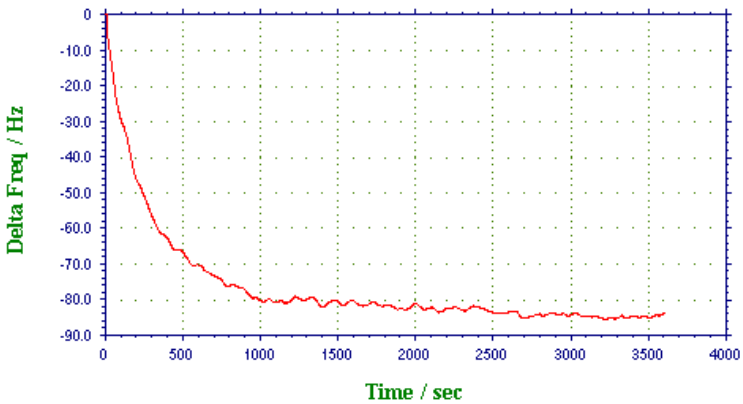

Figure 3. Frequency shifts of the quartz crystal to complementary oligonucleotide.

4. CONCLUSION

In this study, we developed a gene sensor based on quartz crystal microbalance system, which enabled real-time measuring of the hybridization reaction between probe immobilized on the electrode of a piezoelectric crystal and the complementary strand without using any labels.

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ÖZET
Bu çalışmada, alfa-fetoprotein (AFP) genine özgü tek zincirli oligonükleotit dizisinin belirlenmesine yönelik olarak geliştirilen, kuartz kristal mikrobalans (KKM) sistemine dayalı bir gen sensörü anlatılmıştır. Kuartz kristaller, poli(etilenimin) (PEI) polimeri ile kaplanmış, ardından glutaraldehit (GA) ile çapraz bağlama işlemi gerçekleştirilmiştir. Oligonükleotit probu, hazırlanan PEI/GA yüzeyi üzerine immobilize edilmiştir. Yüzeylerin kaplanması atomik kuvvet mikroskobu ile doğrulanmıştır. Tamamlayıcı ve prob dizileri arasında gerçekleşen hibridizasyon reaksiyonu, KKM sistemi ile eş-zamanlı olarak görüntülenmiştir. Bu çalışma ile, KKM sensörünün, kristal yüzeyi üzerinde gerçekleşen hibridizasyon reaksiyonunu, herhangi bir işaretleyici kullanmadan, eş-zamanlı olarak belirleyebildiği gösterilmiştir.

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