# DNA Hybridization 센서의 제작과 전기화학적 검출 특성

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# Fabrication and Electrochemical Detection Property of Single Strand DNA Hybridization Sensor

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**Abstract** - A synthesized 21-mer single-stranded DNA(ss DNA) was covalently immobilized onto a self-assembled aminoethanethiol monolayer modified gold electrode onto QCM. The covalently immobilized ssDNA was hybridized with complementary ssDNA. The interaction between surface immobilized ssDNA and complementary 21-mer DNA in solution was also examined. Each step was followed by monitoring changes in the QCM frequency with time. Also, PBS with pH 7.0 was selected as a supporting electrolyte in order to get maximum sensitivity and good bioactivity.

## 1. Introduction

The key issue in biosensor applications is more efficient by comparison to DNA hybridization investigations performed on membranes that are less sensitive and selective, time consuming and not time resolved [1]. Moreover it has been observed that surface hybridization is faster [2] and more selective [3] than hybridization in bulk solution. DNA biosensors are useful tools due to the high sensitivity and selectivity of biologically active materials. The applications include disease diagnostic devices, miniaturized biosensor arrays, and DNA driven assembly of nanostructures [5].

The quartz crystal microbalance (QCM) is a powerful tool for biosensor applications.It has become one of the most effective techniques of molecular biology and microchemistry because it enables the advantages of simple, rapid and real-time monitoring, as well as high sensitivity and specificity [4].

In this paper, we fabricated the DNA hybridization sensors by immobilization of 21-mer oligonucleotide using QCM Au-electrode. Firstly, aminoethanethiol as a hybridization indicator were evaluated electrochemically. A synthesized 21-mer oligonucleotide was covalently immobilized onto a self-assembled aminoethanethiol monolayer modified gold electrode onto QCM. The single-stranded DNA formed a phosphoramidate bond with primary amino group of aminoethanethiol monolayer. And then, we also investigated the hybridization of oligonucleotide using the frequency dependent techniques of QCM. Probe immobilization was confirmed by cyclic voltammetry. Finally, we determined that the detection of the DNA sequence was carried out with a DNA sensor system consist of an immobilized and hybridized DNA gold electrode.

## 2. Experiment

Both oligonucleotide (5`-GAA AAA AAA TTG CGC AAT CCG-3`) and its complementary target DNA (3`-CGG ATT GCG CAA TTT TTT TTC-5`) with a length of 21-mer oligonucleotides were synthesized by Bioneer Corporation (Daejeon, Korea). Aminoethanethiol and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were obtained from Sigma. Other reagents were commercially available with analytical grade.

Fig. 1 shows the schematic process for the preparation of DNA sensor. At first, the gold electrode was cleaned by piranha solution ($H_2SO_4$:$H_2O_2$=3:1) subsequently cleaned by cycling between potential windows from 0 to + 1.5 V versus Ag/AgCl in 0.05 M $H_2SO_4$ solution at scan rate of 100 mV/s for nearly 20 minutes until stable scans were observed. Then, the electrode was thoroughly rinsed with the ultra pure water. After pretreatment, the gold electrode was immersed in 5 m M aminoethanethiol in ethanol solution for 24 h at room temperature with pure Ar gas. The surface of the electrode was washed with distilled water and ethanol and was purged with pure Ar gas. After these treatments, self-assembled monolayer with amino group was formed on the gold electrode surface as electrode 1.

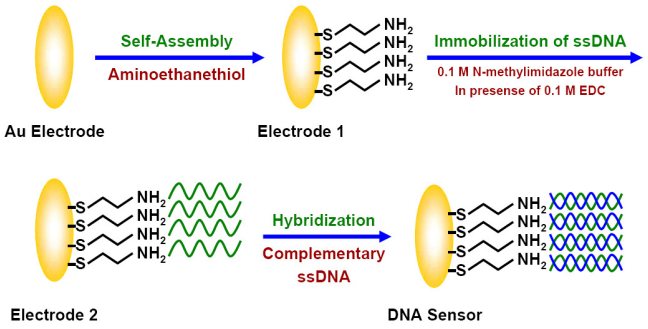


**Fig. 1 The process for the preparation of DNA hybridization sensor.**

The electrode 1 was immersed in pH 6.0, 0.1 M N-methylimidazole buffer containing 0.1 M EDC and 0.1 mg/ml single-strand DNA (ssDNA). The electrode was incubated for 2 h at room temperature. These conditions allow immobilization through the 5`-phosphate groups of ssDNA by the formation of a phosphoramidate bond with the amine groups of the electrode 1 surface. The electrode immobilized ssDNA monolayer is denominated as electrode 2. Different concentrations of DNA, which was complementary to the immobilized ssDNA, were added. The electrode 2 was immersed into the hybridization buffer and incubated at 40 °C for 1 h with shaking [5].

The 9 MHz QCM (AT-cut, 0.5 mm diameter), which was provided with gold-coated electrode, were obtained from Seiko EG&G (Japan). The cyclic voltammetry response have been measured with QCA 922 (Seiko EG&G, Japan) and potentiostat 263A (PerkinElmer, USA). The immobilized and hybridized DNA-modified QCM gold-electrode was used as the working electrode. The Pt wire and KCl saturated Ag/AgCl electrodes were used as counter and reference electrodes, respectively. The stock solutions were made by phosphate buffer solution (PBS, pH 7.0)

## 3. Results and Discussion

Fig. 3 shows the time (t) dependence of the resonant frequency shift (ΔF) in ethanol solution. Fig. 3 (a) is the frequency shift of self-assembled aminoethanethiol, (b) is the immobilization property onto self-assembled gold electrode after injection of 21-mer oligonucleotide, (c) is the subsequent hybridization phenomenon after adding a complementary 21-mer oligonucleotide.In Fig. 3, the measured frequency shifts were about 328 Hz, 192 Hz and 168 Hz, respectively. From the results, we calculated that the adsorption mass was about 351 ng/cm2, 205 ng/cm2 and 180 ng/cm2, according to the Sauerbrey equation [6]. So, we can know the immobilized and hybridized amount on the self-assembled gold electrode.

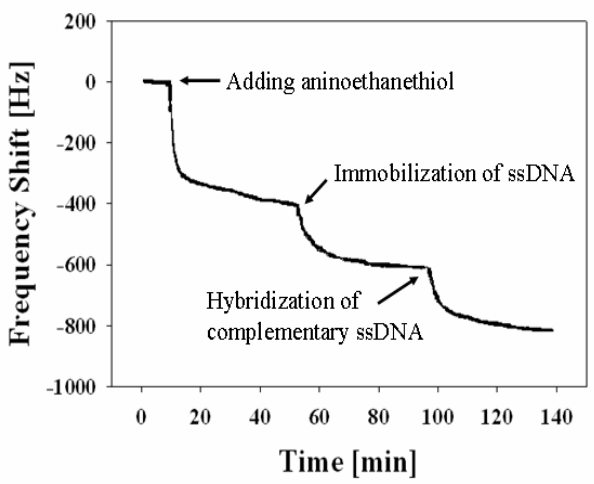


**Fig. 2 The dependence of the resonant frequency shift (⊿F) on time (t).**

After finishing the hybridization of the gold electrode, the cyclic voltammetry was carried out as working electrode in PBS solution (pH 7.0). Fig. 4 shows the cyclic voltammograms (CV) of DNA sensor by different scan rates. In Fig. 4, the anodic peak current was observed clear around 0.4 V on the DNA sensor. Inset figure is the relationship of DNA sensor between scan rate and oxidation peak current. When the scan rate was increased, the peak current also increased linearly. This fact signified that the reaction was reversible [7].

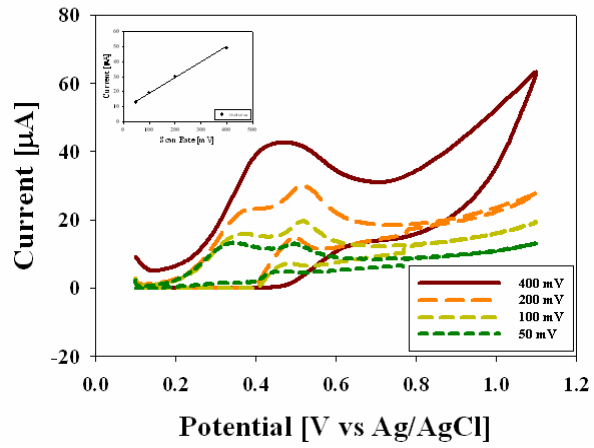


**Fig. 3 The cyclic voltammograms of DNA hybridization sensor in the 0.1 M PBS (pH 7.0).**

The effect of the pH in the buffer solution on the DNA sensor response is one of the most important factors. The maximum current response attained at pH 7.0. In strong basic or strong acidic solution, the biocompounds were denatured. Therefore, PBS with pH 7.0 was chosen as a supporting electrolyte in order to get maximum sensitivity and good bioactivity. We also investigated the amperometric response of the DNA sensor (0.1 M PBS with pH 7.0).

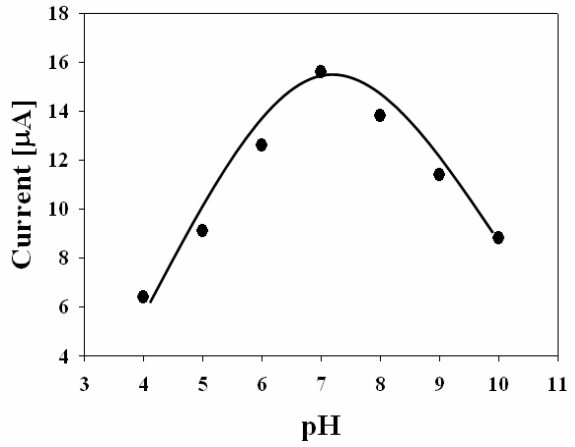


**Fig. 4 The dependence of anodic peak current of the sensor on solution pH.**

## 4. Conclusion

In this work, we investigated the electrode modification of DNA sensor using QCM. The immobilized DNA on the gold electrode can be applied to prepare a DNA electrochemical sensor by the hybridization with a complementary DNA. The proposed biosensor showed nice sensitivity and stability, even though its detection limit is not enough for practical use [8]. Finally, easy fabrication, low cost, fast response time, nice sensitivity, and stability are obvious advantages for this newly proposed modified electrode.

## [Acknowledgement]

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