

An Impedimetric Nanosensor Based on Large-Scale Nanowell Array Electrode for Single Nucleotide Polymorphism of Leptin

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Abstract: *Recently, we reported that the nanowell array (NWA) enhances electrochemical responses during the binding event of molecule to molecule through the binding site control of captured molecule. In this paper, we have demonstrated a method, with high sensitivity and selectivity for single nucleotide polymorphism detection of leptin, which is an established obesity biomarker. A 21-mer probe was immobilized on a nanowell array surface (500 nm diameter), which was coated with streptavidin and a self-assembled monolayer. The surface properties of probe oligomer hybridization with complementary target DNA were characterized using electrochemical impedance spectroscopy (EIS). Significant electric signal changes were observed at SNP oligomer compared with complementary target oligomer. We demonstrated that this SNP detection encompasses a range from 1 pM to 100 nM for quantitative analysis.*

Keywords: *Nanowell arrays (NWA), Electric Impedance Spectroscopy (EIS), Single Nucleotide Polymorphism (SNP), Leptin, Electrochemical sensor.*

1. INTRODUCTION

Single nucleotide polymorphism (SNP) detection technologies are used to scan for new polymorphisms and to determine the allele of a known polymorphism in target sequences. As genetic markers, SNPs can be used to follow the inheritance patterns of chromosomal regions from generation to generation and are powerful tools in the study of genetic factors associated with human diseases. As the demand for genetic information tracking increases, SNP detection technologies are developed at an accelerated pace, fueled by recent advances in enzymology, DNA synthesis and analytical instrumentation.

A number of electrochemical DNA sensors for SNP analysis have been recently reported [1-5]. For example, Barton *et al.* described a system that detects changes in long-range charge transport through the π -stack of duplex DNA by combining redox-active intercalators with exogenous electrocatalytic species [1]. In this approach, targets that alter base-pair stacking, such as a mismatched base within the DNA duplex, are identified via reduced charge transfer relative to perfectly-matched targets. However, these approaches offer accurate room-temperature SNP detection, but are often susceptible to false positives arising from nonspecific binding of redox reporters, and require exogenous reagents and post-hybridization washing steps.

To overcome the limitations of the above-described approaches, we developed a simple electrochemical SNP sensor based on nanowell array electrode (NWA). We already demonstrated that NWA optimized the opportunity for molecular binding events by controlling the molecular binding sites [6, 7]. Also due to their small size, sensors provide sensitive detection and faster mass transport, which takes place predominantly on the electrode surface, enabling kinetic measurement and accelerating electrochemical reactions.

By using these NWA electrodes, the 21-mer probe was immobilized on a streptavidin/SAM coated nanowell array electrode with a diameter of 500 nm. The surface properties of probe DNA hybridization were characterized using electrochemical impedance spectroscopy (EIS). To detect SNP

efficiency between the probe and SNP oligomer, charge transfer resistance (R_{ct}) in EIS was analyzed and plotted as a function of the amount of DNA. The function of the SNP electrochemical sensor was demonstrated by detecting a target DNA of leptin, which is biomarker for induced endothelial dysfunction in obesity.

2. EXPERIMENTAL

2.1. Materials

A biotinylated 21-mer ssDNA oligonucleotide, 5'-biotin-AAC AAA TGT CTT TCC TTC AAT-3', was used as biotinylated probe-ssDNA so it was bound to streptavidin molecules. 5'-ATT GAA GGA AAG ACA TTT GTT-5', 5'-AAC AAA TGT CTT TCC TTC AAT-3' were used as target-ssDNA and negative matching-ssDNA to confirm specificity and sensitivity of impedimetric sensor, respectively. SNP oligomer has sequence of 5'-ATT GAA GGA AGG ACA TTT GTT-3', Adenine was substituted to Guanine.

All ssDNA synthesis reagents were obtained from Bioneer Co. (Daejeon, Korea) Streptavidin, ethanolamine hydrochloride, 11-mercapto undecanoic acid (11-MUA), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC), N-hydroxysuccinimide (NHS) and all other chemicals in analytical grade were bought from Sigma-Aldrich (St. Louis, USA).

2.2. Electrochemical Characterization

NWA electrode as working electrode was fabricated as described in our previous paper [7]. Briefly, a glass Ag/AgCl electrode with a diameter 6 mm and a length of 5 cm and Pt coil counter electrode from BASi analytical instruments (West Lafayette, USA) was integrated into the electrode holder. The NWA electrode, the counter electrode and reference electrode were situated into the electrode holder. The liquid reagents were introduced to the electrode environment and electrochemical analysis was performed.

EIS was carried out in a 5 mM $K_3Fe(CN)_6$ in DI water (resistivity : 18 M Ω -cm) at room temperature by using a potentiostat/galvanostat from IVIUM Co (Netherlands). The impedance spectroscopy were recorded from 1 MHz to 0.1 Hz at 50 mV ac signal amplitudes. Frequencies each decade are 10 points and total measurement points are 71 points. After impedance measurement, data was fitted by using Z-view program.

2.3. Procedures

Prior to beginning the experiment, all electrodes were treated acetone and cleaned with ethanol and DI water. After N_2 blowing, the Self-Assembled Monolayer (SAM) was prepared on a NWA electrode by incubating 10 mM 11-mercaptoundecanoic acid solution in anhydrous ethanol for 1 hour at room temperature. Then, 50 mM EDC and 50 mM NHS in pH 5.5 sodium acetate buffer treated to form an active ester functional groups. 10 μ g/mL of streptavidin in PBS was immobilized on SAM for 30 min at room temperature. The unreacted functional ester groups were blocked by treating 1 M ethanolamine for 30 min. Then immobilization of probe ssDNA molecules on the electrode was performed via the streptavidin-biotin interaction. 10 nM biotinylated probe ssDNA was dispersed onto the streptavidin-modified substrate and incubated at room temperature for 30 min to allow the streptavidin-biotin interaction to complete. In a humidity-controlled experiment, the electrode was located in a highly humidified incubator at RT, which can control the humidity and the temperature. After the immobilization procedure, the electrodes were rinsed three times with PBS buffer and N_2 blowing for further treatment.

3. RESULTS AND DISCUSSION

Figure 1 shows the electrochemical impedance response changes of the perfect matching and SNP matching DNA oligomer on the NWA electrode. As previously said, we treated 1 μ M probe DNA oligomer on the electrode by using streptavidin-biotin interaction. For the perfect matching (10 nM treated), the magnitude of impedance at the low frequency below 10 Hz was increased as shown Figure 1(a). Figure 1(b) is the nyquist plot (Z' versus Z'' ; Z' =real impedance and Z'' =imaginary impedance), it also shows the charge transfer resistance (R_{ct}) that is the diameter of the semicircle is increased. However, for the SNP oligomer, the signal was not significantly changed with base signal (probe signal). Therefore, we can observe SNP signal changes by calculating the difference in the impedance spectra for each electrode before and after hybridization.

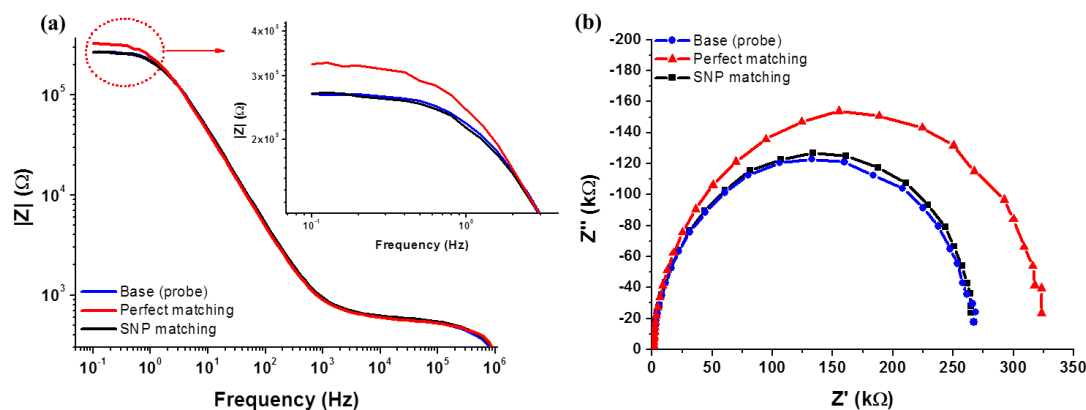


Fig1. (a) Bode plot and (b) Nyquist plot of probe and target DNA hybridization on the NWA electrodes. Perfect matching and SNPs oligomer were used for target DNA.

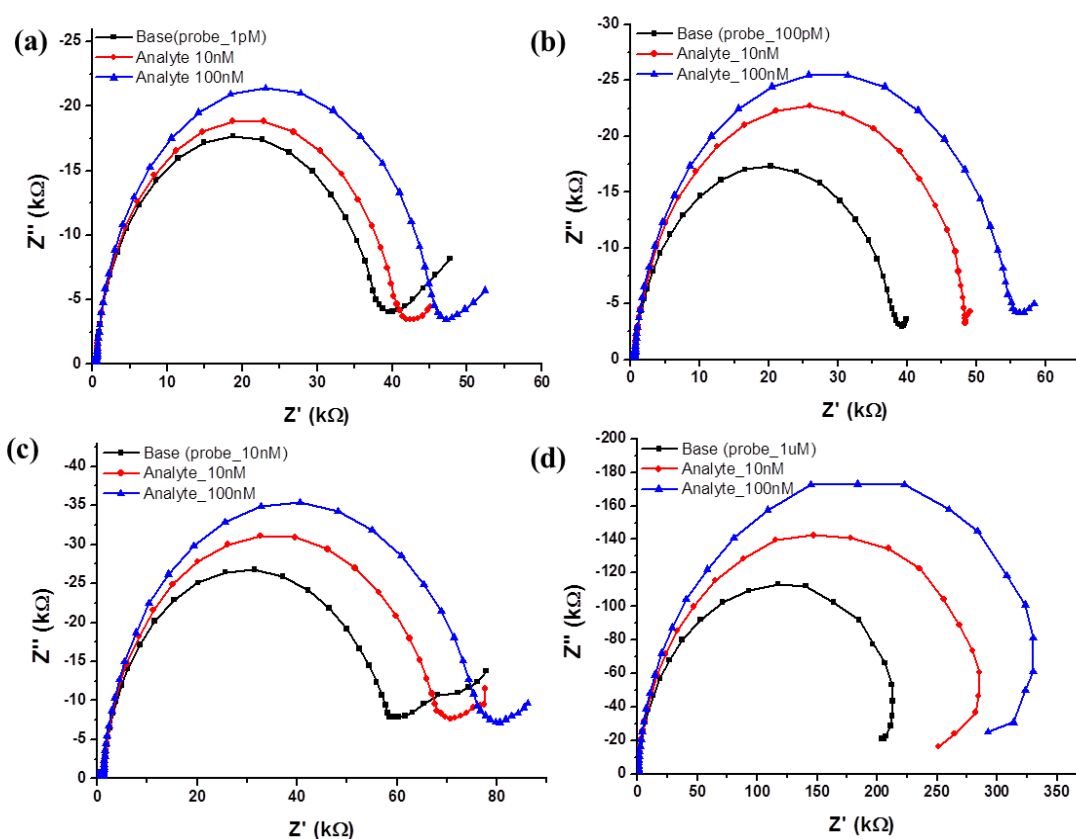


Fig2. Nyquist plots for probe and target DNA hybridization on NWA electrode. Various concentrations of probe DNA were immobilized for hybridization. (a) 1 pM, (b) 100 pM, (c) 10 nM, (d) 1 μ M. 10 nM and 100 nM of target DNA were used for analysis.

One of the most important issues to address in the area of non-labeled DNA detection is the sensitivity of the electrode. In an effort to address this issue, we measured $\Delta R_{ct} / R_{ct}$ (base) at probe oligonucleotide DNA concentrations in the 1 pM to 1 μ M range. Figure 2 shows the EIS spectra of various target DNA samples after hybridization at 10 nM and 100 nM. An increase in charge transfer resistance was observed when the target DNA concentration was increased. The R_{ct} increase can be explained by the accumulation of negative charge from the DNA backbone after hybridization. This causes a higher barrier for the negatively charged ferri/ferrocyanide anions and results in reduced charge transfer ability on the NWA electrode surface. And $\Delta R_{ct} / R_{ct}$ (base) ratio also increased with probe concentrations. At 100 nM analyte, 1 pM probe's ratio is 1.26 (a) 100 pM probe's ratio is 1.35 (b), 10 nM probe's ratio is 1.41 (c), 1 μ M probe's ratio is 1.72 (d) each. Therefore, we found that optimization of the signal can be obtained when we treated 1 μ M of probe oligomer on the NWA

electrode. By using this condition, we observed SNP's detection at a wide range of target concentrations.

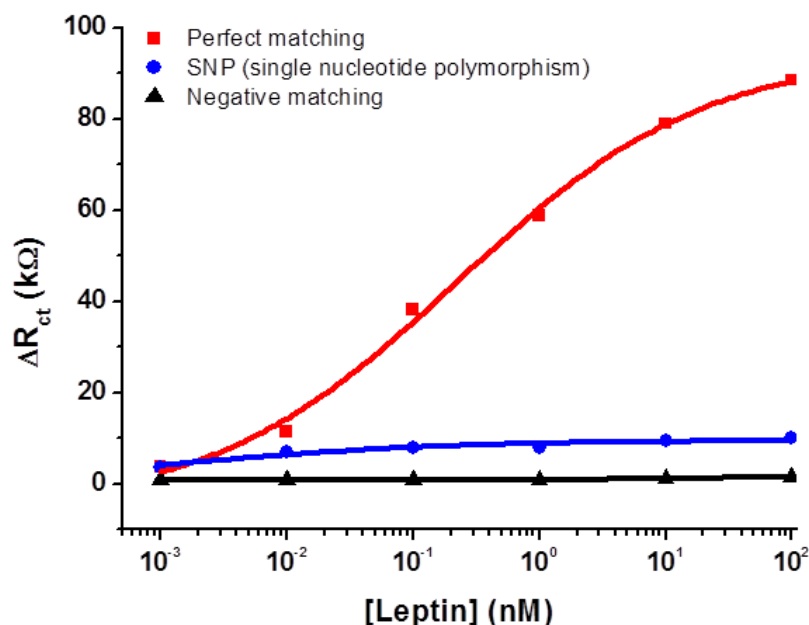


Fig3. Quantitative analysis of probe and target DNA hybridization. ΔR_{ct} (R_{ct} target DNA – R_{ct} of probe DNA) was plotted as a function of target DNA concentration.

As shown Figure 3, a quantitative curve for the detection of complementary target DNA ranging from 1 pM to 100 nM was observed. Shown on the Y-axis, ΔR_{ct} is difference between R_{ct} after hybridization and before hybridization. ΔR_{ct} was increased with complementary probe concentration. In contrast, ΔR_{ct} demonstrated a negligible increase in the case of non-complementary DNA. The most important target, SNP DNA demonstrated no difference when compared with complementary DNA. Although SNP's signal change is larger than non-complementary DNA, but this can be ignored compared with complementary DNA. This result shows that impedimetric NWA electrode sensors can be applied to the non-labeled DNA hybridization detection for the use in SNPs detection.

4. CONCLUSIONS

The Nano Well Array, electrode-based, electrochemical quantitative system for the detection of SNPs in leptin was developed using the EIS method. Non-complementary DNA and DNA with SNPs yielded negligible changes in ΔR_{ct} compared to complementary target DNA at the wide range from 1 pM to 100 nM. This detection method based on NWA electrodes can be applied to the non-labeled DNA hybridization detection for the use in SNPs detection.

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