SELF-ASSEMBLED MONOLAYER-ASSISTED SILICON NANOWIRE BIOSENSOR FOR STUDYING PROTEIN-DNA INTERACTIONS

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ABSTRACT

A self-assembled monolayer (SAM)-assisted silicon nanowire (SiNW) biosensor for studying protein-DNA interactions is presented. The SiNW surface was coated with a vinyl-terminated SAM, and the termination of the surface was changed to carboxylic acid via oxidation. DNA modified with amino group was subsequently immobilized on the SiNW surface. Protein-DNA binding was finally investigated by the functionalized SiNW biosensor. We observed that ERα had high sequence specificity to the SiNW biosensor which was functionalized with three different ERE including perfect, mutant and scrambled DNA sequences. We also demonstrated that the specific DNA-functionalized SiNW biosensor was capable of detecting ERα as low as 10 fM.

KEYWORDS: Self-assembled monolayer; Silicon nanowire; Biosensor; Protein**;** DNA

INTRODUCTION

Silicon nanowires (SiNWs) biosensor, as a label-free and highly sensitive electronic detection methodology, has been extensively developed for detection of nucleic acids and proteins [1-4]. However, much is unknown about studying of protein-DNA interactions using the SiNW biosensor.

Self-assembled monolayer (SAM) is an organic interface, and has several advantages such as easy of preparation, precision of surface control, and a spectrum of possible surface chemistries. It was also proven that the densely packed organic SAM as the gate insulator shows reduced leakage current density, which indicates the compatibility of these SAMs with semiconductor nano-FET device and their wide applications in nanometer-scale electronics[5]. Very recently, organophosphonate-based SiNW biosensor functionalized with peptide nucleic acid (PNA) was used to detect DNA. The organophosphonate SAM is not only stable, but elegant to bond biological system to native silicon oxide surface[6]. The developed SiNW biosensor was applicable to DNA detection using the PNA-functionalized SiNW biosensor via PNA-DNA hybridization.

In this work, a SAM was formed on the SiNW surface, and the SAM-assisted SiNW biosensor was capable of characterizing the protein-DNA interactions with good specificity and sensitivity. The formed SAM as a desirable interface will not only promote surface receptor conformational control for good accessibility of the target molecule binding, but also provide good passivation properties on the native silicon oxide.

EXPERIMENTAL

The SiNWs were formed in an array format through conventional optical lithography, etching and oxidation, and thus are fully compatible with CMOS technology. Figure 1a shows optical image of the working SiNW chip and Figure 1b illustrates SEM image of individual SiNW sensor. The nanowire chip for bio-sensing fabricated in the work is designed with 36 clusters of 5 nanowires each. The SiNW arrays are 90 μm in length and 2 μm spacing in between two wires. The typical resistance of the SiNW used for the work ranges from 10 to 25 MΩ.

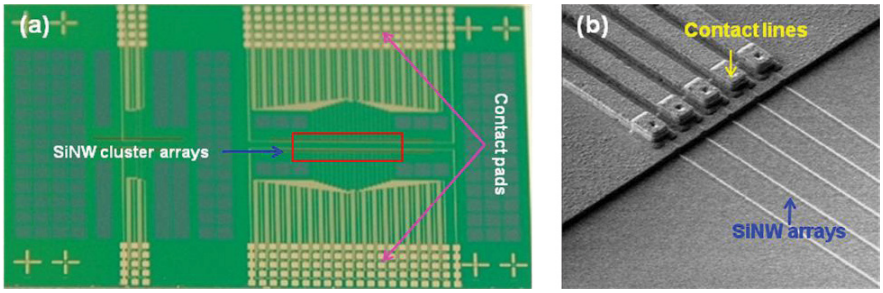

Figure 1. (a) Optical image of SiNW array sensor chip. (b) SEM image of one cluster having 5 individual SiNW array sensors.

A self-assembled monolayer of vinyl group was formed on SiNW surface by immersing the chip in a solution of 0.01M tetradecyltrichlorosilane in bicyclohexyl for 40 mins. Oxidation of the vinyl group to carboxylic group was achieved by placing the SiNW in a mixture of 0.5 mM $KMnO_4$, 19.5 mM $NaIO_4$ and 1.8 mM K_2CO_3 for 24 h. The SiNW was then rinsed with a series of solutions, in the order of 0.3 M $NaHSO_4$, water, 0.1 M HCl, water and ethanol. The SiNW was placed in a freshly prepared mixture of 0.2 M EDC and 0.05 M NHS for 30 mins. 10μM of amine-modified ERE was then applied directly onto the surface and incubated in a moist environment for 2 h. Excess ERE was washed

away with 1×PBS. To prevent non-specific binding of target, the ERE modified SiNW surface was then passivated with 1 mg/ml of amino-PEG.

Electrical measurements were taken in 10 μM HEPES buffer containing 1.5 mM KCl, 100 μM $MgCl_2$ and 10 μM EGTA (pH 7.4). Directly after the application of the ERE probe, the first electrical measurement was taken using Alessi REL-6100 probe station by probing the source (S) and drain (D) electrodes. Currents at 0.1V for 15 wires were recorded. Subsequently, the ERα target was applied onto the surface and incubated in a moist environment for 1 h. The unbounded ER was washed away using HEPES buffer. Using the same measurement method, currents at 0.1V for the same 15 wires were recorded again and the difference in currents was analyzed by using the formula [(I-Io)/Io]×100.

RESULTS AND DISCUSSION

The schematic diagram of the SAM-assisted SiNW biosensor for studying protein-DNA interactions is illustrated in Figure 2. As an example, estrogen receptor element (ERE) and estrogen receptor alpha (ERα) binding was adopted in the work [7]. A vinyl-terminated SAM was formed on the SiNW surface. Change of the termination moiety from vinyl to carboxyl was carried out by oxidation. The aminated ERE after annealing was subsequently immobilized to the carboxyl-terminated SAM. Finally ERα was specifically bound to the immobilized ERE. The ERα contains positive charges in neutral HEPES buffer because PI of ERα is approximately 8. The contribution of positive charge to the n-type SiNW surface induces an increase in current.

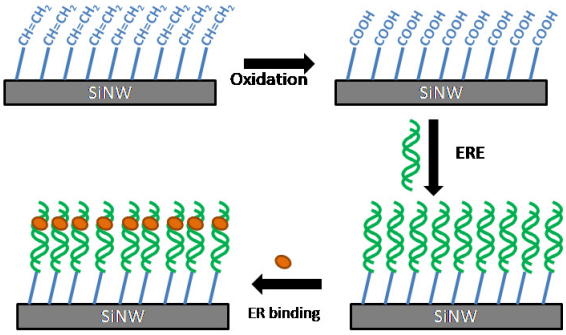

Figure 2. Schematic diagram of a SAM-assisted SiNW biosensor for studying protein-DNA interactions. ERE is immobilized on the surface via a carboxyl-terminated SAM. Positively charged ER is bound to the specific ERE-functionalized SiNW biosensor, resulting in increase in current in the n-type SiNW biosensor.

Table 1 shows sequences of the EREs involved in this study. The wild-type ERE (wt-ERE) carries the palindromic GGTCA half-site (underlined) with a 3-bp separation. The mutant ERE (mut-ERE) contains a symmetric base substitution in each of the ERE half-sites. The scrambled-ERE (non-ERE) has the sequence in the ERE arms scrambled. The three DNA sequences modified with amino group at 5 end for immobilization.

Table 1 Sequences of EREs Involved in This Study

Name (Denoted as)	Sequences
Wild-type ERE (wt-ERE)	5'-H_2N-CAACTGGGTCATTCTGACCTAGAAG-3'
Mutant ERE (mut-ERE)	5'-H_2N-CAACTGGGT***T***CATTCTGA***T***CTAGAAG-3'
Scrambled ERE (non-ERE)	5'-H_2N-CAACTGATCGGTTCCTCGATAGAAG-3'

Specificity of ERα-ERE binding was obtained by applying ERα to the three ERE-functionalized SiNW surface. Figure 3 shows that a remarkable current increase was obtained when the ERα was bound to the wt-ERE-functionalized SiNW biosensor. A negligible response was observed when the ERα interacted with the non-ERE. To evaluate the capability of the SiNW sensor for discrimination between mut-ERE and wt-ERE, the mut-ERE-functionalized SiNW sensor was incubated with ERα. As seen from Figure 3, the increase in current was much lower than that of wt-ERE. The high specificity suggests that the unique SAM-assisted SiNW biosensor allows for label-free discrimination between the wild type and mutant EREs interacting with ER alpha.

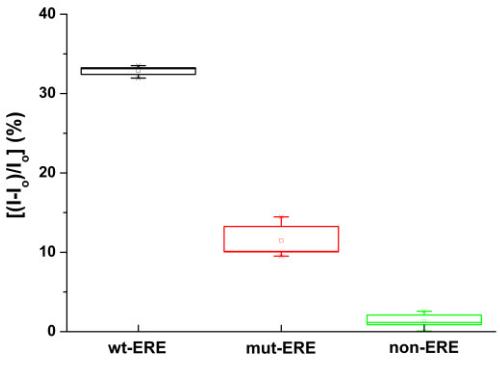

Figure 3. Specificity of ERα with various EREs immobilized on the SAM-assisted SiNW biosensor surface.

Subsequently, sensitivity was investigated by applying various concentrations of ERα from 1 pM to 1 fM to the wt-ERE-functionalized SiNW sensor. Figure 4 demonstrates concentration-dependent characterization of ERα-ERE interaction by the SiNW biosensor. It was observed that the current change dropped as a function of varying concentration of ERα. Almost negligible response was found when 1 fM ERα was bound to the DNA. 1 pM ERα bound to the surface gave rise to a ~33% increase in current. Moreover, a ~10.3% current increase was obtained when 10 fM ERα was used. It was observed that the ultralow concentration of ERα down to 10 fM could be effectively detected with the SAM-assisted SiNW biosensor.

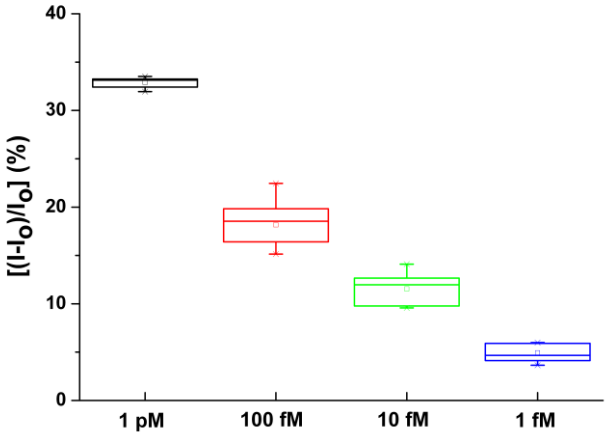

Figure 4.Response of the wt-ERE-functionalized SiNW biosensor to various concentrations of specific ERα.

CONCLUSION

In this presentation, a SAM-assisted SiNW biosensor has been developed for the specific and high sensitive characterization of protein-DNA interactions. The SiNW biosensor is capable of distinguishing the base substitutions in ERE half-sites by binding them to ERα, and the wt-ERE-functionalized SiNW biosensor is proven to detect 10 fM of ERα. This protocol provides a successful demonstration of the monolayer-assisted SiNW biosensor for studying protein-DNA interactions.

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