A DNA POTENTIOMETIC FET SENSOR BASED ON THE DIRECT CHARGE ACCUMULATION

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ABSTRACT

This paper reports a label-free field effect transistor (FET) sensor for the detection of DNA hybridization, which measures the surface potential on oligonucleotide modified electrodes using a direct charge accumulation method. The sensor directly and repeatedly transfers the charges that correspond to the surface potential induced by intrinsic negative charges in immobilized molecules, resulting in an improved signal-to-noise ratio (SNR) through the oversampling effect of accumulation and differential architecture. Our sensor offers a stable, robust and reproducible measurement, unlike the previous ion-sensitive field effect transistors (ISFETs). The proposed device is integrated with working electrodes, a reference electrode and readout circuits into one package via a 0.35 μm CMOS process. Our electrical biosensor has great potential as a bio analytical tool for point-of-care diagnoses.

KEYWORDS: DNA Hybridization, Negative Charge, FET Sensor, Surface Potential

INTRODUCTION

Recently, silicon devices based on the field-effect phenomena offer label-free and direct electrical detection when used to quantify the hybridization of DNA molecules, resulting in a rapid, robust and inexpensive measurement and compatibility with commercial microfabrication technology [1]. With respect to the field-effect device, ion-sensitive field effect transistors (ISFETs) have demonstrated the capability to detect net surface charges via the intrinsic negative charge in immobilized molecules through a shift of the threshold voltage or a change in the drain current [2]. However, these technologies are affected largely by the conversion efficiency in the charge density at the semiconductor space-charge region corresponding to the net surface charge at the electrolyte-electrode interface [3]. These devices are also limited by their minimum detectable level (several mV) of the surface charge because small fluctuating signals are buried by the noise components of FET [4]. In this paper, we propose a method that directly and repeatedly transfers the charges that correspond to the changes in the surface potential, thus improving the signal-to-noise ratio (SNR) through an oversampling effect by accumulation using active components.

METHODS

The proposed device includes metal electrodes and readout circuits on a silicon substrate. The electrodes, which are exposed to the analyte solution, are built into the top surface of a p-type substrate. Readout circuits are located below the substrate, interfacing with the metal electrodes and the external device.

Figure 1 shows a conceptual view and electrical models of the proposed device. The built-in electrodes are working electrodes that are modified with DNA strands and a reference electrode that determines the potential of the analyte solution. Silicon nitride (Si_3N_4) isolates and protects readout circuits from the electrolyte solution. The differential architecture in this device, which detects the relative difference in the surface potential between the only probe-functionalized working electrode and the hybridized working electrode, excludes the disturbing or interfering common-type of noises such as thermal fluctuations, drifts, nonspecific binding and changes in the electrolyte composition, thus enabling accurate measurement. Our device uses gold (Au) as the material for the working electrodes, as gold binds strongly to organic molecules containing thiol group. Aluminum (Al) is used for the reference electrode.

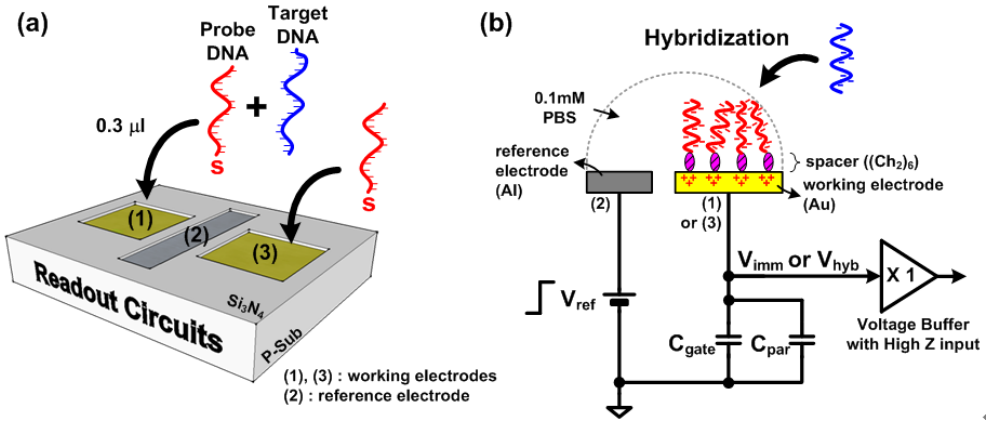

Figure 1: (a) A conceptual view of the device, (b) Electrical models of the device

Figure 2 shows a simplified description of the readout circuits. The readout circuits are composed of three parts: a voltage buffer circuit, an integrator and an analog-to-digital convertor (ADC). The voltage buffer circuit has the input characteristic of high impedance due to the use of a transistor with isolated gate. Thus, it ideally transfers charges induced in the working electrodes to the succeeding stage of the integrator without any leaky charges. The difference in the transferred charges is then accumulated by the fully differential switched-capacitor type integrator [5]. As the signal integration cycle is repeated, the differential information in the surface potential increases and expands the readable signals. Through this integrating operation, it is expected that the signal-to-noise ratio (SNR) of the device increases [4]. The accumulated signal is then transformed into a square signal and interfaced with external digital devices through a one-bit analog-to-digital converter which is composed of a comparator, a counter and a shift register.

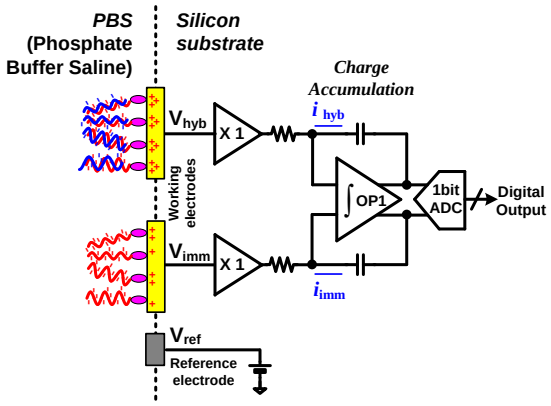

Figure 2. Simplified description of the readout circuits

RESULTS AND DISCUSSION

Figure 3 shows the measured performance from the functional operation of the designed biosensor system. It shows the target range and the detection limit that our biosensor system can measure. The detectable range was investigated while varying the voltage difference, ΔV ($V_{probe} - V_{target}$), between the two differential electrodes using discrete off-chip components. The accumulation method enlarges the infinitesimal input signal to a readable level while suppressing random noise, resulting in a noise-immune measurement. The sensor achieves a detectable range of 88.3 dB and a detection limit of 36 μV for the surface potential. This performance demonstrates that the proposed device is capable of detecting wide concentrations of targets. The accumulation time (t_{acc}) decreases as the difference between the two inputs, V_{probe} and V_{target}, increases. Thus, it is expected that the signal induced by the increased surface charge after hybridization would result in a gradual decrease in the accumulation time.

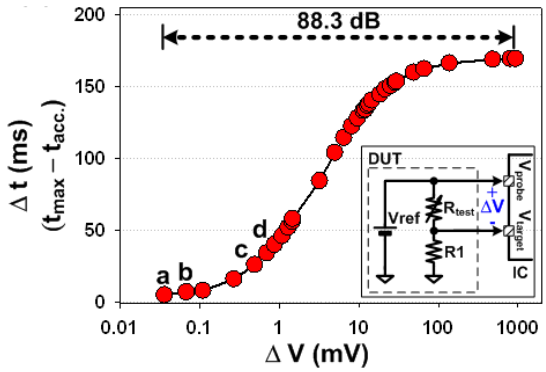

Figure 3. The measured performance of the designed biosensor in terms of the target range and detection limit. t_{acc} denotes the accumulation time generated by the difference in the input voltages, and t_{max} is the maximum accumulation time under the same inputs, as caused by the mismatched components in the readout circuit.

In this paper, three conditions required to verify the proposed biosensor system are as follows: (1) an electrode immobilized with probe DNA, (2) an electrode hybridized specifically with complementary DNA strands, (3) an electrode bound to non-complementary DNA strands. The experiment was carried out by immersing the surface of the electrode in the buffer solution using single-use syringes. Figure 4 (a) shows the experimental results for the hybridization of the probe DNA and the target DNA with four target oligonucleotide concentrations. The relative variation of the difference in the accumulation time was normalized before and after the hybridization of the probe DNA and the target DNA. As expected, the increased surface charge after the hybridization process increases the potential difference between the two differential inputs. The time t_{acc} required to reach the predefined window by accumulating signal then decreases. Therefore, the relative variation of the

difference in the accumulation time is larger with high concentration of targets than that with low concentration of target DNA. The figure shows that specific DNA binding with complementary samples leads to more significant changes compared to that with non-complementary samples. We observed that the induced charge in the working electrode increases as the DNA strand concentration increases. The detection limit of the sensor used in this study was 100 pM, and the normalized variation of the accumulation time was changed linearly with the target concentration. Although our detection method is less sensitive than the state-of-the-art label-dependent methods, our detection limit compares favorably with many label-free methods. With this device, we propose that electrical measurement device can function as a label-free transducer for specific and inexpensive DNA biosensing applications. To demonstrate the specificity of our sensor, we verified that the device can identify a single-base mismatch in 18-mer oligonucleotides (1 nM). In Figure 4(b), the smaller variations of the difference in the accumulation time from a single-base mismatched sample were observed as compared to a fully matched sample. This observation demonstrates that our device can differentiate between fully matched complementary and single mismatched DNA sequences. The sequences used in the single-base mismatch test are shown in the supplementary material. These data demonstrate that the proposed biosensor is capable of robustly detecting and quantifying the hybridization of probe and target DNA.

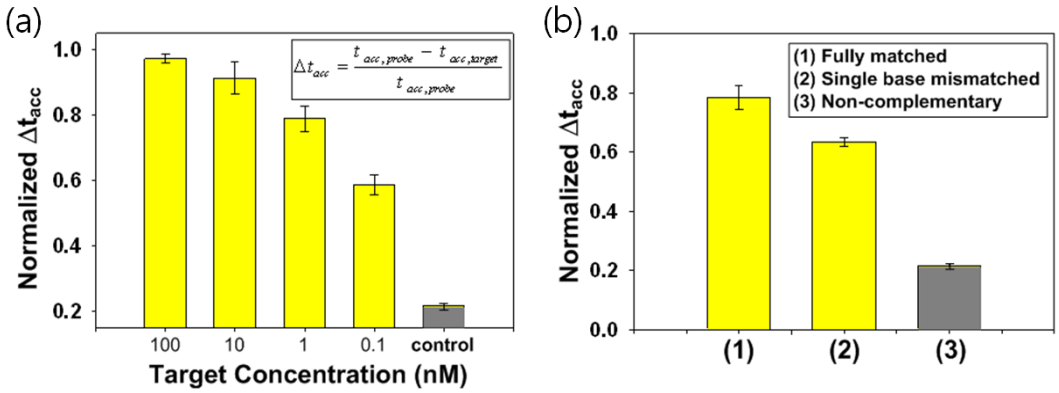

Figure 4. (a) The measured results of the normalized variation of the accumulation time (Δt_{acc}) for hybridization with different target concentrations (100 nM, 10 nM, 1 nM, and 100 pM). The control sample used here was 1 μM of non-complementary DNA ,(b) Measured result after hybridization with single-base mismatched DNA. (1) 1 nM target DNA (18-mer), (2) single-base mismatched DNA (18-mer), and (3) 1 μM non-complementary DNA.

CONCLUSIONS

The FET sensor offers label-free and electrical detection when used to quantify the hybridization of DNA strands, resulting in rapid, robust and inexpensive measurements. This paper introduced a biosensor based on the direct measurement of the surface potential on bio-modified electrodes using the electrostatic induction of molecular charges. Built-in electrodes and readout circuits were integrated into one package with a standard CMOS process. The method that accumulates the charges that correspond to the surface potential repeatedly results in an improved SNR of the device through the oversampling effect of accumulation and the use of a differential architecture. In this study, a quantitative evaluation of an oligonucleotide derived from an avian influenza on a chip was demonstrated successfully. Our electrical biosensor is thought to have great potential for use as a bioanalytical tool in point-of-care diagnoses.

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