Biosensors and Bioelectronics 35 (2012) 456–460

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Short communication

Ultrasensitive and label-free detection of pathogenic avian influenza DNA by using CMOS impedimetric sensors

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ARTICLE INFO

Article history:
Received 3 January 2012
Received in revised form 13 February 2012
Accepted 23 February 2012
Available online 3 March 2012

Keywords:
Avian influenza virus
Interdigitated microelectrode
CMOS
Integrated circuit

ABSTRACT

This work presents miniaturized CMOS (complementary metal oxide semiconductor) sensors for non-faradic impedimetric detection of AIV (avian influenza virus) oligonucleotides. The signal-to-noise ratio is significantly improved by monolithic sensor integration to reduce the effect of parasitic capacitances. The use of sub-μm interdigitated microelectrodes is also beneficial for promoting the signal coupling efficiency. Capacitance changes associated with surface modification, functionalization, and DNA hybridization were extracted from the measured frequency responses based on an equivalent-circuit model. Hybridization of the AIV H5 capture and target DNA probes produced a capacitance reduction of $-13.2 \pm 2.1\%$ for target DNA concentrations from 1 fM to 10 fM, while a capacitance increase was observed when H5 target DNA was replaced with non-complementary H7 target DNA. With the demonstrated superior sensing capabilities, this miniaturized CMOS sensing platform shows great potential for label-free point-of-care biosensing applications.

1. Introduction

Electrical bioassays hold great promise for numerous decentralized clinical applications ranging from emergency-room screening to point-of-care diagnostics due to their low cost, high sensitivity, specificity, speed, and portability. A miniaturized biosensing platform can be achieved through monolithic integration of sensing devices and detection circuits in an integrated-circuit process, such as the CMOS technology. Arrays of sensors fabricated on a CMOS chip can enhance sensing resolution and accuracy through statistical analysis of the collected data. CMOS biosensors have been implemented based on the electrochemical (Schienle et al., 2004), impedimetric (Lee et al., 2010), capacitive (Stagni et al., 2006; Lu et al., 2010), conductive (Li et al., 2003), ion-sensitive (Li et al., 2010), magnetic (Sun et al., 2009), optical (Eltoukhy et al., 2006), and micromechanical (Shekhawat et al., 2006) approaches. Some of the methods require labeling by using, for example, magnetic microbeads (Sun et al., 2009), and many are label-free for biosignal transduction. Sensing resolution at the fM level has been reported by using CMOS ion-sensitive field effect transistors (Li et al., 2010). The CMOS impedimetric sensors presented in this work are intended to demonstrate highly sensitive detection of AIV DNA.

Changes in the electrical properties of a sensing interface (e.g., capacitance, resistance) occur when a target biomolecule interacts with a probe-functionalized surface. An impedance change can be correlated to detection of DNA hybridization, antigen-antibody reaction, or biological cells. A conventional impedimetric biosensor measures the electrical impedance of an electrode-solution interface in a.c. steady state with constant d.c. bias conditions. This approach, known as electrochemical impedance spectroscopy (EIS), is accomplished by imposing a small sinusoidal voltage over a range of frequencies and measuring the resulting current. The impedance consists of both energy dissipation (resistor) and energy storage (capacitor) elements. In addition to the frequency-domain measuring method, interface impedance changes can be measured by the potentiostatic step method where small potential steps are applied to the working electrode and the transient current responses, as determined by the time constant of the interface resistance and capacitance, are measured accordingly. By this time-domain approach, a CMOS impedimetric sensor array (Lee et al., 2010) has achieved a sensing resolution of 10 nM for label-free DNA detection.

The faradaic EIS requires the addition of a redox species which is alternately oxidized and reduced by the transfer of charges to and from a metal electrode. In contrast, no additional reagent is required for non-faradaic impedance spectroscopy. The

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doi:10.1016/j.bios.2012.02.049

associated impedance change is predominantly capacitive (Hedström et al., 2005; Limbut et al., 2006; Loyprasert et al., 2008; de Vasconcelos et al., 2009; Qureshi et al., 2010) with the charge transfer resistance being omitted. The reported detection limits are generally between pg/ml to ng/ml. A low detection limit down to 7 fg/mL has been demonstrated by a microcystin-LR immunosensor (Loyprasert et al., 2008). Immobilization is a critical part in non-faradic impedimetric biosensors since the electrode surface has to be electrically insulated. This can be achieved by coating a dielectric thin film on electrode surface as in this work, or by using insulating self-assembled monolayers of sulfur compounds on gold electrodes (Berggren et al., 2001).

Avian influenza viruses, especially those highly pathogenic types (H5 and H7), are known to produce a significant morbidity rate and a mortality rate. All known viruses that cause influenza in birds belong to the species influenza *A* virus as most strains of all subtypes of influenza *A* virus are adapted to birds. Avian influenza viruses that cause diseases in both human and poultry are subtyped according to the antigenic subtype of the hemagglutinin (HA) and neuraminidase (NA) glycoproteins (e.g., H1N1). A pandemic may happen when a novel HA gene spreads rapidly among infected humans who do not have immunity to the virus. According to the report of World Health Organization in 2011, the total number of H5N1 human cases is 562 with 329 deaths. Therefore, development of highly sensitive, accurate, rapid, and cost-effective diagnostic tools is needed for surveillance programs as well as routine laboratory tests. For example, label-free AIV detection at fM level was reported by using functionalized poly-crystalline silicon nanowire field-effect transistors (Lin et al., 2009).

This study presents integrated CMOS impedimetric sensors for detection of the AIV H5 DNA. The impedance change is essentially capacitive due to a dielectric thin film on top of interdigitated microelectrodes for non-faradic detection. Signal transduction is achieved through a CMOS readout circuit. MOS transistors possess a high input impedance which is ideal for transducing the high-impedance bio-signals. Monolithic integration significantly promotes the signal-to-noise ratio by reducing the parasitic capacitances observed at the sensing node. The small gap (<μm) between interdigitated sensing electrodes also improves the sensing resolution by promoting signal coupling efficiency.

2. Materials and methods

2.1. Materials

3-Aminopropyltriethoxysilane (APTES) and ethanolamine were purchased from Sigma-Aldrich (USA). Glutaraldehyde in aqueous solution (25%) was purchased from Fluka (USA). AIV HA DNA sequences were designed based on prior works (Wang et al., 2008; Townsend et al., 2006). All synthetic oligonucleotides were purchased from MDBio Inc. (Taiwan) including 5′-aminomodified complementary AIV H5 capture DNA probe (5′-NH_2-CAA ATC TGC ATT GGT TAT CA-3′), 5′-Cyanine 3 (Cy3) modified target DNA (5′-Cy3-TGA TAA CCA ATG CAG ATT TG-3′), AIV H5 target DNA (5′-TGA TAA CCA ATG CAG ATT TG-3′), and AIV H7 target DNA (5′-TAC TCA ATT TGA CTG GGT CAA TTT G-3′). Phosphate buffer solution (PBS) was prepared in deionized (DI) water (pH = 7).

2.2. Surface functionalization and immobilization

The cross-sectional view in Fig. 1A illustrates the operating principle of impedimetric AIV H5 DNA detection. Interdigitated microelectrodes covered by the CMOS silicon dioxide thin film were utilized as the sensing interface to perform surface functionalization and immobilization. The CMOS sensors were firstly

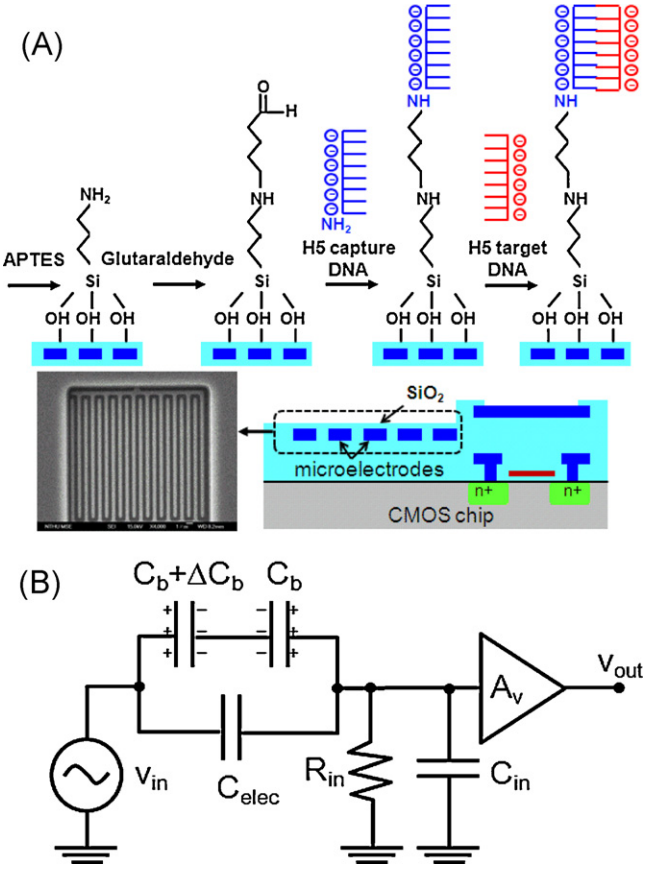

Fig. 1. (A) Surface modification and functionalization for impedimetric detection of AIV H5 DNA hybridization using CMOS interdigitated microelectrodes. The scanning electron micrograph shows the top view of the electrodes. (B) The equivalent-circuit model for impedimetric detection.

washed by ethanol and acetone solutions for 10 min respectively to remove contaminants and introduce–OH on the oxide surface before they were immersed in 2% APTES ethanol solution for 30 min to introduce amino groups on the oxide surface. The devices were then washed with ethanol (99.5%) for three times. In the following step, the device surface was immersed in solution containing 2.5% glutaraldehyde in 10 mM PBS (pH = 7.0) for 2 h to produce aldehyde groups, and then washed by PBS. Finally, the 10-μM 5′-aminomodified capture DNA probes mixed in 10-mM PBS were immobilized on the sensor surface after 10 h. The un-reacted aldehyde groups were blocked by mixing with 50-mM ethanolamine and 4-mM sodium cyanoborohydride in 10-mM PBS for 30 min.

2.3. Device fabrication

A two-polysilicon-four-metal (2P4M) 0.35-μm CMOS process was used for sensor fabrication. The CMOS aluminum layer (metal-3) was used as the electrode material and the inter-metal silicon dioxide on metal-3 was used for immobilization of the DNA capture probes. The passivation thin films on top of microelectrodes were removed by dry etch in the foundry. The remaining oxide thickness on the metal-3 electrodes was 0.35 μm as measured by a surface profilometer (Kosaka ET4000a). A thin silicon dioxide is desired in this study to promote the signal-coupling efficiency. The area of interdigitated microelectrodes is $20 \times 20\ \mu m^2$. The electrode length, thickness, separation, and width are 18, 0.64, 0.5, and 0.6 μm, respectively. The thickness of silicon dioxide beneath microelectrodes is 4.4 μm. The electrode capacitance is calculated