Sensors and Actuators B 175 (2012) 201–207

Contents lists available at SciVerse ScienceDirect

Sensors and Actuators B: Chemical

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

A novel biosensor based on hafnium oxide: Application for early stage detection of human interleukin-10

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ARTICLE INFO

Article history:
Available online 22 May 2012

Keywords:
Biosensor
Hafnium oxide
Aldehyde
Electrochemical impedance spectroscopy
Microcontact printing
Interleukin-10

ABSTRACT

Measurement of interleukin-10 (IL-10) has subsequently become a crucial tool to identify end-stage heart failure (ESHF) patients prone to adverse outcomes during the early phase of left ventricular assisted device (LVAD) implantation. In this context, label-free detection using a novel substrate based on hafnium oxide (HfO_2) grown by atomic layer deposition (ALD) on silicon was applied. Here, we studied the interaction between recombinant human (rh) IL-10 with the corresponding monoclonal antibody (mAb) for early cytokine detection of an anti-inflammatory response due to LVAD implantation. For this purpose, HfO_2 has been functionalized using an aldehyde–silane ((11-(triethoxysilyl) undecanal (TESUD)) self-assembled monolayer (SAMs), to directly immobilize the anti-human IL-10 mAb by covalent bonding. The interaction between the antibody–antigen (Ab–Ag) was characterized by fluorescence patterning and electrochemical impedance spectroscopy (EIS). Confirmation for the bio-recognition of the protein was achieved by fluorescence patterning, while Nyquist plots have shown a stepwise variation due to the polarization resistance (R_p) between the Ab activated surfaces with the detection of the protein. For early expression monitoring, commercial proteins of rh IL-10 were analyzed between 0.1 pg/mL and 50 ng/mL. Protein concentrations within the linear range of 0.1–20 pg/mL were detected, and these values formulated a sensitivity of 0.49 $(ng/mL)^{-1}$. These preliminary results demonstrated that the developed biosensor was sensitive to the detection of rh IL-10, and the measured limit of 0.1 pg/mL in phosphate buffered saline (PBS) was clearly detectable, which displays the high sensitivity of EIS. On analysis of an interference attributable to non-specific binding of other cytokine biomarkers; tumor necrosis factor-α (TNF-α) and IL-1β were analyzed without causing an interference to the IL-10 mAb. This established that selective sensitivity was responsive only to rh IL-10. To our knowledge, this is the first biosensor that has been based on HfO_2 for Ag detection by EIS. In time, the HfO_2 insulator will be incorporated into the gate of silicon-based ion-sensitive field-effect transistors (ISFETs) and developed as a portable real time detection system for the IL family of biomarkers in human serum.

1. Introduction

Heart failure (HF) has become a huge problem in the western world. Every year, approximately 1 million new patients are diagnosed with this illness, which makes it one of the fastest growing cardiovascular diseases (CVDs). Various commonly available methods for the detection of biomarkers related with CVDs have been developed, such as immunoaffinity column assay, fluorometric, and enzyme-linked immunosorbent assay (ELISA) [1–5]. However, these laboratory techniques are based on sophisticated instrumentation that requires qualified personnel, while sample preparation and analysis are also time consuming. Therefore, there is an urgent need for easier use of diagnostic tools with high sensitivity and selectivity, which enable identification of the severity of the inflammatory state in the early stages and thus permit early therapeutic intervention. Such tools would be of great help to treat HF before the patients quality of life is compromised and hence, aid in the development of new pharmacotherapeutic options.

Biosensors based on electrical measurement are devices that employ biochemical molecular recognition for desired selectivity with a specific biomarker of interest. Such biosensors present a

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http://dx.doi.org/10.1016/j.snb.2012.04.090

system that is sensitive, label-free, rapid, reproducible, portable, and at low production costs can eliminate applications that utilize time-consuming laboratory equipment [6–10]. Recently, it has been used for the detection of IL-6 and IL-8 with very low concentrations measured [11,12].

In general, biosensor fabrication is based upon the semiconducting properties of silicon with thermally grown silicon dioxide (SiO_2) and silicon nitride (Si_3N_4) being most favored over the past decades, when applied as transistor gates within field effect transistors (FETs) [13–17]. With the thickness reduction of SiO_2 complementary metal oxide semi-conductor (CMOS) devices, high gate oxide leakage becomes apparent as the reliability of the SiO_2 layer is jeopardized. Capacitance can be improved by increasing the dielectric constant (K) without having to reduce the dielectric thickness to leaky dimensions. Many other materials have been considered as potential alternatives for high-K gate materials instead of SiO_2 as they present the necessary capacitance due to physical thickness and a reduction of the gate leakage current [18]. These include: aluminum oxide (Al_2O_3), tantalum pentoxide (Ta_2O_5), titanium dioxide (TiO_2), zirconium dioxide (ZrO_2) and hafnium oxide (HfO_2) [19–25]. One of these extensively researched materials is HfO_2. When compared to the aforementioned high-K dielectrics, HfO_2 has increased thermal stability on silicon by using atomic layer deposition (ALD) as deposition method (Al_2O_3 has also improved stability on Si, but a lower K of 9 does not render it beneficial for capacitor applications in the continued CMOS advancements), and a higher K for reduced leakage and enhanced gate capacitance when compared with SiO_2 [23]. Therefore, HfO_2 can be considered a promising high-K gate material. On application to a biosensor, these properties can be addressed when considering the charge effect of a material. For instance, improved thermal stability creates a good interface for electrical performance, since consistent capacitance switching behavior of the semiconducting channel correlates to the gate oxide layer that is deposited on the channel [25], while, relative activation requirements upon functionalization of a surface can also be improved by application of a high-K material that obtains a highly polar surface (e.g. low K materials require aggressive activation to improve chemical bonding of the required silane, e.g. piranha solution).

Recently, Chen et al. have applied HfO_2 as a highly sensitive bio-field-effect transistor (bioFET) for biotin functionalization using capacitance–voltage measurements [25]. Streptavidin binding was reproducible by application of a linker molecule; (3-aminopropy)triethoxysilane (APTES). The authors have demonstrated that HfO_2 can be applied for functionalization with biomolecules and, therefore, we propose the first novel HfO_2 biosensor that has been functionalized for antibody (Ab) deposition with detection of a human antigen (Ag) by electrochemical impedance spectroscopy (EIS).

IL-10 is an anti-inflammatory cytokine with an important role in modulating the inflammatory processes in several diseases related with inflammation. An exacerbated increase of IL-10, in addition to uncontrolled release of pro-inflammatory mediators, was proposed as a peculiar pattern of adverse inflammatory response related to the magnitude of multi-organ dysfunction in end-stage heart failure (ESHF) patients supported by left ventricular assisted device (LVAD) [2]. In the early phase of LVAD support, patients are more susceptible to adverse events and are at a higher risk of multiple organ failure syndrome (MOFS). The impact of MOFS, ultimately leads to mortality of the patient and it is influenced by the degree of immuno-inflammation [2]. As a cause, this factor is independent of infection, and the inflammatory response is most dangerous during the first month, especially, within the first few hours after implantation. Early expression of IL-10 within the pg/mL range can discriminate patients of high risk, and since EIS is rapid, early therapeutic intervention can be provided by the clinicians to assist in preventing MOFS from developing to a critical stage. Stumpf et al. reported CHF IL-10 plasma cytokine levels for 50 patients at 2.3 ± 1.9 pg/mL, while controls were measured at 5.2 ± 2.3 pg/mL ($P < 0.01$) [26]. Finally, Bolger et al. studied plasma IL-10 cytokine levels where CHF patients were recorded at 3.7 ± 1.1 pg/mL and control patients at 4.9 ± 1.5 pg/mL ($P = 0.50$) [27]. These results demonstrated plasma samples from CHF patients that had not undergone LVAD implantation though were classified with this condition. Here, the severity of CHF levels was classified as New York Heart Association (NYHA) class II to IV [26], though on both accounts, the control patients recorded an increase in IL-10 plasma levels.

In a study of patients who underwent surgery for LVAD implantation, Caruso et al. have reported detectable human IL-10 plasma levels for 23 LVAD implanted patients between a range of 0 and 1558 pg/mL in the first 30 days of LVAD support using ELISA [2]. Interestingly, the authors analyzed samples within certain time frames to quantify exactly when patients were susceptible to higher levels of inflammation after surgery. Before LVAD implantation, patients obtained minimal IL-10 inflammation (pre-implant) due to the circulating cytokine (1-month survivors, 1.8 pg/mL and non-survivors, 5.6 pg/mL). Following LVAD implantation, plasma samples were taken after 4 h. At this critical stage, early expression of human IL-10 peaked in comparison with other plasma samples analyzed within the 30 day period for survivors and non-survivors. Levels for IL-10 non-survivors (177.8 pg/mL) were also higher than that of the survivors (56.2 pg/mL). Therefore, the authors have established that the elevated IL-10 levels in parallel with other cytokines can play an important role in the development of adverse events early after LVAD implantation [2,3].

Here, we present a novel substrate of HfO_2 where the surface has been functionalized with 11-(triethoxysilyl) undecanal (TESUD) by chemical vapor deposition in a saturated medium [28,29]. Surface activation has enabled direct monoclonal antibody (mAb) bonding, where no other reagents were required that could in essence denature the primary capture Ab [28]. Surface treatment was analyzed by contact angle measurements (CAM) that were based upon cleaned, UV/Ozone activated and TESUD surfaces. The Ab–Ag–Ab bio-recognition on HfO_2 TESUD activated substrates was characterized by fluorescence microscopy, while application of EIS aided in the evaluation of this novel biosensor for early stage detection of human IL-10 inflammation for LVAD recipients.

2. Materials and methods

2.1. Process for substrate fabrication

In the ALD technique, very thin layers can be deposited by sequential self-terminating gas–solid reactions. The cyclic nature of this deposition process results in a layer-by-layer deposition, which exhibits a very important advantage in terms of both thickness and composition control. Typically, a deposition cycle in that sequence, consists on the introduction of the first appropriate precursor gas into the reaction chamber in the form of a very short time pulse, producing the chemisorption of the precursor onto the surface of the substrate, followed by a purge step with an inert gas to remove the precursor excess and the reaction by-products; next, the second precursor gas is pulsed and introduced into the chamber and reacts with the first precursor present on the substrate; and, finally, another purge step is done with the same purpose of the first one. This constitutes one cycle of the process and a monolayer growth by cycle is obtained due to the self-limiting nature of the reactions.

The samples structures were made on 100 mm-diameter p-type silicon wafers (1 0 0), oriented with a resistivity of 4–40 Ω cm.