A NOVEL CORTISOL BIOSENSOR BASED ON THE CAPACITIVE STRUCTURE OF HAFNIUM OXIDE: APPLICATION FOR HEART FAILURE MONITORING

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ABSTRACT

Assessing cortisol level in human bodies has become an essential tool to recognize heart failure (HF). In this work, the label-free detection of cortisol using a novel capacitive substrate based on hafnium oxide (HfO_2) was accomplished. In this context, the interaction between the cortisol with its corresponding polyclonal antibody (pAb) has been studied, and the detection event was followed by electrochemical impedance spectroscopy (EIS). Cortisol was detectable between a wide range of concentration from 2 ng/mL to 50 ng/mL. Moreover, the cross-selectivity study showed that the developed biosensor demonstrated to be highly selective toward cortisol when compared to some related biomarkers such as Tumor necrosis factor (TNF-α) and N-terminal pro b-type Natriuretic Peptide (Nt-ProBNP). To the best of our knowledge, this is the first biosensor that has been based on capacitive HfO_2 for cortisol detection by EIS.

KEYWORDS

Biosensor, cortisol, hafnium oxide, Electrochemical impedance spectroscopy (EIS), silane aldehyde.

INTRODUCTION

Cortisol (MW 362.46 g/mol) is an important glucocorticoid hormone which is produced by the zona fasciculate of the adrenal gland. It's well known as "stress hormone" and it takes part on the regulation of various physiological functions including energy metabolism, electrolyte balance, blood pressure and cognitive function [1], [2]. In addition, it contributes to the homeostasis of the adrenal [3], cardiovascular [4], [5], immune [6], and endocrine system [7]. Moreover, it plays a key role in brain regions that are important for cognitive learning, retrieval, encoding, and memory consolidation [8]. This steroid hormone thus represents a potential biomarker for numerous pathological conditions and diseases [9], as well as a useful clinical indicators for relapse vulnerability in chronic alcohol use.

Nowadays, cortisol is usually determined by enzyme immunoassay (EIA) [10], [11], enzyme-linked immunoassay (ELISA), and radioimmunoassay (RIA) [12], as well as chromatographic techniques coupled with mass spectrometry (MS) or tandem MS/MS have been developed [13]–[15]. Commonly, the principal limitations of these steps lie in their high costs, the long run-time, and the requirement of sophisticated technical skills.

Herein, a novel and highly selective approach is proposed for the detection of cortisol. To the best of our knowledge, this is the first biosensor that uses HfO_2 substrate for cortisol detection using electrochemical impedance spectroscopy.

MATERIALS AND METHODS

Biosensor fabrication

For the fabrication of the bio-sensing platform, HfO_2 were firstly functionalized using silane aldehyde. For this purpose, bare HfO_2 substrates were cleaned by sonication in acetone, followed by thorough rinsing in ultrapure water (Millipore Milli-Q). Surface activation of the HfO_2 substrates was performed using an UV/Ozone ProcleanerTM (BioForce, Germany). The objective here was to create -OH groups at the hafnium oxide surface for the grafting of silane aldehyde. Afterwards, the substrates were thoroughly rinsed and sonicated in Milli-Q water. The active HfO_2 substrates (with –OH) were functionalized by a SAMs (Self Assembled Monolayer) of TESUD ((11-triethoxysilyl) undecanal) (abcr, Germany) using vapor-phase method [16]. Then, the substrates were placed into an oven at 100°C. After baking, they were rinsed with absolute ethanol and dried with nitrogen. Subsequently, the functionalized substrate surface was incubated with pAb-cortisol (10 µg/mL) already diluted in PBS. Finally, the substrate was treated with ethanolamine (1% v/v) in PBS buffer. This step is crucial to prevent nonspecific bonding phenomenon at the detection stage of Cortisol (fig.1).

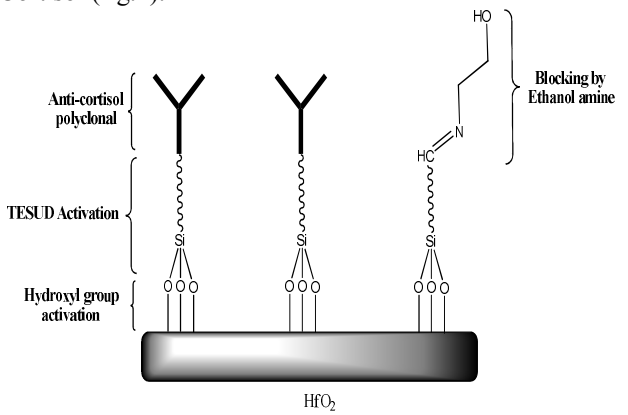

Figure 1: functionalization of HfO_2 substrate

Magnetic nanoparticles bio-functionalization

The magnetic nanoparticles were prepared having a magnetic core of Fe_3O_4 surrounded by styrene/DVB/ACPA polymers that included COOH as terminal functional group [17]. Initially, they were washed two times with PBS buffer (pH 7.4) and subsequently –COOH group were activated using a mixture of EDC/NHS at 100mM in PBS. A magnetic field was used to separate the nanoparticles from the storage solution. Ab-cortisol monoclonal (10 µL) at 100 µg/mL was added to the mixture and incubated with slow stirring at room temperature for 90 min. The non-reacted active carboxylic acid groups were blocked with Bovin Serum albumin (0.1%) in PBS buffer for 30 min. The antibody-coated magnetic nanoparticles were then separated from the mixture, re-suspended in 1 mL of PBS buffer, and used for the incubation. The procedure described above is summarized in Fig .2.

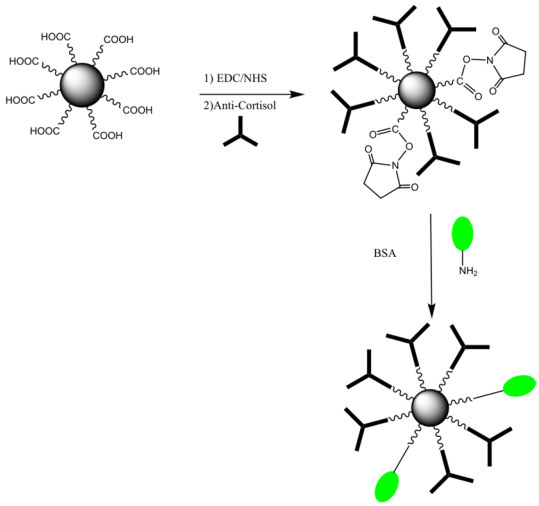

Figure 2: MNPs-COOH bio-functionalization with Anti-Cortisol

RESULTS AND DISCUSSIONS

Scanning Electron Microscopy (SEM)

Fig.3. shows a SEM image of MNPs onto HfO_2 substrate using FEI Quanta FEG 250. Here the successful formation of complex (Ab-Ag-Ab-MNP) was confirmed since it remains on the surface of the electrode after aggressive rinsing of the surface with PBS. Moreover, it can be seen an homogenous distribution of MNPs.

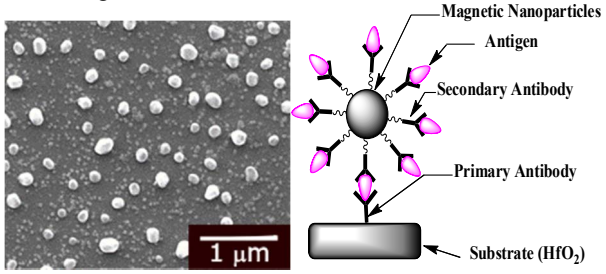

Fig 3: Image SEM of functionalized TESUD substrates with Ab-Ag-Ab-MNP (Magnetic nanoparticles) bio-recognition

Biosensor calibration by EIS measurements

EIS is an efficient technique for investigating interfacial properties on surface modified working electrodes. The biosensor developed was calibrated using EIS. For this purpose, the antibody-modified electrode was incubated with cortisol at different concentration. Nyquist plot obtained are shown in Fig. 4. An additional incubation of the electrode with the MNPs functionalized with the complementary antibody was carried out. This served to study the signal exaltation due to the presence of a larger complex.

The Nyquist plots of HfO_2 modified with cortisol polyclonal-Ab, followed by increasing cortisol concentrations were analyzed. A variation increase of the Rct (load transfer resistor) can be seen from the initial cortisol polyclonal-Ab (p-Ab) (Fig. 4) at 90 kΩ to cortisol (2 ng/mL) at 320 kΩ. The change in resistance demonstrates the bio-recognition of the Ag to the fixed p-Ab on the HfO_2 substrate. The Rct for cortisol increased with 10 ng/mL at 640 kΩ; 15 ng/mL at 720 kΩ; 50 ng/mL at 770 kΩ and MNP-Ab at 825 kΩ. The increase of the electrochemical impedance demonstrated that functionalization on HfO_2 with p-Anti-cortisol was successfully achieved as it permitted to establish a linear relationship between the Rct and cortisol concentration. The ~230 kΩ variations between the pAb-modified electrode and the first concentration of Ag-cortisol at 2 ng/mL, demonstrates that the biosensor was sensitive to low concentrations. Detection from 2 ng/mL to 50 ng/mL increased the Rct, forming a clear distinction between each observable concentration analyzed.

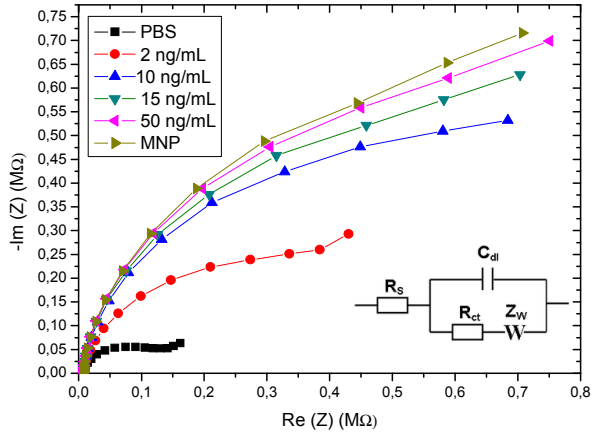

Fig 4: Nyquist plot for the detection of different concentrations of cortisol and after coupling with MNP. EIS measurements were carried out in PBS using the conditions: frequency range from 5 Hz to 200 KHz, amplitude of 25 mV

From the impedance spectra, a Z fit calculation can be obtained what provides information about the equivalent circuit. Therefore, one can obtain the variation of the Rct with cortisol at different concentrations. The equivalent electrical circuit Rs + Cdl/ (Rct + Zw) applied for the simulation that formulated the best fit for the data is shown at the inset in Fig. 4. Heiren, the components can be explained as follows: Rs corresponds to the resistance of the electrolyte solution (Rs). Cdl is the double-layer

capacitance that is in parallel with Rct which is the load transfer resistor and (Zw) Warburg impedance.

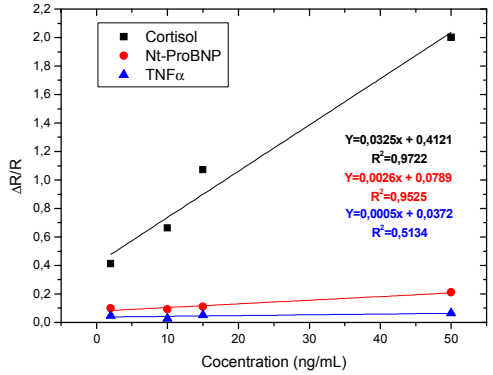

Fig 5: Normalized curve by EIS detection of (black) cortisol and selectivity of (red) Nt-ProBNP and (green) TNFa with concentrations ranging from 2 ng/mL to 50 ng/mL against the variation of the Rct calculated by ΔR/R

In Fig. 5 (black), the Rct of the cortisol concentration plot produced a linear relationship ranging from 2 ng/mL to 50 ng/mL at R^2 = 0.9722 with a slope of 0.03 (ng/mL). A cross-selectivity study was performed to assess the level of with non-specific binding. For this purpose, some cytokines structurally related to cortisol were used, namely TNF-α, and Nt-ProBNP using the same conditions and concentrations as for cortisol. From Fig .5 it can be seen that the biosensor demonstrated to be highly selective toward cortisol when compared to both TNF-α and Nt-ProBNP. The Rct of TNF-α (green) (R^2 = 0.5134 with a slope of 0.0005 (ng/mL)) and Nt-ProBNP (Red) (R^2 = 0.9525 with a slope of 0.0026 (ng/mL)) are both much lower than that of cortisol; the biosensor was 65 times more selective for cortisol than TNF-α, and 13 times more selective for cortisol than Nt-ProBNP what proved the high overall performance of the biosensor developed.

Mott–Schottky results

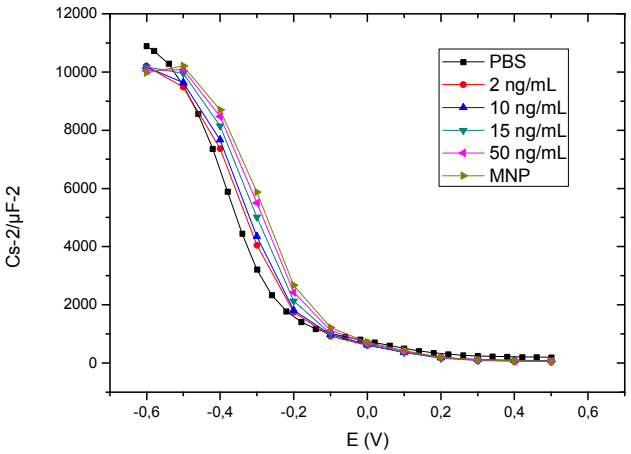

Fig 6: Mott–Schottky plots for cortisol detection using the capacitance biosensor

Complementarily, Mott-Schottky analyses were performed in order to characterize the semi-conducting behavior of HfO_2 for each concentration of cortisol. The electrochemical parameters applied were optimized to use the appropriate frequency. The biosensor was maintained in the electrochemical cell and incubated in PBS containing cortisol at different concentrations. The biosensor was then rinsed with PBS in order to remove any adsorbed proteins and analyzed afterward by Mott-Schottky using PBS as electrolyte. This procedure of the biosensor incubation was made for all cortisol concentrations (2ng/mL to 50 ng/mL). The detection of cortisol antigens at various concentrations is shown in Fig. 6. Herein, the Cs/Cmax shows a shift in the X-direction when increasing cortisol concentrations, which confirms a built-in potential difference, equivalent to a flat band voltage variation, and thus an increase in the conduction current value. All these findings demonstrates the high overall performance of the capacitive biosensor developed.

CONCLUSION

In this study, the label-free detection of cortisol using a novel capacitive substrate based on hafnium oxide (HfO_2) was accomplished. In this context, HfO_2 was functionalized by formulating SAMs of TESUD by vapor-phase method in a saturated medium. Subsequently, the functionalized substrate surface was incubated with pAb-cortisol. Then, the interaction between the cortisol with its corresponding polyclonal antibody (pAb) has been studied, and the detection event was followed by electrochemical impedance spectroscopy (EIS) and mott Schottky. Cortisol was detectable between a wide range of concentration from 2 ng/mL to 50 ng/mL. Moreover, the developed biosensor demonstrated to be highly selective toward cortisol when compared to some related biomarkers such as Tumor Necrosis Factor (TNFa) and N-terminal pro b-type Natriuretic Peptide (Nt-ProBNP). This work represents the first novel biosensor that has been based on HfO_2 for cortisol detection by means of EIS and Mott-Schottky.

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