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Novel capacitance biosensor based on hafnium oxide for interleukin-10 protein detection

Michael Lee[a]*, Nadia Zine[a], Abdellatif Baraket[a], Miguel Zabala[b], Francesca Campabadal[b], Maria Giovanna Trivella[c], Nicole Jaffrezic-Renault[a] and Abdelhamid Errachid[a]**

[a]*Laboratoire des Sciences Analytiques (LSA),Université de Claude Bernard-Lyon1, 43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne Cedex, France.*

[b]*Instituto de Microelectrónica de Barcelona (IMB), Centro Nacional de Microelectrónica (CNM) - CSIC, 08193 Bellaterra, Barcelona, Spain.*

[c]*CNR (Consiglio Nazionale Ricerche) Clinical Physiology Institute and University of Pisa, Via Moruzzi 1, 56124, Pisa Italy.*

Abstract

In this present study, we have analyzed one cytokine, interleukin-10 (IL-10), that plays a pivotal role in patients with chronic heart failure. For this purpose, a novel capacitance substrate, based on hafnium oxide (HfO_2) grown by Atomic Layer Deposition on silicon, was applied to study the interaction between IL-10 with the corresponding antibody. HfO_2 has been functionalized using an aldehyde monolayer, to directly immobilize the anti-human IL-10 monoclonal antibody. The interaction between antibody-antigen (Ab-An), was characterized by fluorescence patterning and electrochemical impedance spectroscopy (EIS). The preliminary results for fluorescence patterning, demonstrated bio-recognition of the recombinant protein, while Nyquist plots showed variation when we changed the concentration from 1-10 ng/mL. This demonstrated that the developed biosensor was sensitive to the detection of IL-10.

Keywords: Hafnium Oxide; interleukin-10; silane; capacitance biosensor; microcontact printing

1. Introduction

Cardiovascular diseases are a predominant cause of mortality in developed countries. Post-operative death due to cardiac surgery can be expressed by high elevated levels of varying cytokines and immune cells.

* Corresponding author. Tel.:+33-4-72-43-14-44; fax: +33-4-72-44-84-79.

E-mail address: michael.lee@etu.univ-lyon1.fr; ***abdelhamid.errachid@univ-lyon1.fr*

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This fatal acute rejection can be determined by measuring specific biomarkers that provide clinical inflammation in both cellular and biochemical events. The anti-inflammatory cytokine, interleukin-10, (IL-10) contributes to this process when patients experience a cytokine storm [1].

Hafnium oxide (HfO_2), presents an alternative substrate to silicon dioxide, due to its high thermal stability, and high dielectric constant, when compared to silicon [2,3]. This high dielectric constant, requires no surface activation of hafnium oxide, and thus differs upon silicon, where piranha activation is a requirement for hydroxyl group formation.

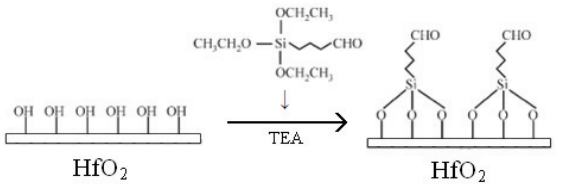

Fig. 1. Surface activation with triethoxysilane aldehyde (TEA) on hafnium oxide.

Immobilization of antibodies onto the immunosensor surface is a requirement, for the detection and analysis of bio-recognition processes [4,5]. By activation with triethoxysilane aldehyde (TEA), antibodies can be covalently bonded to a substrate surface, without additional activation steps that may affect the activity of the antibody [6].

On activation of these surfaces, microcontact printing is a rapid technique that enables pattern transfer [7]. Fabrication of a polydimethylsiloxane (PDMS) stamp produced by soft-lithography, creates a pattern that can transfer biomolecules, over a large surface area on the printed substrate. These biomolecules are physisorbed onto the PDMS stamp, and transferred by conformal contact with the substrate, to form a biolayer. Application of a fluorescent biomarker, subsequently, denotes the Ab-An-Ab complex due to the visible fluorescent pattern.

In this research, we present a novel capacitance substrate of hafnium oxide (HfO_2) where the surface has been functionalised with TEA. We also include TEA activation results of silicon dioxide, silicon nitride and aluminium oxide for comparison. This activation enables direct antibody bonding where no other reagents were required.

2. Method

A self-assembled monolayer of 11-(triethoxysilyl)undecanal was activated onto HfO_2 surfaces, by vapor-phase for 1 hr. This formulated the aldehyde (CHO) groups. Afterwards, the substrates were heated in an oven at 90°C for 1 hr. The substrates were then rinsed in ethanol, and dried with nitrogen. Using a positive 10 μm PDMS stamp, the anti-human IL-10 monoclonal antibody (10 μg/mL) was incubated for 10 min, and then dried with nitrogen. By μCP, the PDMS stamp was brought into contact with the aldehyde activated substrate surface (10 min). Non-functionalised areas from the PDMS stamp were blocked in a solution of α-Methoxy-ω-amino poly(ethylene glycol) (PEG-NH_2) (1 mM) in triethylamine for 30 min, to prevent non-specific binding. The sample was then incubated with the rh IL-10 (0.25 μg/mL) for 1 hr. Finally, the Ab-An-Ab bio-recognition was formulated, with the incubation of anti-human IL-10 fluorescein monoclonal antibody (2.5 μg/mL) for 1 hr.

3. Results

Contact angles using deionised water demonstrated a higher hydrophilic nature on cleaned SiO_2 and Si_3N_4 substrates, in comparison to Al_2O_3 and HfO_2. Both the latter contain higher dielectric constants than

silicon, while silicon substrates require piranha activation to ensure hydroxyl groups are present on the surface. By vapor-phase with TEA, the angles on all four substrates increase in hydrophobicity, due to the formation of aldehyde groups (Table 1).

Table 1. Contact angles of cleaned substrates followed by oxygen and TEA activation.

	SiO_2	Si_3N_4	Al_2O_3	HfO_2
Cleaned substrates	54.8° ± 0.7	57.3° ± 1.0	64.4° ± 1.1	76.5° ± 1.3
Activation in piranha solution (H_2SO_4: H_2O_2 – 3:1, v/v)	29.9° + 1.4	9.0° + 1.1	-	-
Activation in triethoxysilane (TEA)	65.0° ± 0.6	67.3° + 1.1	85.5° + 1.0	88.7° ± 1.4

The functionality of the immobilised receptor by the fluorescent pattern, visible in Fig. 2, shows the Ab-An-Ab bio-recognition. The labelled fluorescent tags, formulate 10 µm positive structures, where non-printed regions have been blocked with PEG-NH_2.

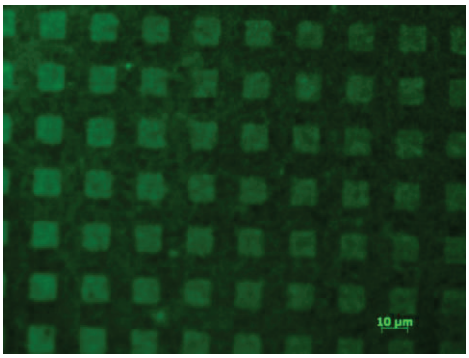

Fig. 2. Fluorescent image of IL-10 Ab-An-Ab recognition on HfO_2, using a positive 10 µm PDMS stamp. After formation of the -CHO layer, IL-10 Ab was microcontact printed onto the surface, blocked with PEG-NH_2, then incubated with the rh IL-10, followed by the IL-10 Ab tagged with fluorescein.

The interaction between antibody and antigen was also analysed by EIS. Primary results have indicated a distinction between the formation of the -CHO layer onto the HfO_2 substrate, when compared to bare HfO_2. By covalent bonding of the antibody onto the surface, we followed the detection of the recombinant protein by Nyquist plots (Fig. 3). With the incubation of the antigen to form an Ab-An complex, the substrate shows a variation and shift of impedance, with detectable concentrations ranging from 1-10 ng/mL. Though concentrations are within the ng/mL range, we aimed to ensure primarily that HfO_2 was capable of modification. Future applications will concentrate on the development of biosensors based on field-effect transistors (FETs) using HfO_2 as a gate.

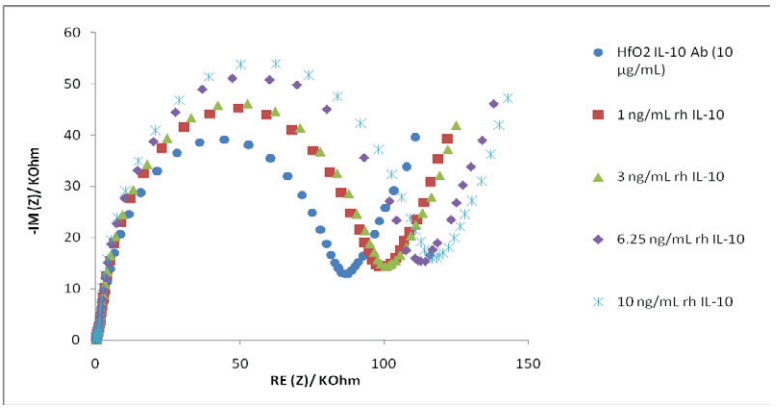

Fig. 3. Nyquist plot demonstrating detection of varying rh IL-10 concentrations in comparison with IL-10 Ab. EIS measurements were carried out in PBS using the conditions: Frequency range from 50 mHz to 100 KHz, AC amplitude voltage at 25 mV and DC amplitude voltage at -100 mV.

4. Conclusion

In this work, we have achieved direct patterning of anti-human IL-10 monoclonal antibodies, onto HfO_2 grown by Atomic Layer Deposition on silicon, that have been activated with aldehyde groups. By µCP, the patterned antibodies have detected the recombinant human protein, as observed by fluorescence. EIS has shown that detection can be made with varying protein (IL-10) concentrations, though at this preliminary stage, analysis of the recombinant protein was acquired within the ng/mL range to ensure that detection was possible. Silicon substrates have been used extensively in microelectronic applications. By application of HfO_2, a novel type of capacitance biosensor can be fabricated for biosensor applications. Future experiments are on-going to analyse within the pg/mL range that will establish detectable concentrations. This will include sensitivity and selectivity that is attributable to non-specific binding of inactive proteins.

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