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Reusable chromium-coated quartz crystal microbalance for immunosensing

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ABSTRACT

The application of oxidized chromium as a reusable platform for the development of immunosensors is presented. Chromium films were deposited on quartz crystal microbalances to study the affinity interaction between rabbit immunoglobulin G (IgG) and goat anti-rabbit IgG. A covalent approach, based on the silane chemistry, was followed for the grafting of either the rabbit IgG or the anti-rabbit IgG on the silane-modified chromium surface. Next the differences between the deposition of rabbit IgG on immobilized anti-rabbit IgG and the deposition of anti-rabbit IgG on immobilized rabbit IgG were investigated. The chromium layer could be reused between experiments, after the proper removal of the organic layers with piranha etch, obtaining a high repeatability in the steps of the functionalization protocol.

1. Introduction

Nowadays there is an increasing interest in the development of biosensors in many different applications [1,2]. These devices consist in a transducer and the biomolecular receptors on the transducer surface that interact with the corresponding analyte. Such interaction is converted by the transducer into a detectable signal. The material of the transducer surface can be of many different types: oxidized silicon [3,4] or H-terminated silicon [5,6], silicon nitride [7,8], GaN and AlN [9], carbon related materials [10,11] or metals such as gold [12,13].

Among the different transduction mechanisms, the quartz crystal microbalance (QCM) is a very well-established technique that allows for a quantitative determination of the process taking place on its surface due to the mass-dependence of its resonance frequency. Such dependence is governed by the Saurbrey equation [14]. It is also important to point out the possibility of measuring the change in the dissipation factor of the QCM after any deposition step and correlate this change with the rheologic properties of the deposited layer [15]. Due to the QCM mass sensitivity, the molecular recognition between antibodies and the corresponding antigens can be easily monitored with this transducer [16,17].

The sensing material for standard QCMs is the gold of the electrodes. The modification of gold is based on the thiol chemistry. The thiol chemistry is very well established due to the ease of preparation, the uniformity of the films and the large range of available ending groups. Despite of all these advantages, the search of other alternatives to the thiol/gold combination should not be unconsidered, as thiol films are affected by drawbacks, such as limited stability [18,19]. In our case, we propose the use of oxidized chromium. This approach would allow us the use of the different functionalization strategies available for oxide surfaces, such as silanes, phosphonates and phosphates, which might overcome the limitations of the thiol chemistry [20,21].

In this work, we deposited chromium thin films on QCMs and, after the oxidation of the chromium layer, studied the interactions between an antibody such as polyclonal goat anti-rabbit immunoglobulin G (IgG) and the corresponding antigen, rabbit IgG. Oxidized chromium surfaces have already been reported for the study of surfactants adsorption [22] and the deposition of organosilanes [23]. Here we go a step further and demonstrate the reliability of oxidized chromium as a support for immunosensors.

A covalent approach, based on silane chemistry and already used in the literature [24–27], was used for the immobilization of either the antibodies or the antigens. We examined the binding of antigens in solution to the antibody-immobilized layer under varying antigen concentration, as well as the capture of the antibody by the immmobilized antigens. The deposition of antigens on the immobilized antibodies resulted in a saturation monolayer coverage as the antigen concentration was increased. On the contrary, the deposition of antibody on the antigen-immobilized layer presented no saturation.

After the experiments, the chromium-modified QCM was cleaned in order to remove the organic layers and reused again. After reusing the QCM for 6 times, a specific immobilization step of

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the protocol had a standard deviation as low as 1.4 Hz, demonstrating the reusability of oxidized chromium for biosensing purposes.

2. Materials and methods

2.1. Materials

Rabbit IgG (I8140); polyclonal goat anti-rabbit IgG (R2004); mouse IgG (I5381); anti-mouse IgG (M8642); bovine serum albumin (BSA) (B4287); 3-aminopropyltriethoxysilane (APTES) (A3648); glutaraldehyde (GA) (340855); potassium phosphate monobasic and dibasic were purchased from Sigma–Aldrich; toluene, hydrogen peroxide, sulphuric acid, acetone, 2-propanol were obtained from VWR. Water was purified with a Direct-Q 3 UV Millipore system.

2.2. QCM

Gold-terminated QCMs from KSV Instruments were used. The frequency of the first resonance mode was 5 MHz and the active area was 10 mm. 40 nm thick chromium films were deposited on one side of the QCM by DC-pulsed reactive magnetron sputtering in a home built system [28]. Chromium-coated QCMs are also available commercially [29], not being necessary in that case the chromium deposition step.

The impedance response of the QCM, from the third to the eleventh overtones, was registered using a KSV Instruments QCM-Z500 microbalance system, which determines the resonance frequency and the dissipation factor. The QCM cell was temperature-controlled at 25 °C thanks to a Peltier element.

2.3. Elaboration of the sensors

2.3.1. Oxidation

First, the oxidation of chromium was accomplished by immersion of the QCM in fresh piranha etch (H_2SO_4:H_2O_2, 3:1, v/v) for 5 min. After rinsing in Millipore-Q water, the chromium surface was hydrophilic, as it was completely wet after removal from water. Next the QCM was dried with nitrogen.

2.3.2. Silanization

The grafting of APTES was performed as follows. The QCM was immersed in freshly prepared 20 mM APTES solution in toluene for 1 h in sonication, followed by a thorough cleaning in toluene and isopropanol. The APTES step covered the surface with amine-terminated silane organic molecules for the subsequent steps.

2.3.3. Glutaraldehyde activation

Next the APTES-modified surface reacted with a 20 mM GA solution for 1 h, followed by rinsing with water and drying with nitrogen. GA was used as a homo-bifunctional cross-linker between the amine groups of the APTES and the primary amines of the immunoglobulins.

2.3.4. Biomolecules immobilization

After the previous step, the QCM was mounted in a cell designed to work in liquid media that allowed the on-line tracking of both the resonance frequency and the dissipation factor of the QCM during the subsequent steps. Once mounted in the cell, the first step was the covalent binding of either the antibody or the antigen, followed by the affinity interaction with the complementary biomolecule. In between the two previous steps, 1 mg/ml bovine serum albumin (BSA) was used as blocking agent for remaining free binding sites [30]. The rinsing steps between the modification steps were done with PBS. Reactions were considered as complete when equilibrium was reached. Fig. 1 shows the different steps of the process.

3. Results and discussion

3.1. APTES grafting

The achievement of an APTES-modified chromium surface was checked by measuring the resonance frequency of the QCM after oxidation and after the APTES step. Both measurements were taken in air. A frequency decrease of 15 Hz was measured. Our result is

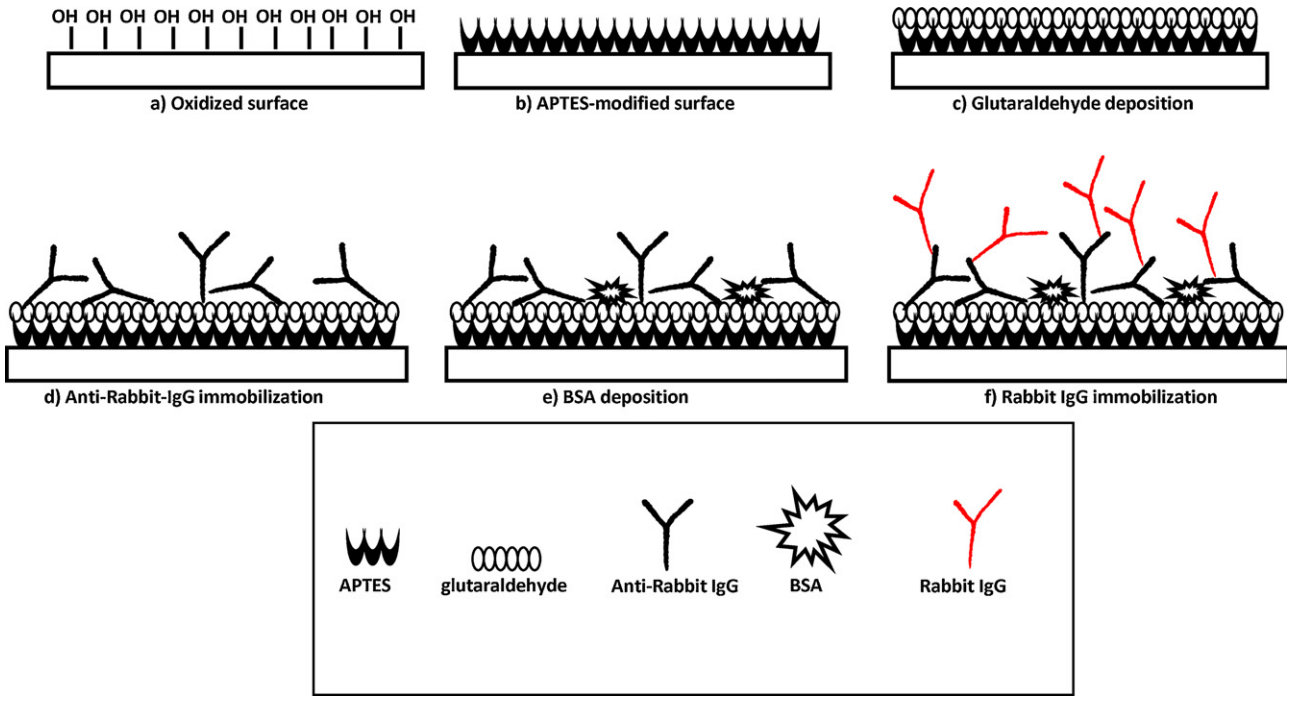

Fig. 1. Steps of the functionalization protocol.