Sensors and Actuators B 222 (2016) 682–687

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Ni–Co alloy coated QCM immunosensor to immobilize multiplex histidine-tagged proteins for label-free immunoassays

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ARTICLE INFO

Article history:
Received 11 May 2015
Received in revised form 27 August 2015
Accepted 29 August 2015
Available online 1 September 2015

Keywords:
Quartz crystal microbalance (QCM)
Electrodeposition
Immunosensor
Histidine

ABSTRACT

This paper presents a QCM immunosensor to immobilize multiplex histidine-tagged proteins for immunoassays. On the silver-plated electrode of QCM, a Ni–Co film was coated as the metal-affinity immobilization layer by electrodeposition. Ni–Co film can provide specific binding with histidine-tagged proteins based on the method of immobilized metal affinity chromatography (IMAC). The adsorption is through the histidine, based on the technique of IMAC and irrelevant to the species of proteins. Thus, multiplex immunoassays of histidine-tagged proteins can be conducted on the same QCM immunosensor. The experimental results show that the Ni–Co layer possesses reliable immobilization ability for different protein concentrations. After immunoreaction, frequency shift caused by low abundance of antibody (133 ng/mL) was measured, while the fluorescence intensity was too low to be detected. Moreover, the experiments using 0.5 μL droplets, with each droplet containing one kind of histidine-tagged protein and various antibodies, revealed that His-tagged protein and its corresponding antibody can be immobilized on the Ni–Co coated QCM for immunoassay and measurement. All experiments showed linear relationship between concentration and frequency shift. This feature provides practical implementations for the users to carry out multiplex label-free immunoassays on the same QCM.

1. Introduction

Many important physical and chemical processes can be observed by the associated mass changes of the analyte. Quartz crystal microbalances (QCM) are mass-sensitive devices on which mass accumulation and/or changes can induce the oscillation frequency shift. Because of its high sensitivity, QCM gained much attention and has been implemented in many fields. In recent years, a lot of studies have used QCM as the immunosensor for performing immunoassays [1–5].

The conventional method of immunoassay is to label either the antigen or antibody with fluorescent material (i.e. fluoroimmunoassay, FIA), enzyme (i.e. enzyme linked immunoassay, ELISA), radioisotopes (i.e. radioimmunoassay, RIA), colloidal gold, or magnetic bead, so that the target molecule can be detected. These existing immunoassay techniques are laborious and expensive. Alternatively, QCM can provide a label-free analysis of biological molecules through the mass changes. On the electrode surface of QCM, the immobilization either of protein or antibody samples as capture agents is a prerequisite for the investigation of molecular interactions. For different capture agents, different surface treatments are needed. Immobilization of capture agents on QCM surface via covalent bonding is a common method and various approaches have been reported in the literatures, for instance, chemical modification [6,7], surface modification to form a mixed self-assembled monolayer (SAM) [8–10], immobilized aptamers [11,12], photonics immobilization technique [13–15], and other manners [16–22]. Obviously, the implementation of QCM immunosensor with specific surface treatment is often restricted to detect a particular type of protein only. An appropriate electrode surface to immobilize multiplex types of proteins for label-free immunoassays deserves to be explored.

In this study, a QCM immunosensor with Ni–Co alloy film fabricated by electrodeposition on the electrode of crystal oscillator was investigated. The Ni–Co film functions as the metal-affinity immobilization layer to provide specific binding with a variety of histidine-tagged proteins, based on the principle of immobilized metal affinity chromatography (IMAC) [23–29]. Moreover, the blocking step used in the conventional immunoassay procedure is not required due to the specific binding between the histidine-tagged proteins and the Ni–Co film [29]. After immunoassay,

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http://dx.doi.org/10.1016/j.snb.2015.08.124
0925-4005/

binding of target analytes onto capture agents immobilized at the sensor surface is directly transduced into a resonance frequency variation signal. This signal is related to the concentration of target analytes. The Ni–Co interface of the QCM surface provides the capability to immobilize multiplex protein samples for the investigation of molecular interactions and biological reactions.

Two biomaterials were adopted as examples of bioassays: tumor suppressor p53 and kinases ERK1. The tumor suppressor p53, characterized primarily to regulate gene expression as a sequence-specific transcription factor, is one of the most studied proteins. It plays a role in activating DNA repair protein, inducing cell-cycle arrest, and initiating apoptosis, to upregulate growth arrest and apoptosis-related genes in response to stress signals. Hence, tumor protein 53 serves to protect the organism from stimuli that lead to DNA damage and to prevent cancer. Moreover, the functions of kinases ERK1 include the regulation of meiosis and mitosis in differentiated cells. Growth factors, G protein-coupled receptor ligands, carcinogens, and other stimuli can activate the ERK pathway. ERK activation influences a host of responses including proliferation, differentiation, and transcription regulation.

2. Materials and methods

2.1. Materials

2.1.1. Substrate

This proposed QCM immunosensor utilized the commercially available single quartz crystal oscillator as substrate. It is fashioned from a thin AT-cut quartz crystal with diameter of 9 mm, thickness of 0.17 mm, sandwiched between two silver-plated electrodes used to establish an electric field across the crystal. The diameter and thickness of electrode are 5 mm and 100 nm, respectively. This crystal oscillator is made to oscillate at 10 MHz.

2.1.2. Plating chemicals

The necessary chemicals for electrodeposition included $Ni(NH_2SO_3)_2{\cdot}4H_2O$, $Co(NH_2SO_3)_2{\cdot}4H_2O$, H_3BO_3, $NiCl_2{\cdot}6H_2O$, $(NH_2SO_3)_2{\cdot}4H_2O$, and $NiCO_3$, among which $(NH_2SO_3)_2{\cdot}4H_2O$ and $NiCO_3$ were used to adjust the pH of the plating bath to 4.1. Additionally, a low-stress additive (sodium saccharin, $C_6H_4SO_2NNaCO{\cdot}2H_2O$) was used. It is a surfactant to assist the progress of electroplating. All these materials were purchased from Blue Giant, Inc. (Taiwan).

2.1.3. Bio-materials

The recombinant proteins His-tagged p53 (human) and His-tagged ERK1 (human) were purchased from Enzo Life Sciences, Inc. (Lausen, Switzerland), while antibody p53 (DO-1) FITC and antibody ERK1 (K-23) Alexa FluorR 647 were obtained from Santa Cruz Biotechnology, Inc. (CA, USA). The usage of antibody with fluorescence allows us to compare the experiment result of QCM immunosensor with the detected fluorescence intensity. Phosphate buffered saline (PBS) was prepared by dissolving 10.9 g of K_2HPO_4 and 3.2 g of KH_2PO_4 in 1000 mL of DI water. Then, the solution was mixed into coating buffer, dilute buffer, and washing buffer which were essential for immunoassay experiment.

2.2. Fabrication of QCM biosensors

Electroplating is a technology for surface treatment. By means of the electrochemical principle, nickel and cobalt can be co-deposited on the surface of quartz crystal oscillator. The apparatus consists of a W 18 cm × L 25 cm × H 18 cm electroplating tank, an anode net, a cathode plate, a power supply, and a pump with filtering system. Thus, Ni–Co electrodeposition was performed in the tank. A 100 V/35 W pump which was equipped at the bottom of the tank provided a flow upward to the substrate in order to enhance the turbulence for promoting the uniformity of the plating bath. To eliminate impurities and crystalline solids, a filter with 1 μm pores was installed above the pump. Furthermore, a digital power supply controlled by a PID controller supplied a 100 V/100 W output to a quartz heater for temperature control of the plating bath.

Before electrodeposition, the surface of crystal oscillator was washed with degreaser and dried using the nitrogen gas. Since the electrode of crystal oscillator can serve as plating seed layer, the fabrication process of the QCM immunosensor can be simplified. In this study, only one side of silver-plated electrode was electroplated to form the metal-affinity immobilization layer by connecting the anode to the metal Ti net and the cathode to the electrode of crystal oscillator.

The crucial properties of the electrode coating must be uniform, rigid, and as thin as possible. The thickness of electrodeposited Ni–Co film can be precisely controlled if the cathode substrate is big. Hence, the quartz crystal oscillator was mounted on a printed circuit board (PCB), which has a conductive layer typically made of thin copper foil, to increase the cathode area. The thickness of the deposited Ni–Co layer was set to be 1 μm. After the electroplating process, the QCM immunosensors were kept in a vacuum container for two weeks before usage.

2.3. Bio-assays

The QCM immunosensor was placed horizontally and connected to a frequency counter (Agilent 53131A, Santa Clara, CA, USA) for measuring the frequency. Before proceeding to the bio-assays, repeated measurements of fundamental resonance frequency of Ni–Co alloy coated QCM immunosensor must be carried out under a stable temperature. This step is very important to ensure that there is no environmental disturbance. This resonance frequency must keep stable within a certain tolerant range in order to ensure the credibility of the follow-up measurements and the overall experimental error. The experimental setup is illustrated in Fig. 1.

Bio-assays were performed utilizing the principle of protein–antibody binding specificity to verify the performance of QCM immunosensor. The same experimental procedure was adopted for both His-tagged recombinant proteins used in this study. A droplet of diluted His-tagged protein was dropped on the surface of sensor using Pipetman, followed by incubation for immobilization in a 37 °C hot convection oven for 2 h. After the immobilization step, the QCM immunosensor was washed with washing buffer (PBS Tween-20) two times for 2 min per wash in order to remove the residual but not immobilized proteins. Next, thirty-minute baking in a 37 °C oven aimed to dry QCM surface. The induced frequency shift by the immobilized His-tagged protein was then measured.

The subsequent step is to add the diluted antibody onto the top of recombinant protein layer. One-hour incubation in a 37 °C chamber was followed by two washes. Dehydration bake in a 37 °C oven for thirty minutes was performed again to dry QCM surface. Finally, the frequency shift caused by antibody was measured.

The electrode of QCM immunosensor was carefully removed and stuck on a 1″ × 3″ glass substrate for fluorescence scan. It was performed on GeneTAC LS IV Microarray Scanner manufactured by Genomic Solutions Inc, and the intensity was analyzed by GenePix-Pro 6.0 software.

3. Results and discussion

In this study, three groups of experiments were carried out. The first group was designed to examine the immobilization ability of Ni–Co QCM immunosensor. The second group was planned