Sensors and Actuators B 189 (2013) 123–129

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Comparison of two types of acoustic biosensors to detect immunoreactions: Love-wave sensor working in dynamic mode and QCM working in static mode

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ARTICLE INFO

Article history:
Available online 11 February 2013

Keywords:
Love wave
Microfluidic
QCM
Immunodetection
Biosensor
Antibody conjugated

ABSTRACT

A Love-wave device and a PDMS microfluidic chip have been developed in order to measure immunoreactions in liquid media, operating in dynamic mode (continuous flow-through). The obtained results with the above system for the detection of rabbit immunoglobulin by means of an antibody of goat anti-rabbit have been compared with those obtained by the most used acoustic wave device, the quartz crystal microbalance (QCM), which has worked in static mode. It has been demonstrated once again that QCM is an excellent tool as an immunosensor, although the Love-wave device is a good alternative due to its higher sensitivity.

The dynamic mode improves the response time and the limit of detection. In addition, the use of microchannels has allowed working with a small sample volume for a long period of time in dynamic mode.

On the other hand, a secondary antibody conjugated with gold nanoparticles has been used as a different method to measure the desired concentrations of antigens, obtaining in this case, a greater frequency shift.

1. Introduction

The identification of biomolecules involved in early disease states may very well provide a new approach to clinical diagnostics and an efficient route to the development of new drugs and disease treatments. On the other hand to avoid the spread of diseases it is possible to determine the source of infection of bacteria, virus or toxins, in water, food or humans.

Several investigators have reported on biosensors as being an alternative method to the conventional analytical methods (high-performance liquid chromatography (HPLC) or enzyme-linked immunosorbent assay (ELISA)) [1–16]. The systems based on immunoreaction of antibody–antigen binding recognition (also called "immunosensors") generally rely on highly sensitive devices to translate the biological recognition process into a physical magnitude variation in real time.

The acoustic wave (AW) sensors are reusable, with high sensitivity [15–21] and cheap systems of measurement. Therefore an immunosensor based on AW devices with very high sensitivity can be a good solution for the detection of antigens in real time and "on line". In the field of AW biosensors, quartz crystal microbalance (QCM) immunosensor is very well-established for the detection of immunological reactions [15,22,23]. The QCMs are suitable transducers for chemical and biochemical sensing in general, they are used to detect the micro mass changes and physical properties of thin layers deposited on the crystal surfaces and are capable of detecting in real-time. Due to its simplicity and low cost, this method has been important for the detection of biomolecules. The QCM immunosensor comprises a quartz crystal with an antigen or antibody immobilised on its surface. Particularly, among AW immunosensors, a Love wave device is very promising as an alternative because of its high sensitivity compared with other acoustic wave devices such as QCM, since almost all of its energy is concentrated in the guiding layer [16,24–27]. It is well known that a Love wave has a pure shear horizontal polarisation and a small attenuation of the wave is caused in liquid media.

The use of the microfluidic [28] is envisaged in the field of the medicine and security due to its inherent advantages: small volumes of sample without the need to waste expensive reagents, and easy fluid control using pumps to automate fluid handling working in dynamic mode. The reaction between antigen and antibody is thus promoted due to the antigens being transported by the movement of the liquid medium. In the case of the static mode, however, the immunoreactions are due to the diffusion of the antigens. Both

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http://dx.doi.org/10.1016/j.snb.2013.02.003

modes have been studied in this work. Furthermore, the Love wave devices are easily integrated with a microchannel chip.

2. Materials and methods

2.1. Materials

The reagents used in the experiment were:

Rabbit IgG (I8140); polyclonal goat anti-rabbit IgG (GAR) (R2004); bovine serum albumin (BSA) (B4287); 3-aminopropyltriethoxysilane (APTES) (A3648); glutaraldehyde (GA) (340855); polyclonal goat anti-rabbit IgG conjugated with gold nanoparticles (G7402); potassium phosphate monobasic (P0662) and dibasic (P3786) to prepare phosphate buffered saline (PBS); tris buffered saline (TBS) (T-6664); all supplied by from Sigma–Aldrich. Water was purified.

2.2. Love wave device

Our Love-wave devices with dimensions 30 mm × 40 mm × 0.5 mm, contain two delay lines. They are based on a shear horizontal surface acoustic wave (SH-SAW) propagated on the ST-cut quartz perpendicular to the *x* crystallographic axis. This SH-SAW, with a wavelength $\lambda = 28\ \mu m$, is generated and detected by interdigital transducers (IDTs), which are made by standard lithographic techniques depositing a 200 nm thickness aluminium layer by means of RF sputtering. A double electrode structure is repeated 75 times to form each IDT. The spacing centre to centre between IDTs is 225λ and the acoustic aperture is 75λ. Finally, the SH-SAW is guided in a film of SiO_2 deposited by PECVD in order to obtain a Love wave. The highest sensitivity is found for a SiO_2 thickness of about 3.5 μm, being the synchronous frequency around 163 MHz.

The sensors were electrically characterised by means of a vector network analyser (Agilent 5070B) and the oscillator through a spectrum analyser (Agilent 9320A).

2.2.1. PDMS chip

The manufacture of the PDMS chip was made by means of a mould of SU-8 with the microchannel shape. The negative photoresist SU-8 was spun on a clean wafer with a 150 μm thickness, and then it was exposed to an optical lithography process and baked to obtain the master. The silicone base and curing agent were mixed in a 9:1 ratio in weight. The mixture was degassed to remove any bubbles and poured over the master and degassed again, ensuring all entrapped gases were evacuated. After baking and cooling, the PDMS was easily peeled and cut. The microstructure of PDMS and the Love wave device were joined by pressure, thus forming microchannels of 150 μm of height without leakages (Fig. 1a). Previously, Comsol software was used to simulate the flow of the liquid due to microchannel shape in order to achieve a uniform velocity in the path between the IDTs (Fig. 1b).

2.2.2. Experimental setup

The system of measurement is based on a continuous circulation of fluid through the microchannel, therefore a cone is placed on the inlet of each microchannel, in which the sample is deposited. On the outlet, a microtube is connected that links the syringe pump (KDScientific 210) with the microchannel. This pump works in suction mode, producing a constant movement of the liquid contained in the cones. The liquid circulates through the microchannel and finally arrives via the microtubes to the syringes, in which all the samples used in the experiment are stored as residues. The velocity of fluid through the microchannel is set by the flow rate that is selected in the syringe pump.

Love wave sensors are really sensitive to the temperature. Therefore in order to keep it constant at 30 °C (close to the temperature of the human body) a Peltier device controlled by a PID programme from the computer is used.

Each delay line works in an oscillator system, which includes two amplifiers and a directional coupler. Therefore, when the sensor is perturbed, the oscillating frequency is shifted in order to satisfy the law of Barkhausen, what means that the phase of the closed loop is $2\pi n$ and the gain is 0^+ dB. The measured quantity is the output frequency. The circuits transmit part of signal energy to each one of the channels of the frequency counter (Agilent 53131A). The frequency counter, the multimeter that measures the resistance of Pt100, the power supply which controls the Peltier, and data acquisition are controlled in real time through a GPIB protocol by software made at home specifically developed for this experimental setup.

2.3. Quartz microbalance device

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^2. 40 nm thick chromium films were deposited on one side of the QCM by DC-pulsed reactive magnetron sputtering in a home built system. The chromium-coated QCMs are also available commercially, not being necessary in that case the chromium deposition step.

2.3.1. Experimental setup

The impedance response the third overtone of the QCM was registered using a KSV Instruments QCM-Z500 microbalance system, which determines the resonance frequency. The QCM cell was temperature-controlled at 25 °C with the assistance of a Peltier element.

2.4. Protocol for functionalisation of the sensor surface

The surfaces of the QCM and Love-wave devices were functionalised according to the following steps:

I. First, the oxidation of SiO_2 surface was performed depositing fresh piranha etch (H_2SO_4:H_2O_2, 3:1, v/v) for 5 min. After rinsing in water, the surface was hydrophilic.
II. The Love-wave device was immersed in a freshly prepared 20 mM 3-aminopropyl triethoxysilane (APTES) solution in toluene for 1 h (15 min in sonication, 15 min was left in rest, 15 min in sonication and 15 min was left in rest) 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.
III. The APTES-modified surface reacted with a 20 mM glutaraldehyde (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.

2.5. Protocol for measurement of antigens

After the functionalisation of the device surface and system configuration, measurements were made follows:

I. The buffer solution was introduced untill the frequency was stable.
II. Once the frequency was stable, a solution 100 $\mu g\ ml^{-1}$ of antibodies GAR was grafted.
III. When the antibodies were fixed to the functionalised surface, a rinsing with buffer solution was carried out in order to remove the non-covalently bonded antibodies.