Biosensors and Bioelectronics 54 (2014) 151–157

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Development and validation of a novel leaky surface acoustic wave immunosensor array for label-free and high-sensitive detection of cyclosporin A in whole-blood samples

Kai Chang [a,1], Feng Wang [a,1], Yi Ding [b], Feng Pan [a], Fake Li [a], Shuangrong Jia [a], Weiping Lu [a], Shaoli Deng [a], Jianfeng Shi [b], Ming Chen [a,*]

[a] Department of Clinical Laboratory Medicine, Institute of Surgery Research, Daping Hospital, The Third Military Medical University, Chongqing 400042, China

[b] The 26th Research Institute, Chinese Electronic Scientific and Technical Group Company, Chongqing 400060, China

ARTICLE INFO

Article history:
Received 5 August 2013
Received in revised form
23 October 2013
Accepted 23 October 2013
Available online 11 November 2013

Keywords:
Leaky surface acoustic wave
Immunosensor
Array
Cyclosporin A

ABSTRACT

This manuscript described a novel 2 × 3 model of leaky surface acoustic wave (LSAW) immunosensor array for label-free and high-sensitive detection of Cyclosporin A (CsA) in whole-blood samples. In this technique, every resonator crystal unit of the LSAW immunosensor array had an individual oscillator circuit to work without mutual interference. The LSAW immunosensor was first immobilized with protein A from Staphylococcus aureus and monoclonal anti-CsA antibody on the gold electrode surface of 100 MHz $LiTaO_3$ piezoelectric crystals, which then captured the CsA. The CsA increased the mass loading of LSAW immunosensor and leaded to phase shifts of LSAW. Consequently, under optimal conditions, the designed LSAW immunosensor exhibited a detection limit of 0.89 ng/mL, quantification limit of 2.96 ng/mL, and wide dynamic linear range from 1 ng/mL to 1000 ng/mL for CsA detection. Application of the LSAW immunosensor array to clinical sample revealed that consistency and comparability between LSAW immunosensor and the enzyme multiplied immunoassay method were good. Moreover, the immunosensor could be regenerated for ten times without appreciable loss of activity. Therefore, the self-designed LSAW immunosensor array provided a rapid, accurate, label-free, easy handling, and dynamic real-time method for the detection of immunosuppressive drugs in clinical laboratory.

1. Introduction

The immunosuppressive properties of Cyclosporin A (CsA) have been used for organ transplantation by inhibiting the activity of immune system (Lee and Gabardi, 2012). In addition, CsA has also been indicated for the treatment of new clinical applications such as immune-mediated diseases (Malnick et al., 2012), antiviral (Liu et al., 2012), and type I diabetes (Sobel et al., 2010). Due to narrow therapeutic spectrum, variable pharmacokinetics, and lack of a reliable correlation between dose and drug exposure, the application of CsA requires regular immunosuppressant therapeutic drug monitoring (TDM) in order to arrive at the optimal dose for therapeutic efficacy as well as minimizing toxicity (Muller et al., 2013).

Currently, there are two main analytical methods for determination of immunosuppressive drugs: immunochemical methods (enzyme multiplied immunoassay (EMIT), cloned enzyme donor immunoassay (CEDIA), chemiluminescent microparticle immunoassay (CMIA), radioimmunoassay, etc.) (Grundmann et al., 2011; Hamwi et al., 1999) and liquid chromatography based methods (reverse phase high-performance liquid chromatography (RP-HPLC), HPLC, liquid chromatography mass spectrometry (LC–MS), and LC–tandem mass spectrometry (LC–MS/MS)) (Mohamed et al., 2012; Said et al., 2012). Although immunochemical methods have many advantages, such as high sensitivity and low cost, the fluorescent or radioactive-labeled molecular is required and analytical precision is not ideal. Furthermore, the liquid chromatography based methods also have several major disadvantages, such as complicated procedures, sophisticated facilities, and rigidly matched reagents, all of which limit its clinical applications. Thus, a label-free, selective, and sensitive high-throughput bioanalytical method for immunosuppressive drugs quantification is urgently needed for clinical use.

Biosensors that combine the unique specificity of biological reactions and the high sensitivity of physical sensors, have been widely used in a variety of fields, such as food industry, environment protection, and clinical analyses (Holford et al., 2012). Additionally, biosensors have also been used in the real-time analyses of specific proteins because they do not rely on radioactive or fluorescent labels

* Corresponding author. Tel.: +86 236 875 7601; fax: +86 236 871 6530.
E-mail address: chenming1971@yahoo.com (M. Chen).
[1] Both the authors contributed equally to this work.

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.10.066

(Luppa et al., 2001; Schneider and Clark, 2013). With the progress of the microelectronic and acoustic technique, a novel leaky surface acoustic wave (LSAW) biosensor has been developed recently (Belovickis et al., 2012; Inoue et al., 2007). The LSAW biosensor, which consists of a special orientation of a piezoelectric crystal exciting a LSAW mode, such as the 36° rotated Y-cut X propagation lithium tantalate ($LiTaO_3$) piezoelectric single crystal. In this case of crystal configuration, the LSAW is characterized by a minimal vibration component perpendicular to the surface and more than 90% of its energy localizes in near the crystal surface, which lead to high energy density along the surface. Moreover, the LSAW has the higher velocity and electromechanical coupling coefficient values than the conventional Rayleigh waves. For these reasons, the wave is potentially sensitive to any change in the surface, such as mass loading and conductivity changes (Gronewold, 2007; Meng et al., 2011). Compared with our constructed piezoelectric quartz crystal microbalances (Chen et al., 2005; Luo et al., 2006), the basic resonance frequency of LSAW biosensor is up to 100 MHz, and can achieve substantially higher sensitivities.

In our previous study, we have set up a LSAW biosensor and successfully detected human papilloma virus and Japanese encephalitis virus (Wang et al., 2009; Xu et al., 2012). In this study, we constructed a LSAW immunosensor which allowed label-free and high-sensitive detection of CsA in the whole-blood samples. To meet with the requirement of assay speed and efficiency in clinical laboratory, we designed a 2×3 model of LSAW immunosensor array. Subsequently, the main experimental conditions were optimized, and analytical parameters of the LSAW immunosensor were investigated in detail. Finally, the LSAW immonusensor was used to detect CsA in the clinical whole-blood samples compared with those given by EMIT applied in current clinical laboratory.

2. Materials and methods

2.1. Reagents

An anti-CsA monoclonal antibody was purchased from Abcam Ltd. (Cambridge, UK), Staphylococcal protein A (SPA) was the product of Sigma-Aldrich (St Louis, MO, USA). The standard substance of CsA, FK506, valproic acid, digoxin, carbamazepine, and theophylline were obtained from EMIT 2000 kits of Dade Behring Inc. (Cupertiono, CA). We used a blank blood solution containing none of the CsA as the negative control. High concentration of CsA quality control reagent (Dade Behring Inc., Cupertiono, CA) was used as the positive control. The blocking reagent used in all experiments was bovine serum albumin (BSA) from Sigma-Aldrich. Piranha solution (1:3 mixture of 30% hydrogen peroxide and 98% sulfuric acid) and phosphate buffered saline (PBS) were prepared in the laboratory. All chemical reagents used were of analytical reagent grade.

2.2. Apparatus

A 100 MHz LSAW biosensor was obtained from the 26th Research Institute, Chinese Electronic Scientific and Technical Group Company (Chongqing, China). The oscillating signals from LSAW biosensor were automatically input into a NI-PXI data collection system (National Instruments, Austin, TX, USA). The self-developed biosensor monitor system software BSMS 2.0 was used to collect, store, and analyze the experiment data (phase shift) by a personal computer (Fig. 1C). The real-time monitoring signals for the LSAW immunosensor detection were shown in Fig. 1D.

2.3. Fabrication and assembling 2×3 model of LSAW immunosensor array

The LSAW immunosensor was designed as 2-port resonators. The device was fabricated using simple side polished 36° rotated, y-cut and x-propagation $LiTaO_3$ crystal as piezoelectric substrate. The $LiTaO_3$ crystal was initially cleaned by rinsing with acetone, isopropyl alcohol, and ultrapure water respectively, and then dried with nitrogen stream. The interdigital transducer (IDT) consisted of a pair of single-phase unidirectional transducers (SPUDT) on both sides of the LSAW immunosensor. The input and output IDT were patterned on the $LiTaO_3$ crystal by metal evaporation, reactive ion etching (RIE), and ion sputtering techniques. The number of fingers in the input and output IDT were 100. The area between input and output IDT was used for biological reaction, which was fabricated from gold by vacuum ion sputtering on $LiTaO_3$ piezoelectric single crystal and separated from the $LiTaO_3$ substrate by a thin layer of chromium. The reflector array was deposited by e-beam evaporation and etched in the outside of IDT. The dimension of LSAW immunosensor was 20×5 mm^2 rectangular, 800 μm thick. The structure of the LSAW immunosensor and IDT were shown in Fig. 1A. The assembling 2×3 immunosensor array (including five detection sensors and one reference sensor) was shown in Fig. 1B. The LSAW immunosensor and array were co-designed and developed by our laboratory and the 26th Research Institute of the China Electronics Technology Group.

2.4. Antibody immobilization

After sputter coating with gold, the gold membrane was mechanically cleaned with ultrasound. Then, the crystal was immersed in piranha solution (30% H_2O_2:98% H_2SO_4=1:3) for 10 min, rinsed three times with ultrapure water, and dried in a nitrogen stream. Subsequently, 10 μl of SPA solution (5 mg/ml) was tiled on the entire surface of the gold electrode, and the crystal was put in 4 °C for overnight. The excessive SPA was removed after overnight incubation. Then, CsA antibody was immobilized on the gold electrode surface of detection sensor through the SPA method (Jiang et al., 2011). The corresponding reference sensor was filled with PBS (0.01 M, pH 7.4). To block unoccupied binding site, the BSA (10 mg/ml) was laid on the electrode surface, incubated 1 h at 25 °C. After being cleaned and dried, the sensor was used for experiment.

2.5. Optimization of detection conditions

2.5.1. Effect of antibody concentration

Antibody, with the final concentration 1 mg/L, 3 mg/L, 5 mg/L, 7 mg/L and 9 mg/L, respectively, was immobilized on the surface of the detection sensor. The corresponding reference sensor was filled with PBS (0.01 M, pH 7.4). Then, the final concentration of 500 ng/mL CsA was added into detection sensor and reference sensor, respectively. The immunosensor was maintained at 37 °C for reaction. The detection procedure was monitored, and the real-time phase shift was recorded and displayed via the software BSMS 2.0. The phase shifts of detection sensor (P_1) and reference sensor (P_2) were determined, and the relative phase shift attributed to the antigen and antibody reaction was calculated by: $\Delta\text{Phase} = P_1 - P_2$.

2.5.2. Effect of pH value of PBS buffer

The detection sensor and reference sensor were dripped into 36 μl of PBS (10 mmol/L) with a range of pH (7.0, 7.2, 7.4, 7.6, and 7.8) and the oscillating signals from detection sensor and reference sensor were monitored. After the phase shifts were stabilized, the final concentration of 500 ng/mL CsA was added on gold electrode