A FULLY-AUTOMATED SURFACE ACOUSTIC WAVE IMMUNOSENSING SYSTEM FOR THE DETECTION OF CARDIAC MARKERS IN WHOLE BLOOD

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ABSTRACT

A fully-automated fluid control system with disposable cartridge was developed for the surface acoustic wave (SAW)-based point-of-care testing (POCT), and the simultaneous detection of three cardiac markers in human blood, cardiac troponin I, creatine kinase (CK)-MB, and myoglobin, was performed. The whole assay, which included plasma separation, solution mixing, air-bubble removal, gold nanoparticle (AuNP)-based sandwich immunoassay, signal enhancement by gold staining, washing, and electric detection, was conducted successfully within 12 min.

KEYWORDS: Surface acoustic wave (SAW), Biosensor, Cardiac marker, Point-of-care testing (POCT), Cartridge

INTRODUCTION

As the importance of POCT increased as a means to give patients a convenient and immediate clinical management decision, the SAW device, especially working in a guided shear-horizontal mode (also known as the Love wave), has been paid attention as one of the promising sensing platforms to detect a variety of target proteins due to its high sensitivity, low cost, and reliability [1]. However, if one sensing mechanism is to take an active role in POCT, it should fulfill two indispensable requirements: speed, and reliability such as accuracy, limit of detection, interference, linearity, and measurement range [2]. In this respect, the SAW biosensor must be equipped with a suitable operating platform together with a prominent sensing quality.

In this study, we proposed a disposable cartridge and driving system to accomplish the complex SAW-based sensing procedure in an automatic and precise way for the simultaneous detection of three cardiac markers of acute myocardial infarction. The AuNP-based sandwich immunoassay was done with a subsequent gold deposition onto the AuNPs leading to a significant signal amplification. The full assay sequence from plasma separation to signal acquisition was carried out on the cartridge and the sensor responses were exploited successfully.

EXPERIMENTAL

The assay procedure is described in Figure 1. Human plasma containing cardiac markers was mixed with AuNPs, which had been conjugated in advance with the detection antibodies for troponin I, CK-MB, and myoglobin, and the cardiac markers were allowed to bind to their corresponding antibodies on AuNPs. Then, the solution was applied to the sensor surface, on which capture antibodies of each cardiac marker had been immobilized, and immunoreaction proceeded. After washing and stabilizing with PBS, gold staining solutions of 20 mM chloroauric acid ($HAuCl_4$) and 40 mM hydroxylamine (NH_2OH) were mixed and incubated on the sensor surface, which resulted in catalyzed deposition of gold onto the AuNPs captured on the sensor surface. To stop the gold enhancing, the surface was rinsed again with PBS.

The SAW sensors were fabricated on a 36˚YX-$LiTaO_3$ substrate. 3000 Å -thick aluminum interdigital transducer (IDT) electrodes were deposited by sputtering, and patterned using a conventional photolithography and wet etching. The input and output IDT electrodes consisted of 72 finger pairs with an electrode width of 5.0 μm to obtain 200 MHz center frequency. A

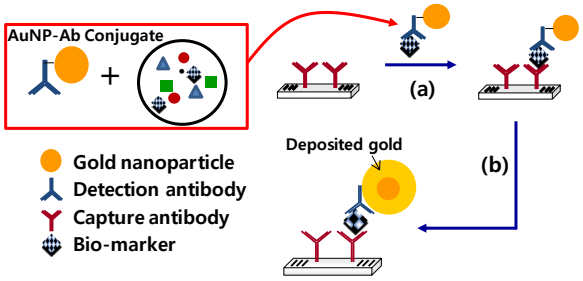

Figure 1: Schematic of sandwich immunoassay in combination with gold staining. (a) Immunoreaction between cardiac marker-AuNP complex and capture antibody. (b) Gold staining process.

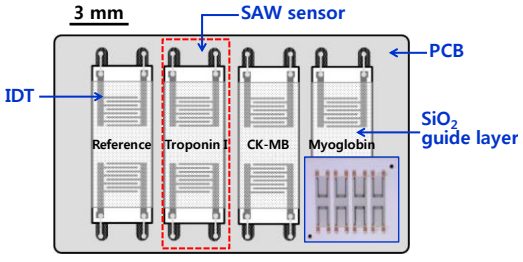

Figure 2: Schematic of the SAW sensor array and the corresponding photograph (inset).

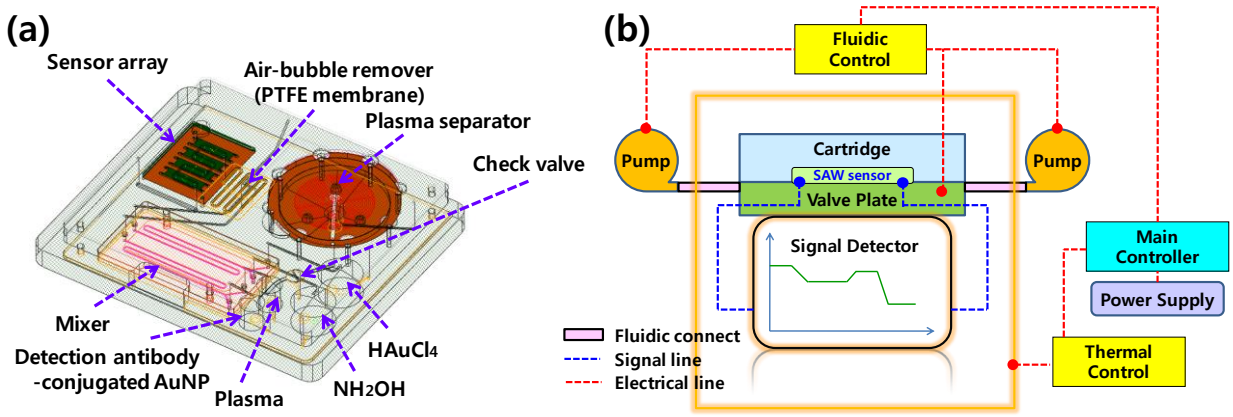

Figure 3: Schematic of the cartridge (a), and the block diagram of the system (b).

5.2 μm-thick SiO_2 layer for guiding Love waves was deposited on the IDT patterned substrate by plasma-enhanced chemical-vapor deposition and contact pads were opened with a buffered oxide etchant for electric contact. After dicing the fabricated wafer, four sensors were mounted on a custom-made printed circuit board and wire-bonded for the electrical connection with detection part as shown in Figure 2.

Fluids in the cartridge (Figure 3a) were controlled pneumatically by two external peristaltic pumps and eight three-way solenoid valves positioned in a valve plate (Figure 3b), and each fluidic sequence was carried out automatically with a circuit board by transmitting control signal to the pumps and the valves. The pump pressure was transferred to the cartridge through tubings and the valve plate. The cartridge consisted of a plasma separator with porous membrane-filter, a plasma reservoir for storing the plasma separated in the plasma separator, three reagent reservoirs for storing AuNP-conjugate solution and two gold staining solutions, two passive mixers for mixing the plasma and reagents, an air-bubble remover, and a four-sensor array. Between the plasma separator and the plasma reservoir, a thin-membrane type fluidic valve was disposed to synchronize the fluid flow of the plasma and the AuNP-conjugate solution. It opened during the plasma separation and closed during the mixing sequence of the plasma and the AuNP-conjugate solution, which prevents the plasma separator from being pressurized by the pushing pressure applied to the plasma reservoir. All air bubbles plugged in liquid flow were eliminated at the air-bubble remover by selective discharge of gas phase into the atmosphere through a hydrophobic PTFE membrane on its top surface. Waste solutions were discarded through an outlet at the backend of the sensor chamber. PBS and waste were stored in external bottles, while the other solutions resided in the cartridge.

Resonance frequency change due to a mass loading on SAW sensor surface was monitored continuously using a laboratory-built oscillator circuit [3] and the periodic signals were acquired from four sensors by switching with two multiplexers (details were not shown). The first multiplexer was connected to the input terminal of the sensor array, and the other was connected to the output terminal. The oscillator was located between the first and the second multiplexers, and transmitted an electrical signal to the SAW devices. The input IDT electrodes generated surface acoustic waves, which traveled toward the output IDT electrodes, and then the received waves were amplified at the oscillator and fed back to the input IDT electrodes again. A channel controller was connected to the multiplexers to supply channel selection signal. A frequency counter measured the frequency of SAW signals using a field-programmable gate array. To exclude the thermal noise of SAW sensors, temperature was kept at 25°C during the assay.

RESULTS AND DISCUSSION

After spiking troponin I, CK-MB, and myoglobin with the concentration of 1, 10, and 10 ng/mL, respectively, the human whole blood was loaded into the cartridge as shown in Figure 4a. By applying air pressure onto the blood, blood plasma was filtered out through the porous membrane and transported to the plasma reservoir (Figure 4b). With a closing of the fluidic valve, the plasma and detection antibody-AuNP conjugates passed through the mixer and incubated on the SAW sensor surface for sandwich immunoreaction (Figure 4c). Following a PBS rinsing, staining solutions were discharged into another mixer and catalyzed gold was deposited on the captured AuNPs for 1.5 min (Figure 4d and e). The gold enhancing was halted by another PBS rinsing step and the frequency changes due to the mass loading on the SAW sensors were measured (Figure 4f). During the operation, all air-bubbles were vented out through the bubble remover, although they were continuously generated in the channel intersections and infused from the pressurizing tubings.

Sensor responses during the fluidic sequence are demonstrated in Figure 5. During the plasma separation, the sensor signals were stabilized under PBS buffer to reach a steady oscillating frequency. Addition of the plasma and AuNP-conjugate

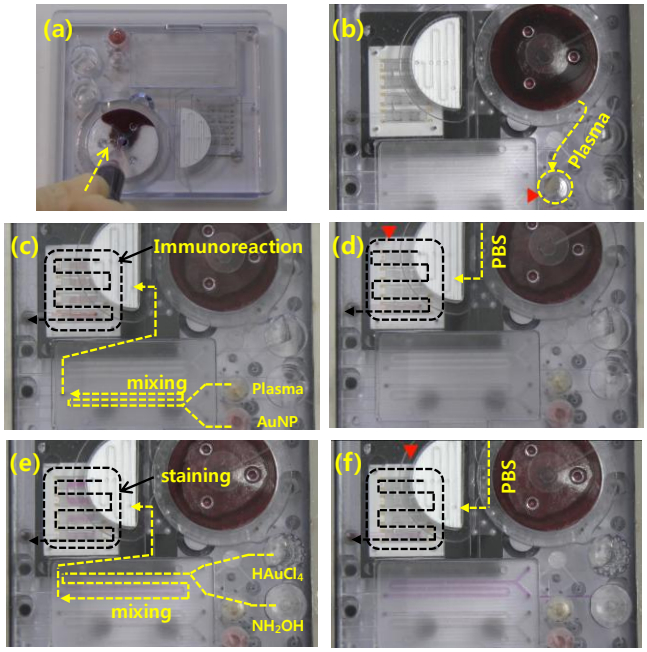

Figure 4: Fluidic sequence. (a) Blood loading. (b) Plasma separation. (c) Mixing of plasma and AuNP solution, and immunoreaction. (d) PBS rinsing. (e) Gold staining. (f) PBS rinsing.

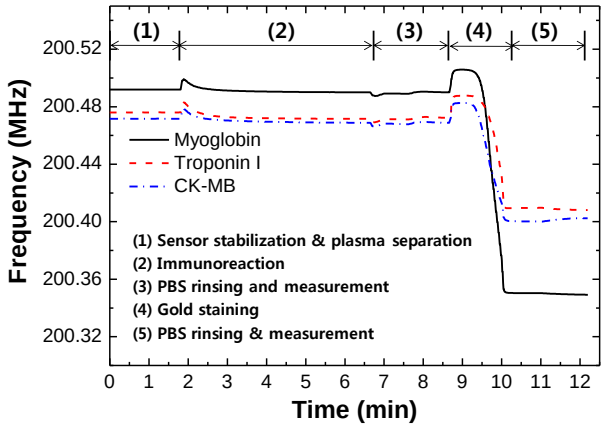

Figure 5: Frequency responses of SAW biosensors during the immunoassay for troponin I, CK-MB, and myoglobin.

mixture onto the sensor surface resulted in a frequency decrease, which was attributed to a specific binding of the cardiac marker-AuNP complexes with the immobilized capture antibodies as well as a non-specific adsorption of plasma proteins and AuNPs onto the sensor surface. Following PBS rinsing caused a minor signal recovery and this is an indicative that loosely adsorbed AuNP-complexes and proteins on the functionalized surface were readily removed by the PBS. Application of gold staining solutions yielded a frequency increase due to a sudden decrease in pH [4]. However, the frequency decreased again as the mass addition resulting from catalyzed gold deposition became dominant. Since the frequency difference between the third and fifth steps in Figure 5 depends proportionally on the concentration of cardiac markers, the contents of troponin I, CK-MB, and myoglobin in blood could be assessed quantitatively.

CONCLUSION

In this study, the diagnostic assay for acute myocardial infarction was demonstrated with the integrated SAW biosensing platform. Even though some improvement is required to guarantee more reliable operation, we expect the proposed system can serve as an enabling tool for point-of-care diagnostics due to its cost- and time-effectiveness. As a promising tool for sensitive and rapid detection, our efforts will be further expanded to detect other disease makers in body fluids.

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