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Surface acoustic wave immunosensor for real-time detection of hepatitis B surface antibodies in whole blood samples

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ABSTRACT

We demonstrate an application of Love wave mode surface acoustic wave (SAW) immunosensor to detect hepatitis B surface antibody (HBsAb) in aqueous conditions. SiO_2 guiding layer was deposited on 36°YX-$LiTaO_3$ piezoelectric single crystal substrate to protect the electrodes and to trap the acoustic energy near the surface, and hepatitis B surface antigen (HBsAg) was immobilized on the sensing area. The resonance frequency shift was monitored to detect specific binding of HBsAb to immobilized HBsAg. To eliminate the effects of other physical factors except for the mass change, the resonance frequency was compared to that of a reference SAW device coated with bovine serum albumin (BSA) to block binding of HBsAb. The guiding layer thickness with maximum mass sensitivity was found to be 5 μm, which was in agreement with the theoretical calculation, and the center resonance frequency was around 199 MHz. The sensor showed binding specificity to HBsAb and a linear relationship between the frequency shift and the antibody concentration with sensitivity of 0.74 Hz/(pg/μl) and detection limit below 10 pg/μl. In addition, our SAW immunosensor successfully detected HBsAb in whole blood samples without any pretreatment, opening up its applicability in fast label-free protein detection methods.

1. Introduction

Hepatitis B virus (HBV) infection occurs worldwide and is one of the major causes of acute and chronic viral hepatitis. In the United States, approximately 300,000 new cases of HBV infection are reported each year, and it is estimated that more than 300 million people worldwide are chronic carriers of the virus (Gitlin, 1997). The antibody response to hepatitis B surface antigen (HBsAg) appears during the late phase of infection. The antibodies to HBsAg (HBsAb) are able to neutralize HBV infectivity and therefore, clear circulating HBsAg and infectious HBV particles from peripheral blood. The presence of HBsAb is considered as an indicator of immunity to HBV infection (Zhang et al., 2007). Since patients infected with the virus can develop life-threatening cirrhosis and liver cancer, there is a compelling need of diagnosis of active infection and immunity through various HBV markers. Traditional detection methods include polymerase chain reaction (PCR) assays and enzyme-linked immunosorbent assays (ELISA), but these methods are expensive and time-consuming (Duan et al., 2005).

More recently, various label-free protein detection methods have been explored, as they are faster, less expensive and allow simultaneous measurement of multiple analytes. Depending on the nature of the transducers, these sensors can be classified into different types, such as electrochemical, optical, piezoelectric and thermal sensors (Shankaran et al., 2007). Most of them, however, require time- and sample-consuming pretreatment processes since high concentrations of various non-relevant proteins along with the biomolecules of interest can serve as noise factors and severely interfere with the device performance.

Surface acoustic wave (SAW) sensor, which is a piezoelectric sensor first introduced by White and Voltmer (1965), is a potential biochemical sensing platform due to its superb sensitivity, speed and reliability. Since Wohltjen and Dessy's application (Wholtjen and Dessy, 1979), SAW sensors based on Rayleigh surface waves have been used as highly sensitive gas sensors (Caliendo et al., 1997; Mah and Thurbide, 2006). However, Rayleigh waves possess a displacement component perpendicular to the substrate and cannot be utilized for sensing in liquid environments because of a strong radiation loss. On the other hand, shear-horizontal (SH) waves can propagate without coupling excess acoustic energy into the liquid (Chang et al., 2006). SH type acoustic waves include surface skimming bulk waves (SSBW; Lewis, 1977), leaky surface acoustic waves (Nakamura et al., 1977), Love waves (Gizeli et al., 1992), thickness shear modes (Collings and Caruso, 1997), acoustic plate modes (Niemczyk et al., 1988), and Bleustein–Gulyaev waves (Zhang et al., 2001).

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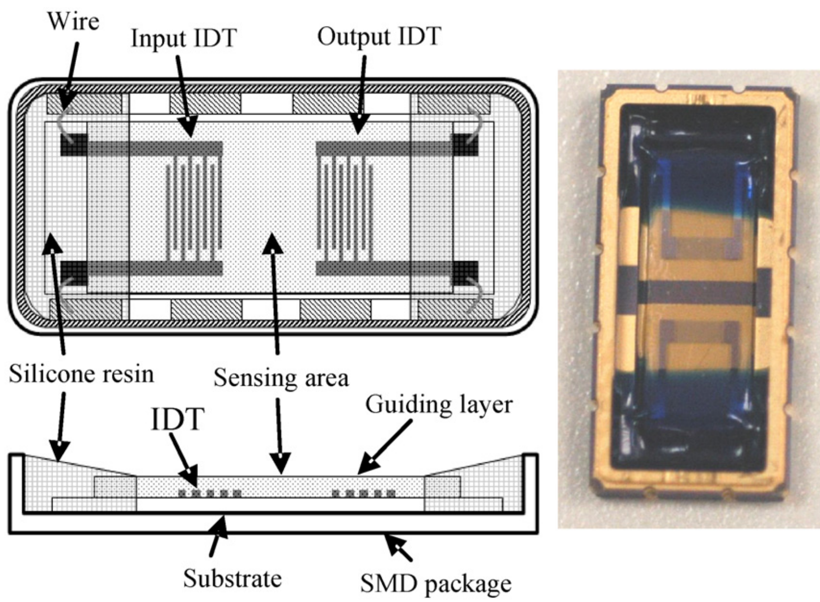

Fig. 1. Top: top-down diagram of the Love wave type SAW device with two Al transducers on $LiTaO_3$ substrate (SAW chip: 9 mm × 3.3 mm; SMD package: 13 mm × 6 mm). Bottom: cross-sectional diagram of the SAW device. Right: photograph of the SAW device.

In particular, Love wave devices, which consist of a piezoelectric substrate supporting a SH wave mode, such as the SSBW of ST-cut quartz or the leaky wave for 36°YX-$LiTaO_3$, have been shown to be the most promising platform for sensing applications. Deposition of a thin acoustic guiding layer or waveguide converts these modes into a Love wave mode, and these devices have been used to detect mass and viscosity change (Du et al., 1996; Jakoby and Vellekoop, 1998). The role of the guiding layer is to trap the acoustic energy near the sensing surface, which leads to an increased sensitivity to any physical changes at the surface, such as changes in the mass density, mechanical stiffness, viscosity, pressure, or temperature (Lee, 1981). The guiding layer can also protect interdigitated transducer (IDT) electrodes from the liquid environments. Waveguides consisting of a single layer of polymethylmethacrylate (Gizeli, 1997; Bender et al., 2000) and photoresist (McHale et al., 2001; Rasmusson and Gizeli, 2001) have been reported. In many cases, SiO_2 has been used as the guiding layer due to its low acoustic loss, high mechanical and chemical resistance, and ease of conjugating bioactive films (Kovacs et al., 1994). Love wave sensors employing polymer and silica guiding layers have been used in biosensing applications to detect antibody–antigen interactions (Gizeli et al., 1997; Harding et al., 1997).

In this paper, we report a successful implementation of a Love wave type SAW sensor to specifically detect HBsAb in aqueous media. Design of our SAW immunosensor and immobilization of HBsAg to the sensing surface are discussed. After investigating the effects of the guiding layer thickness on the mass sensitivity both theoretically and experimentally, binding specificity to HBsAb and antibody concentration dependence were tested. Finally, the immunosensor was used to detect HBsAb in whole blood samples and compared to commercial ELISA.

2. Materials and methods

2.1. Design of the Love wave type SAW device

Configuration of the SAW device in the present study was designed to generate Love waves. Two pairs of the IDT electrodes were patterned on 36°YX-$LiTaO_3$ substrate, which has been widely used for their low insertion loss, very large electromechanical coupling factor (K^2) and low propagation loss (Branch and Brozik, 2004). Input and output IDT electrodes consisted of 72 finger electrode pairs with the width of 5.0 μm and center-to-center separation of 10.0 μm. The spacing between the delay lines was 100λ. The area of the SAW device was 3.0 mm × 9.0 mm, and the aperture of the IDT electrodes was 1.6 mm. SiO_2 guiding layer was deposited on the device to confine the acoustic energy near the surface. These devices supported SH waves with center frequencies at around 200 MHz. The detailed configuration of the SAW device is shown in Fig. 1.

2.2. Fabrication and packaging of the SAW device

The proposed SAW devices were fabricated using single-side polished 4 in. 36°YX-$LiTaO_3$ wafers (Iljin display Co., Gyeonggi-do, Korea) as piezoelectric substrates. The wafers were initially cleaned by rinsing with acetone, isopropyl alcohol, and deionized (DI) water in order, then dried with N_2. Aluminum thin film having a thickness of 3000 Å was sputtered onto the $LiTaO_3$ wafers, patterned with a conventional photolithographic technique, and wet-etched to define the IDT electrodes. The SiO_2 guiding layer was grown to a specified thickness (1–6 μm) by tetraethylorthosilicate plasma-enhanced chemical vapor deposition (P-500, Applied Materials, Inc., CA, USA) on the wafer, and then a patterned chromium layer was applied on the SiO_2 layer as an etch mask. The wafer surface was rinsed with DI water, and the exposed SiO_2 was etched using the Buffered Oxide Etch (J.T. Baker, Inc., NJ, USA) solution to form wire bonding pads.

The diced SAW devices were mounted in individual ceramic Surface Mount Device (SMD) packages and bonded with aluminum wires for electrical connection. Since sample and washing solutions to be loaded on our SAW devices contained electrolytes, the opened bond pads and the wires were protected with silicone resins (Cookson Electronics, GA, USA) in order to prevent current leakage and electrical short circuit. The silicone resins were deposited with an