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A study of temperature dependence of immunoreactions using shear horizontal surface acoustic wave immunosensors
横波型弾性表面波免疫センサを用いた免疫反応温度依存性の研究

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1. Introduction

Portable immunosensor has been required in point of care testing such as home medical care, bedside care, and also accident and emergency. There were various important requirements, such as sensitivity, miniaturization, and rapid detection. A shear horizontal surface acoustic wave (SH-SAW) based immunosensor is one of the candidates of these applications, because of small size, easy to use, wireless connectivity and quantitative. Since SH-SAW has a horizontal polarization in the direction normal to the propagation direction parallel to the substrate, the SH-SAW energy is less radiated into the liquid and then SH-SAW is suitable for immunosensors[1]-[4].

On the other hand, since Rayleigh surface acoustic wave (R-SAW) radiates a longitudinal wave into the liquid, the liquid shows non-linear dynamics, such as vibrating (agitating), streaming, small droplet flying, atomizing, and heating[5]-[7]. We can use an SH-SAW and an R-SAW on the same substrate and some papers have described R-SAW assisted SH-SAW immunosensors. Although R-SAWs can accelerate immunoreactions, very little is known about the mechanism of it. In this paper, we investigate the temperature dependence of immunoreactions.

2. Materials and methods

We designed a reflection type delay-line on a 36-degree Y-cut quartz substrate. A cross section of the SH-SAW immunosensor is shown in **Fig. 1**. The delay-line has a floating electrode unidirectional transducer (FEUDT), a grating reflector, and a

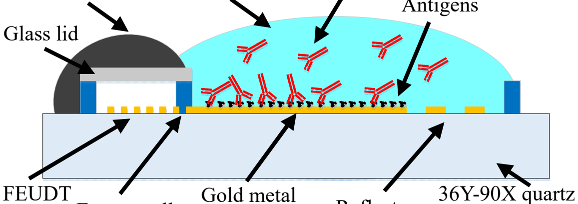

Fig. 2 A cross section of SH-SAW immunosensor.

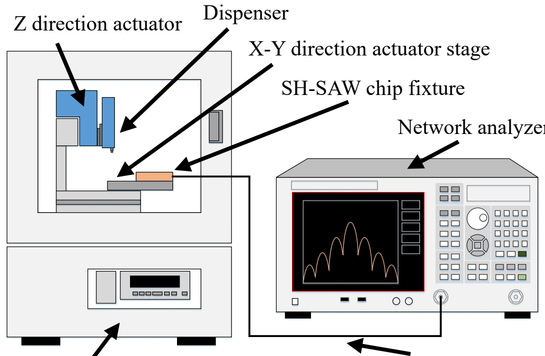

Fig. 1 Block diagram of measurement setup.

sensing area between them. A center frequency of about 250 MHz was designed using a wavelength of 20 micrometers. The number of finger pairs of the FEUDT is 80 and the aperture is 0.4 mm. The number of strips of the grating reflector is 19. The distance between the FEUDT and the reflector is 2 mm and the area is covered with a 92 nm gold metalized layer. An air-cavity is achieved above the FEUDT and protects the FEUDT from the liquid. The insertion losses were 25.4 dB without water and 35.6 dB with water.

The gold surface of the SH-SAW chip was exposed to a solution of dithiobis(succinimidyl propionate) (DSP) at 4mg/ml dilute in dimethyl sulfoxide (DMSO). DSP contains an amine-reactive N-hydroxysuccinimide (NHS) ester and disulfide bridge (SS-bound). NHS esters react with primary amines to form stable amide bonds, and sulfur (S) in the disulfide is coupled to the gold surface. Human serum albumin (HSA) antigens dilute to 100 μg/ml in phosphate buffered saline (PBS) and applied to the chip surface for 15 minutes. Following incubation with HSA, the chip was washed with tris buffered saline (TBS) containing 0.05% Tween 20 (TBS/T) and 100mM of hydrochloric acid (HCl) and deionized water. The chips were dried under a nitrogen (N_2) gas after a washing step.

The block diagram of measurement setup is shown in **Fig. 2**. The velocity changes were measured with a network analyzer. The SH-SAW

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chip is placed in a thermostatic chamber in order to keep the temperatures, 4°C, 23°C and 37°C. The sample liquids were dropped using a dispensing machine with X/Y/Z-axis actuators in the thermostatic chamber.

3. Result

In order to evaluate the immunoreactions of the SH-SAW immunosensor, the human serum albumin (HSA) antigen-antibody was used. The sensing area of the chip was coated with DSP and HSA antigen as described in the previous chapter. In the evaluations, the polyclonal antibodies of rabbit-originated anti-human serum albumin (Anti-HSA) were used. The 10 μl of the anti-HSA antibodies with 10 μg/ml and 100 μg/ml concentrations diluted in PBS was dropped onto the chip. The measured SH-SAW velocity changes at different temperatures are shown in **Fig. 3** after the Anti-HSA solutions were dropped. The SH-SAW velocity changes are resulted by the immunoreactions on the surface.

The velocity changes with time due to immunoreactions can be supposed to exponential functions. We assume that the maximum velocity changes can be the same for any different ambient temperatures. **Figure 4** shows the velocity changes due to immunoreactions for the different ambient temperatures, which are normalized by the maximum velocity change. It was confirmed the HSA immunoreactions can be influenced by the ambient temperatures and higher temperatures can shorten the immunoreaction time.

4. Conclusion

In this paper, the HSA immunoreaction which is the HSA antigen-antibody reaction was investigated at different ambient temperatures. It was confirmed the HSA immunoreactions can be influenced by the ambient temperatures and higher temperatures provided more active reactions. In R-SAW assisted SH-SAW immunosensors, we can raise the temperatures and we can agitate the sample liquid with different input powers of the R-SAWs. Both of them can improve SH-SAW immunosensor performance. Since we made it clear the temperature dependence of the HSA immunoreactions in this paper, we will investigate the agitating effects of R-SAW assisted SH-SAW immunosensors. We will report it in the near future.

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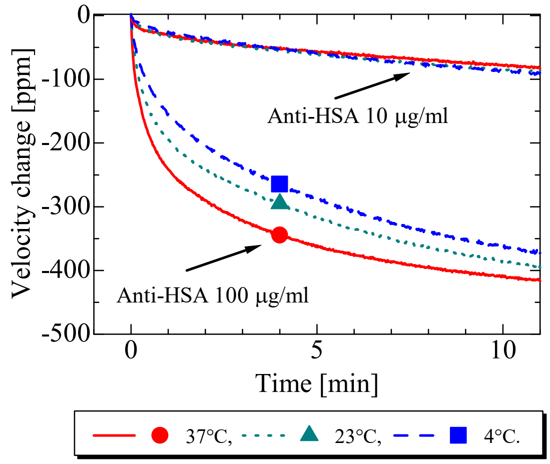

Fig. 3 Velocity change of SH-SAW due to HSA antigen-antibody reaction at different ambient temperatures.

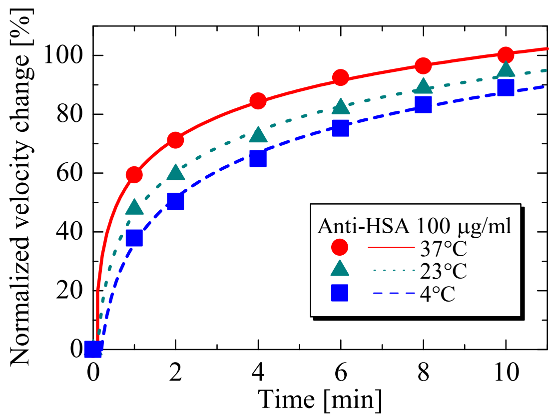

Fig. 4 Normalized velocity changes of SH-SAW due to immunoreactions at different ambient temperatures.