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A novel surface acoustic wave biosensor for detecting human cathelicidin LL-37

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1. Introduction

Cathelicidin LL-37 is unique human antimicrobial peptide with positive charges, which has a capacity to distinguish membrane composed by different lipids in human (1). This unique property enables LL-37 to destroy the negative charged bacteria membranes. The patients who suffer urinary tract infection (UTI) will be found the elevated LL-37 concentration in their urine. To shorten the diagnosis time of UTI, a biosensor for LL-37 is desirable. The biosensor has been designed based on SAW technology. For example, the SAW immunosensor has been prepared by $LiTaO_3$ poezoelectric single crystal substrate to detect hepatitis B surface antibody (2).

In this study the biosensor is designed by integrating inter-digital (IDT) electrodes, which are fabricated on $LiTaO_3$, and a molecularly imprinted polymer (MIP), which recognizing LL-37. MIP has been successfully used to separate and purify small organic compounds (3). The technology is modified and used to prepare sensing zone of SAW device. The Non-covalent molecular imprinting technology creates recognition cavities that are specific to LL-37. When LL-37 presents in the urine sample, it will interact with recognition cavity and adsorb onto the MIP film. The change of weight results in the frequency change of SAW device and can be calculated with the following equation (4):

$$\Delta f = \frac{-\triangle m_s \times f^2}{A \times \rho \times N}$$

Δf ：the change of frequency（Hz）
Δm_s：the change of mass on sensing area（g）
f ：original frequency of SAW device（Hz）
A ：sensing area (cm^2)
ρ：density of piezoelectric film (for $LiTaO_3$:7.456 g/cm^3)
N = $f \times t$ (t：thickness)

2. Materials and Methods

The surface acoustic wave device was prepared by integration of molecularly imprinted polymers and inter-digital electrode. The substrate of biosensor was $LiTaO_3$. Inter-digital electrodes were prepared with lift-off technology. Both the width of electrode and the space between electrodes were 5μm.

The imprint of cathelicidin LL-37 was conducted by micro-contact technology. Briefly, LL-37 was prepared in three concentrations, 0.02, 0.08, and 0.14 mg/mL with phosphate buffer. LL-37 was incubated with clean cover slide for 2 hr to form uniform adsorption film. The sensing area of SAW was then functionalized with 0.4 % methacrylic acid 3-(trimethoxy-silyl) propyl ester (in 6 mM acetic acid) (**Fig. 1**). The cover glass with adsorbed LL-37 was covered on sensing area of IDT, which was loaded with functional monomer, methacrylic acid (MAA), and crosslinker, poly (ethylene glycol) dimethacrylate （PEG400DMA, Mn=550）, in the ratio of 5:95 by volume. The initiator for polymerization reaction was 2,2-dimethoxy-2-phenylacetophenone (2 wt.%). The polymerization reaction was initiated with UV for 10 min at ambient temperature. After washing the polymer film by trypsin and 1% sodium dodecyl sulfate, the identifiable cavities of LL-37 was formed. The recognition capability of MIP was characterized with ELISA as before (5)

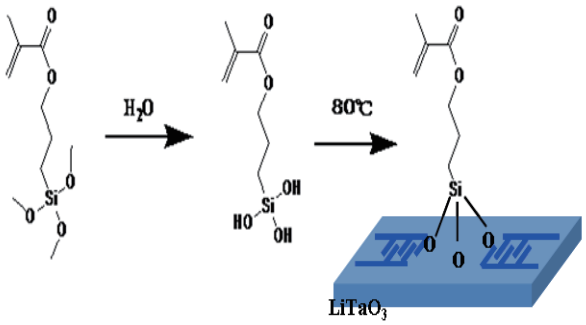

Fig. 1. Modification of IDT electrode.

The start frequency of the prepared biosensor was measured with Agilent 8720ESU network analyzer. The biosensor was then inserted in LL-37 or human serum albumin solution for 2 hr, washed with phosphate buffer, and dried with nitrogen gas. The frequency variation of SAW-IDT after protein adsorption was determined by network analyzer.

3. Results and Discussion

The recognition capability of polymer film

can be analyzed with the imprinting factor, which interprets as better recognition for target molecules. The re-binding amount of LL-37 on MIP and non-imprinting polymer (NIP) were determined and the following equation was used to calculate the imprinting factor.

$$\text{Imprinting factor } (\alpha) = \frac{MIP\,ARB - MIP\,AW}{NIP\,ARB - NIP\,AW}$$

The MIP prepared by cover glass in 0.02, 0.08, and 0.14 mg/mL of LL-37 solution showed the imprinting factor of 2.69, 3.23, and 6.08, respectively. The cover glass in higher LL-37 concentration may result in higher peptide adsorption and create higher imprinting factor.

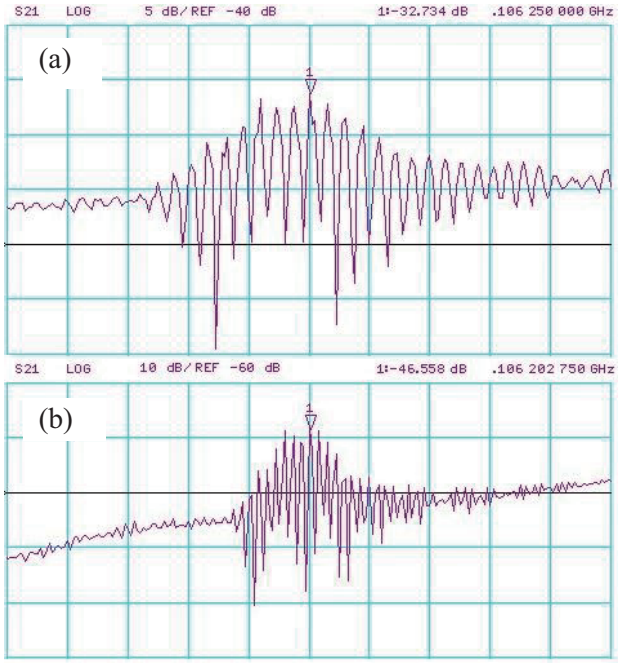

Fig. 2. Frequency response of LL-37 (0.14 mg/mL) adsorbed onto SAW-IDT biosensor. (a) blank sensor; (b) after adsorption of LL-37.

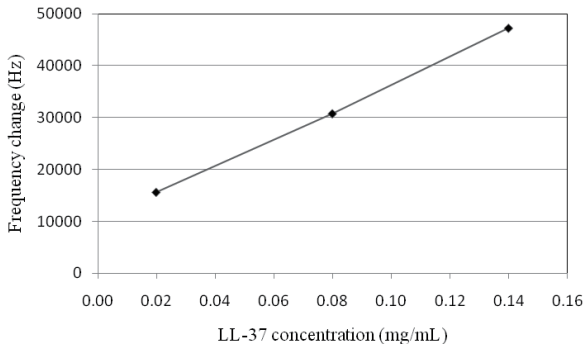

Fig. 3. Frequency change of SAW-MIP in different LL-37 concentrations.

The start frequency of SAW-IDT biosensor prepared with cover glass which made in 0.14 mg/mL LL-37 was 106,250,000 Hz. The frequency decreased to 106,202,750 Hz after LL-37(0.14 mg/mL) rebinding (**Fig. 2**). The response of frequency decrease upon the increase of LL-37 concentration was shown in **Fig.3**. The biosensor was also examined with human serum albumin and the result didn't show frequency change. Based on those findings, we can ascertain that the SAW–IDT biosensor can be used to determine LL-37 concentration according to the change of frequency. Because the detection is performed without pre-labeling the target molecules and can be read out with network analyzer directly, it is useful for quick diagnosis of UTI.

Acknowledgment

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