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Interdigitated Electrodes Based on Impedance Biosensor for Sensing Peptide LL-37

Jia-Yi Lee, Chung-Yih Wang, Chi-Fang Huang and An-Ting Cheng

***Abstract*— An antimicrobial peptide, LL-37, is found in an innate defense system of humans. Patients who suffer urinary tract infection (UTI) will generate LL-37 and which is released into urine. LL-37 can be used as an indicator for the diagnosis of UTI. We have designed a biosensor with an interdigitated electrode on a printed-circuit board (PCB). The surface of the electrode was modified with 3-mercaptopropionic acid and immobilized with anti-LL37 antibody to improve the specificity of the biosensor. By de-embedding jig impedance, the impedance associated with the change of LL-37 concentration was calculated. The sensitivity of this biosensor for LL-37 in a urine sample can reach 50 μ g/mL.**

***Keywords*— Biosensor, LL-37, urinary tract infection, interdigitated electrode, impedance**

I. Introduction

HUMAN cationic antimicrobial protein (hCAP-18) is a unique antimicrobial peptide found in humans [1]. In response to bacterial infection, the active form of the peptide, LL-37, is produced by proteolytically cleaving two amino acids at the N-terminal of hCAP-18 [2]. The highly positively charged amino acids in the LL-37 sequence will interact with the negatively charged bacterial membranes and result in the death of the bacteria. Patients who suffer urinary tract infection (UTI) will induce the release of inflammatory materials. Among those molecules, LL-37 can be used as an indicator for UTI [3]. Current analytical assays for LL-37, such as a radial diffusion assay or ELISA, are complicated and labor intensive [4]. It is desirable to develop a biosensor that can detect LL-37 in a urine sample directly.

Recently, many researchers applied electrochemical impedance spectroscopy (EIS) to study the response of adsorption of biomolecules onto the surface of the electrode [5]. The theoretical basis of EIS is to treat biomolecules as polarized dielectrics with a permittivity ε under the supplied field [6]. In terms of circuit theory, such kinds of biomolecules will form a double layer capacitor on the surface of the electrode and exhibit impedance when an electrical signal is applied. Consequently, for building a probe for sensing peptides, the corresponding varying impedance will serve as a good electrical indication for peptide concentration. The interdigitated microelectrodes have been fabricated to detect molecules due to their rapid reaction kinetics and improved signal-to noise ratio [7, 8].

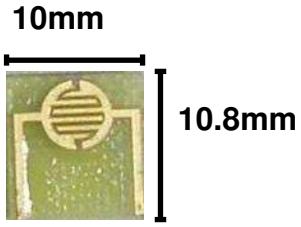

Fig. 1 A probe on a printed-circuit board (PCB) for electrochemical impedance spectroscopy.

To develop a biosensor for the diagnosis of UTI, an electrode on a printed-circuit board (PCB) measuring the impedance associated with the change of LL-37 concentration was designed and fabricated. We have developed a board-level electrode, which is workable to monitor the impedance variation due to the peptide concentration [9]. However, the sensitivity is poor for those large electrodes. In the present work, a reduced size interdigitated electrode, as shown in Fig. 1, has been designed and manufactured as a testing jig for the purpose of measuring the concentration of proteins or other biomolecules directly. To achieve the specificity to capture LL-37 in a urine sample, the gold electrode was first modified with 3-mercaptopropionic acid to form a self-assembled monolayer (SAM). Crosslinkers n-hydroxysuccinimide (NHS) and 1-(3-dimethylaminopropyl) -3-ethylcarbodiimide (EDAC) were then applied to an immobilized anti-LL37 antibody on a gold electrode. The advantages of this biosensor include easy manufacturability, low cost, and to detect LL-37 without pre-labelling the target molecules. Moreover, when LL-37 binding on the surface of electrode, the impedance of the PCB electrode is de-embedded using the equivalent circuit model. The results

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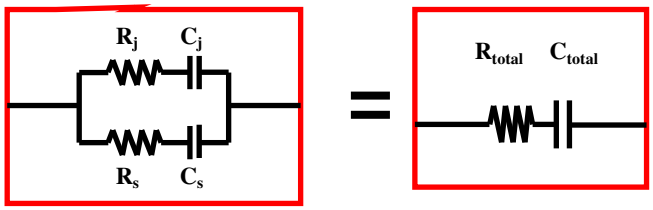
obtained after de-embedded calculation show only the intrinsic impedance resulting from LL-37.

II. Design Theory

Considering that the electrode being filled with peptide in Fig. 1 is a two-port network, the equipment circuit shown in Fig. 2(a) can be proposed to represent their electrical property. In the equivalent circuit, (R_j, C_j) represents the jig (electrode), and (R_s, C_s) represents the tested sample, respectively. Then (R_{total}, C_{total}) represents their integral impedance and they are the measured data from the used equipment or device.



(a) (b)

Fig. 2 Equivalent circuits for the electrode and biomolecules under testing.

By circuit theory, the impedance for a pair of (R, C) is

$$Z = R + \frac{1}{j2\pi f C} \qquad (1)$$

where f is the testing frequency.

Since

$$Z_{total} = \frac{1}{\frac{1}{Z_j} + \frac{1}{Z_s}} = \frac{Z_j Z_s}{Z_j + Z_s} \qquad (2)$$

It can be derived that

$$Z_s = \frac{Z_j Z_{total}}{Z_j - Z_{total}} \qquad (3)$$

It is worthwhile to note that, Z_j and Z_{total} are complex numbers and can be read out when the blank jig and the filled jig are measured respectively by an impedance vector analyzer.

III. Biosensor Fabrication and Measurement

FR406 PCB (purchased from Isola Laminate system, WI) was coated, from bottom to top, with 0.033 mm Cu, 0.15 mm Ni and 0.001 mm Au. An interdigitated electrode of PCB was fabricated by the etching machine ProtoMat C60 of LPKF Company [10] in the Microwave Lab of Tatung University, Taiwan. The electrode was rinsed with ethanol and dried with nitrogen gas. The gold electrode was modified to immobilize an anti-LL37 antibody. Briefly, the electrode was immersed in 99% 3-mercaptopropionic acid for 16 hr at room temperature. The electrode was then rinsed with ethanol and dried with nitrogen. The SAM was further functionalized with a 0.1 M NHS / 98% EDAC (9/1, v/v) solution. Ten μL of anti-LL37 IgG (500 μg/mL) was then applied to the electrode to complete the recognition function of the biosensor. The impedance for the blank biosensor was determined by applying 300 μL of deionized water or phosphate buffer. The applied voltage for the test signal was 0.1 V and the scanning frequency range was from 0.1 to 100 kHz. The impedance was measured by the potentiostat/galvanostat PGSTAT 30. The intrinsic impedance was obtained after de-embedded calculation. The LL-37 was prepared in phosphate buffer or urine to make various concentrations of solution. The interference of salt was studied by adding 150 mM sodium chloride to LL-37 solution and EIS was recorded. To survey the specificity of the biosensor, the LL-37 or human serum albumin (HSA) was prepared in a urine sample and added to the center of the electrode. After a 10 min incubation, the urine was drained and the electrode was washed with deionized water. The equivalent impedance of sample solution was calculated out by the equation (3).

IV. Results and Discussion

The impedance in response of different LL-37 concentrations was shown in Fig. 3. The results display the characteristics of double layer capacitance in terms of varying concentration. The difference in impedance was significant as frequencies ranged from 0.1 Hz to 100 Hz. When frequencies increased above 100 Hz, where the effect of double layer capacitance decreased, the impedance variation between different LL-37 concentrations disappeared.

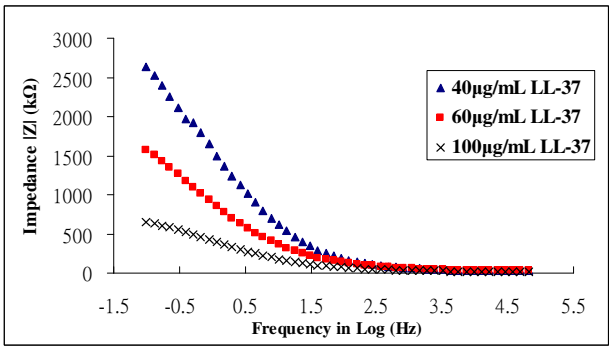


Fig. 3 $|Z_{total}|$ vs. frequency for different concentrations of LL-37.

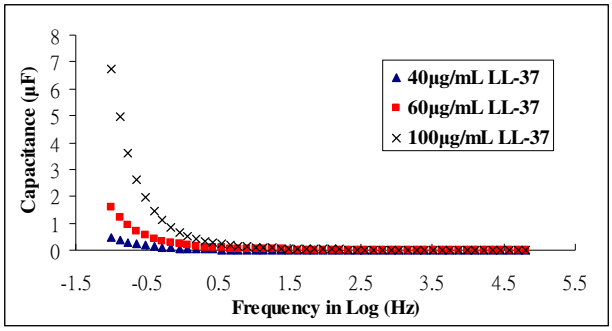


Fig. 4 De-embedded capacitance vs. frequency for different concentrations of LL-37.

The equivalent circuit model (Fig. 2) was used to deduct the de-embedding equation. The impedance of the jig was eliminated after a de-embedding calculation was used. Fig. 4 showed the measured capacitance vs. frequency for different concentrations of LL-37. The data are jig-independent and represent the double layer capacitance formed by the adsorption of LL-37. The advantage of the de-embedding calculation is providing the information of double layer capacitance of biomolecules, which can be used to compared the data measured with different biosensors.

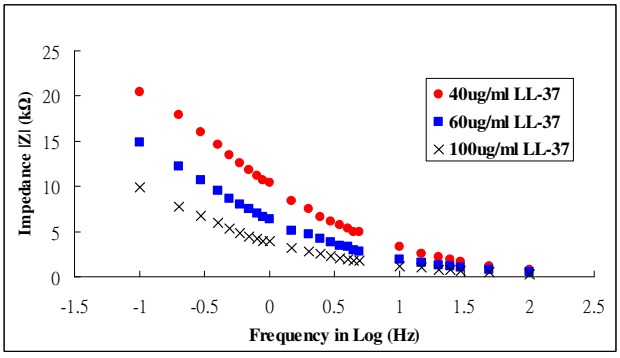


Fig. 5 Impedance amplitude spectroscopy of LL-37 with interference of 150 mM NaCl.

The interference generated by the possible existence of salt and other biomolecules was also studied. Fig. 5 shows the results of EIS of LL-37 in the presence of 150 mM NaCl in urine. The total impedance decreased when the concentration of LL-37 increased in the range of frequencies scanned. It suggests that a high NaCl concentration interfered with the impedance amplitude spectroscopy significantly. The addition of NaCl results in the increase of solution conductance and this kind of interference can not be eliminated by the de-embedding operation. To avoid the interference from salt, the biosensor immersed in urine for LL-37 binding was washed before measured with PGSTAT 30.

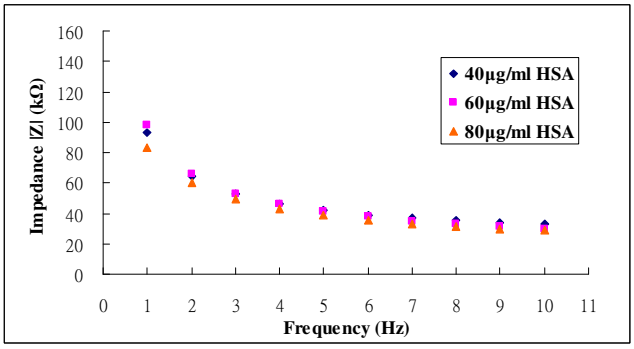


Fig. 6 The interaction of HSA with the biosensor, where an anti-LL37 antibody was immobilized on the surface of the electrode.

HSA was added to urine to survey the interference of biomolecules. After incubation and washing with deionozed water, the impedance was measured. The results showed that in the presence of 40 to 80 μg/mL of HSA, the change of impedance is insignificant (Fig. 6). It suggests that the ant-LL37 antibody immobilized on the surface of the electrode will not capture HSA. Fig. 7 shows the results of LL-37 detection after the de-embedding calculation was used. The capacitance change was linearly dependent on the concentration of LL-37 at different testing frequencies. The detection limit of this board-level biosensor is comparable to Zou's chip-level biosensor [5].

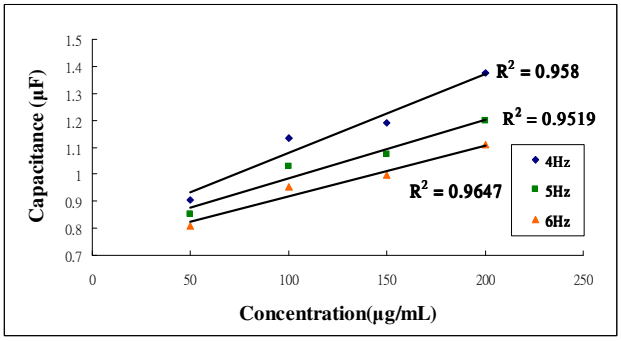


Fig. 7 Measured capacitance vs. concentration of LL-37 at different testing frequencies.

V. Conclusion

This work developed a biosensor composed of board-level interdigitated electrodes with a bio-affinity surface. The biosensor is efficient to detect the concentration of the antimicrobial peptide, LL-37, in urine without the need of pre-labelling the target molecules. A de-embedding technique was also developed to exclude the impedance effect of the PCB jig itself. Generally, the surface of electrode can be modified with bio-affinity molecules and used as a sensitive biosensor to monitor the change of peptides or other biomolecules in a biological sample. A chip-level nano-scale biosensors with similar design is developing to further improve the sensitivity and increase the signal/noise ratio.

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