Electrode structure reducing the variation of the impedance measurement for electrical bio-sensor

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Abstract
In this paper, we investigated the electrode structure of the electrical bio-sensor to reduce impedance measurement fluctuation. Five pairs of the same electrodes were formed on the PCB substrate, and a fluidic channel was constructed with a double-sided tape and polycarbonate cover so that all the electrodes had the same reagent distribution. The electrode structure was manufactured in five types with the same interval between electrodes and increasing the number of electrode layers. As a result of an experiment in which physiological saline was injected into an electrode chip made of five electrode structures, it was confirmed that the change in the impedance between the electrodes was small as the number of electrode layers was increased.

Keywords: Electrode, Impedance, PCB substrate, Saline, Impedance variation Lock-in amplifier.

INTRODUCTION
Measuring DNA or protein using fluorescent labels requires expensive and precise equipment and has unexpected interference with detection systems [1]. On the other hand, electrical sensors enable the label-free detection by measuring electrical signals instead of fluorescence brightness. It is a very promising biosensing method because the limits of detection are very low and the time-to-results are relatively fast [2]. Recently, it is called electrochemical impedance spectroscopy (EIS) because it primarily measures the impedance change depending on the concentration of DNA or the amount of protein captured in the antibody [1, 3-5]. On the other hand, electrical bio-sensing method called electrical cell-substrate impedance spectroscopy (ECIS) is used to measure micro-motion, response to drugs, and assessment of barrier function of cancer cell or stem cell [6-10]. ECIS can be regarded as a kind of EIS using the phenomenon that impedance increases between electrodes as cell mobility increases.

All of the above electrical sensors need electrodes. For example, to determine the concentration of prostate-specific antigen associated with prostate cancer, three gold electrodes were prepared by photolithography on a silicon substrate [3]. There are also cases where an electrode array is made using CMOS technology and used for DNA detection [1]. On the other hand, in order to observe the motion of the cell, an electrode is formed on glass or printed circuit board (PCB) substrate [8-11]. These electrodes are expected to have different properties each time they are made, but research on the variation of the characteristics is hard to find. Instead, calibration is performed taking into account changes in electrode properties before use [1, 9].

In this paper, we investigate the variation of the characteristics of electrodes and investigate how to make the fluctuations small. The electrodes can be constructed in various ways. However, we used the manufacturing process of printed circuit board PCB which is stable. In case of manufacturing by other methods, fluctuation of electrode characteristics may be caused by other factors such as them from the unstable process. Since the PCB fabrication process is the most stable process due to the development of information technology, the fluctuation of the electrode manufactured by this process must cope with each application. It is desirable to experiment with fluctuation between several PCB substrates.

However, since there is concern about fluctuation of reagent distribution due to channel fabrication, five electrodes were manufactured on one PCB substrate and the variation between them was examined. This is because the electrode structure, that minimize the impedance fluctuation between these electrodes, is expected to reduce also the variations among the PCB substrates. The electrodes were arranged side-by-side in order to immerse the electrodes in the same solution. Then, a double-sided tape was used to construct channels of the even width and covered with a polycarbonate cover to form a fluidic chip. If the length of the channel is long enough and the width is fairly even, the same amount of solution will be distributed around all the electrodes.

For measurement of impedance as in the reference [1], saline solution was distributed on electrodes and measured by sinusoidal stimulation using trans-impedance amplifier. Assuming
that the electrode width is main cause of the impedance fluctuation, it is expected that the fluctuation becomes smaller when the electrode is arranged in multiple layers. Assuming that the variation of the electrode is mainly due to the fluctuation of the electrode width, it is expected that the fluctuation is small when the electrode is arranged in multiple layers. Experimental results were similar to those expected, but the frequency characteristics became more and more bumpy as the number of electrode layers increased.

**MATERIAL METHOD**

Experimental setup

In this paper, experiments were conducted using electrodes made of PCB substrate. Five pairs of electrodes with a constant width were arranged side by side on a PCB substrate. To reduce the chemical reaction with the test solution, the electrode material was selected with electroless nickel immersion gold (ENIG). The electrodes were manufactured to a uniform size of 100μm×1mm. The interval of the electrodes was constant at 100μm. Five pairs of electrodes arranged on a PCB substrate were manufactured with five types of electrode structures ranging from one to five layers with a constant width. The type of electrode manufactured is shown in Figure 1.

In order to distribute the saline solution evenly in electrodes on the PCB substrate, a double-sided tape was used to fabricate a constant width channel. The channel of the chamber tape was 23mm×4mm that is longer enough to guarantee the uniform distribution on all the electrodes. And both ends of the channel were rounded to ensure that the saline solution was injected well into the channel. The manufactured channel can be seen in Figure 2(b). Finally, a fluidic chip was manufactured by covering the channel with a polycarbonate cover with inlet and outlet. The finished chip and the assembling diagram of the chip is shown in Figure 2(a) and (c), respectively.

A simple circuit was constructed to carry out the experiment to compare the variation according to the structure of the electrode. The circuit is shown in Figure 3(a). In order to measure the impedance between each pair of electrodes, a sinusoidal stimulation was applied on the voltage divider circuit with the known precision reference resistance (100 kΩ, 1%) using a USB oscilloscope with a function generator (Picoscope2204A, Pico Technology Ltd., England). The saline solution was injected into the inlet hole of the fluidic chip, the inlet and outlet were sealed with a tape, and then applied to the voltage divider. Figure 3 shows the circuit diagram for the impedance measurement, where the 3rd electrode pair e3 is ready to be measured. One end of all pairs of electrodes was connected together and it was probed with the channel B of the oscilloscope. The other ends were probed with the channel A in turn. The impedance ratio between each electrode pair and the reference resistor was estimated with the lock-in amplifier law. Assume that the impedance of the considered electrode is $Z$, then the voltage gain between the voltages probed from the channel A and B is follows:

$$G = \frac{R}{R + Z} \quad (1)$$

where $R$ is the resistance of the reference resistor (100k, 1%).

The voltage gain $G$ can be estimated using the following locked amplifier law:

$$\text{real}(G) = \int_{t=0}^{T} x(t) \cos \left( \frac{2\pi}{T} t \right) dt$$

$$\text{imaginary}(G) = \int_{t=0}^{T} x(t) \sin \left( \frac{2\pi}{T} t \right) dt \quad (2)$$

where $x(t)$ and $T$ are the divided voltage proved by the channel B and the period of the stimulus sinusoidal function. The stimulus voltage is the cosine function in the integral in the equation. The cosine and sine function in the integrals could be accurately acquired with the software development kit for Matlab given by the oscilloscope vendor.
Experimental procedure

The saline solution was injected into the chamber channel through the inlet of the fluidic chip. Injection should have been careful not to have air bubbles near the electrodes. A constant volume of saline solution of 35μg was required to fill up the channel. The saline solution used in the experiment was diluted to a concentration of 1.25ug/ml. After the injection of the saline solution was completed, the inlet and the outlet of chip were sealed before measuring. Then the fluidic chip was set on the measurement circuit as shown in Figure 4. For each fluidic chip with one of the five types of electrode structures, the impedance of each pair of electrodes measured at the frequencies from 10 kHz to 100 kHz in 5 kHz intervals in turn. The impedance of the electrode pair according to each frequency was calculated in MATLAB program we built with the given software development kit. The impedance data of each electrode extracted from the program were analyzed using Microsoft Excel.

RESULTS

In this paper, an experiment was conducted to find an electrode structure with little variation in impedance depending on the electrode structure. For the experiment, electrodes constructed using PCB process were used. Five pairs of electrodes were arranged side by side, and all of the five pairs of electrodes were made to have a constant width of a certain size. A fluidic chip was made for the electrode PCB and the electrode impedance was measured using saline solution.

The impedance variations of 1-layer and 5-layer electrode pairs are shown in Figure 5 and 6, respectively. It can be seen from the graph of Figure 5 that the variation of the impedance of the electrode pairs are large even though they are placed on the same PCB substrate for 1-layer ones. The fluctuation in the 5-layer electrode structure is reduced as shown in Figure 6. Note that the impedance is reduced to about 1/5 for 5-layer case, while the distance of the real impedances for 1-layer electrodes is about 1kΩ contrast to that for 5-layer electrodes is 100Ω. Which means that the relative deviation is about a half for 5-layer electrodes. However, it is interesting that the frequency characteristic of the electrode is bumpier in the 5-layer electrode structure that in 1-layer case.
The results of all kinds of the electrode fluidic chips were collected and the relative deviations of the impedance magnitudes were extracted and plotted in Figure 6. In the graph, it can be seen that the relative deviation decreases as the number of electrode layers increases. However, the results for 4-layer and 5-layer seem to be similar trends, indicating kind of saturation of the relative deviation.

**CONCLUSION**

In this paper, a fluidic chip was fabricated as an electrode on PCB substrate, and experiment was conducted to measure impedance variation according to electrode structure. The structure of the electrodes was made up of five structures, from one to five layers, with increasing the number of electrode layers. Experimental results show that the variation of the impedance value between electrodes is reduced in the structure with many electrode layers. As a result of this experiment, I was able to confirm what was expected. Also, it was confirmed that the frequency characteristic of the electrode becomes bumpy as the number of layers of electrode increases. It was confirmed that the impedance difference between the inlets and the electrodes arranged near the outlet was large in the arranged electrodes. We have discovered a further research topic on whether the solution is entering and causing a chemical reaction with the electrode.

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**REFERENCES**


