Measurement System for Local Electric Impedance of Gel in Cell with Vibrating Electrode

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ABSTRUCT

A new methodology to measure local electric impedance of gel in a cell has been proposed, and a measurement system has been designed with a vibrating electrode. The vibration of electrode was generated with the piezoelectric actuator, and controlled with an electric oscillator. The manufactured system was evaluated in the experiment with endothelial cells *in vitro*. The endothelial cells were exfoliated from the descending aorta of a rat, and cultured on the hydrophilic modified surface of silicon rubber. One of the electrodes was stuck into the cell under the microscope, and vibrated at the frequency of 100 Hz. Another electrode was dipped into the culture medium, and fixed in the close vicinity of the cell. The output signal was amplified and the frequencies were analyzed. The experimental results show that the local impedance in the order of 0.1 kilo-ohm was calculated from the amplitude component of output signal at 100 Hz.

Keywords: Electric Impedance, Electrode, Frequency Analysis, Endothelial Cell, Piezoelectric Actuator, Vibration.

1. INTRODUCTION

A biological cell includes the electrolyte solution. The electric impedance depends on ionic mobility in the solution. There have been many studies about impedance of biological tissue to estimate its functional structure [1, 2]. When the electric properties are measured in biological tissue, an alternating current is usually applied to avoid chemical reactions on electrode [3]. To measure local impedance, the electrode should be minimized in dimension [4]. The micro-electrodes have high value of impedance, which scatters in the shape of electrode. The number of electrodes stuck into the tissue should also be decreased to minimize damage to local structure of biological tissue [5, 6]. To measure local electric impedance of gel in a cell, the present study has proposed a new methodology, in which only one electrode is stuck into biological tissue and the impedance is calculated independently of that of micro-electrode. The performance of the measurement system has been evaluated in the experiment *in vitro*.

2. METHODS

Methodology to measure local electric impedance

The idea to measure local electric impedance of gel was proposed with a vibrating electrode. The impedance between two electrodes dipped into an electrolyte gel varies with the distance between electrodes (Fig. 1). When the distance is sinusoidally oscillated with a mechanically vibrating electrode, the impedance is sinusoidally varied, too. The variation corresponds to impedance of the local space, where the electrode is vibrating (between A and B in Fig. 1). When the frequency of input electric alternating voltage is much higher than frequency of mechanical vibration of electrode, output electric voltage signal fluctuates as that in Fig. 2. Variation, V_{dx}, corresponds to impedance of *dx*. The magnitude of the variation is the product of local impedance and electric current, and does not depend on the impedance of the other part of the electric circuit.

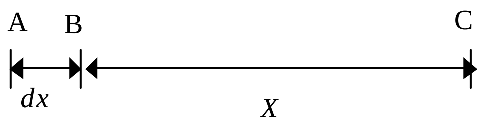

Fig. 1: Distance between two electrodes. One electrode is fixed at point C and another electrode is vibrating between A and B.

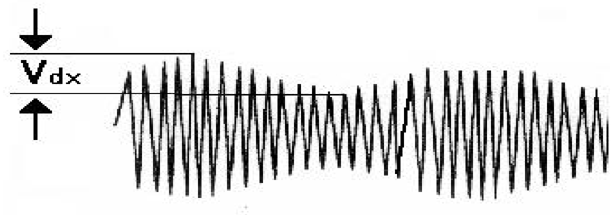

Fig. 2: An output electric alternating voltage signal (V_{out} in Fig. 4) oscillates with time. V_{dx} corresponds to impedance of the local space, where the electrode is mechanically vibrating.

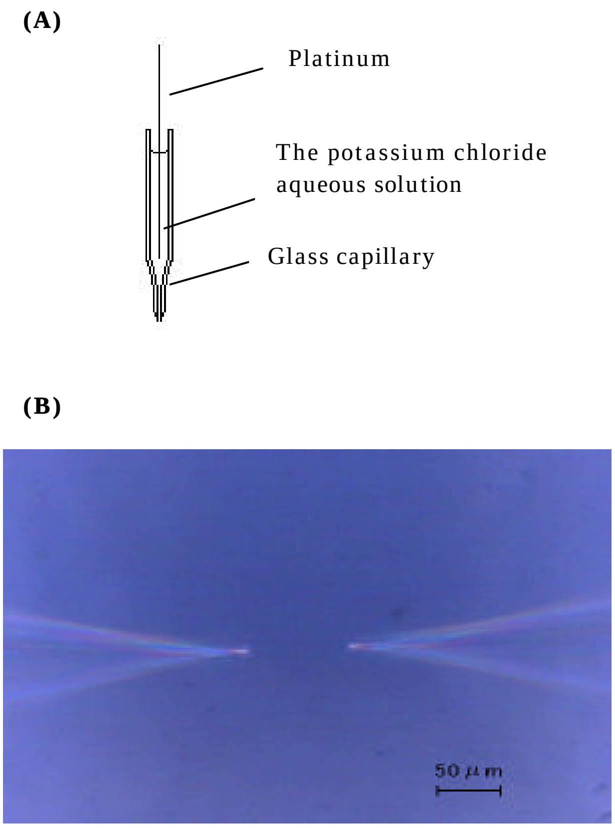

Fig. 3: Micro-electrode. Schematic diagram (A), and the tips of micro-electrodes under microscope (B).

Micro-electrode

The electrode was made of a glass micro-capillary filled with the potassium chloride aqueous solution (3 mol/L). The platinum wire was inserted in the center of capillary (Fig. 3). The diameter of the tip of the capillary is 0.001mm.

Calculation of the impedance

The electric circuit of the measurement system is shown in Fig. 4. When the input impedance of the operational amplifier is much higher than that of feedback resistance of R, output current (I_2) is approximate to input current (I_1).

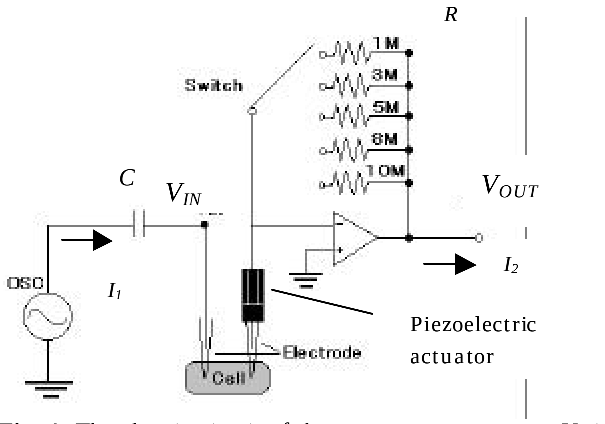

Fig. 4: The electric circuit of the measurement system. V_{in} is input signal applied to the biological tissue and V_{out} is amplified output signal. R is varied with the switch.

$$I_2 = I_1 \qquad (1)$$

The Eq. (1) leads to proportional relationship between impedance and voltage in absolute values.

$$R \,/\, Z_{dx} = V_{out} \,/\, V_{dx}$$
$$= A\ V_{out} \,/\, A\ V_{dx} \qquad (2)$$

In Eq. (2), Z_{dx} and V_{dx} the local impedance and the local voltage of the gel correspond to dx, respectively (Fig. 1). The local voltage (V_{dx}) is measured with the amplified component ($A\ V_{dx}$) of output voltage, where A (= V_{out} / V_{in}) is the amplification constant. The ratio of V_{out} to $A\ V_{dx}$ is calculated with spectra (Fig. 11).

$$\boldsymbol{D} = 20\ \log(V_{out}) - 20\ \log(A\,\boldsymbol{V}_{dx}) \qquad (3)$$

Z_{dx} is calculated with D.

$$Z_{dx} = R \,/\, (A\ 10^{D/20}\ ) \qquad (4)$$

Measurement system for local impedance

The manufactured measurement system consists of an oscillator, an amplifier, an oscilloscope, electrodes, piezoelectric actuators and a microscope.

The input signal was generated with the oscillator (Kenwood, AG-203D). The frequency can be selected between 10 Hz and 1 MHz with the oscillator.

The output signal was detected with the digital oscilloscope (Yokogawa, DL1520L). The oscilloscope has FFT function. The frequency of output signal was analyzed to calculate the local impedance (Eq. (4)). Both the input and output signals were observed with the oscilloscope simultaneously.

The local voltage of gel was amplified with the electric circuit illustrated in Fig. 4.

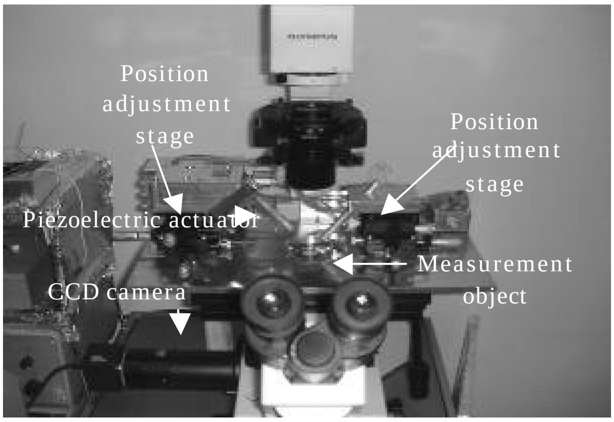

Fig. 5: Microscope and stage.

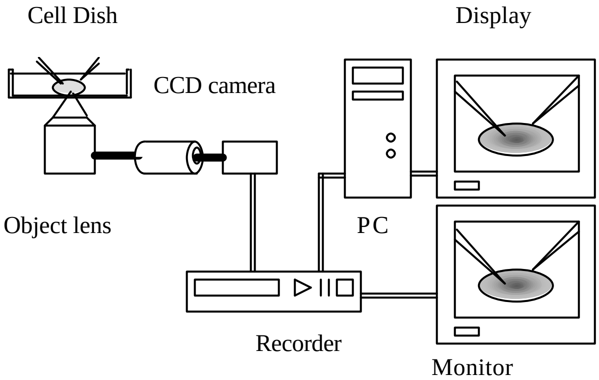

Fig. 6: The diagram of capturing the image of the tips of electrodes.

Microscope and stage
To stick the electrode into a cell, the microscope and stage system was used (Fig. 5). Two electrodes were fixed on the position adjustment stages, respectively. Each stage has three micro-screws to adjust the position in three-dimensional direction, respectively. Two stages fixed on a phase contrast microscope (Olympus, IX70). The positions of each tip of electrode are adjusted with the screws under observation with the microscope.

The state of the tips of electrodes was recorded with CCD camera attached on the microscope, displayed on the monitor, and captured with a personal computer.

Piezoelectric actuator
When voltage is applied to the piezoelectric actuator in polarized direction, it extends in parallel to the direction of the electric field and contracts in perpendicular direction. This piezoelectric effect was applied to vibrate an electrode. The piezoelectric actuators are classified into two types: bimorph type and build up type.

The piezoelectric effect in the bimorph type is shown in Fig. 7(a). Two pieces of piezoelectric stuck in a counter direction of polarization with each other generates flexion. The cylinder type of actuator with the bimorph type (Fig. 7(b)) was used in the vibration unit of the measurement system. This type of actuator generates movement in two directions (X and Y in Fig. 7(b)), when the corresponding voltage is applied. To add movement in Z (Fig. 7(b)) direction, the build up type of piezoelectric actuator was applied.

The build up type of piezoelectric actuator, which has longitudinal piezoelectric effect, consists of alternately accumulated thin films of piezoelectric ceramic (Fig. 8(a)).

To control the position of electrode in three-dimensions and vibration of electrode, two types of piezoelectric actuators were combined in the scanning probe (Fig. 8(b)). The movements of the probe with voltage were 165 nm/V in X and in Y direction (Fig. 7(b)), and 10.4 nm/V in Z direction.

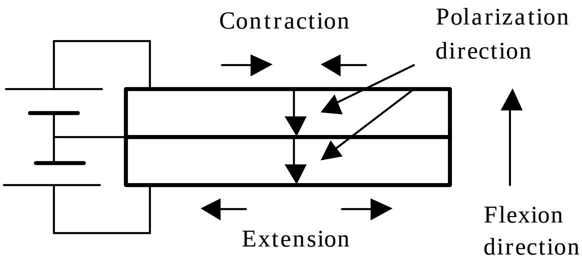

Fig. 7(a): Bimorph type of piezoelectric actuator.

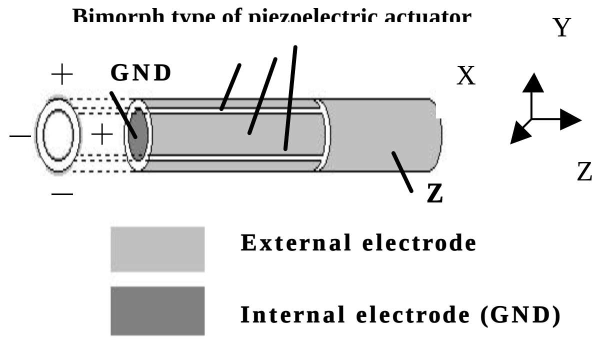

Fig. 7(b): Cylinder type of piezoelectric actuator. This actuator consists of four external electrodes, one internal electrode and build up type of electrode. Movement in X (or in Y) direction is controlled with voltage between opposite external electrodes.

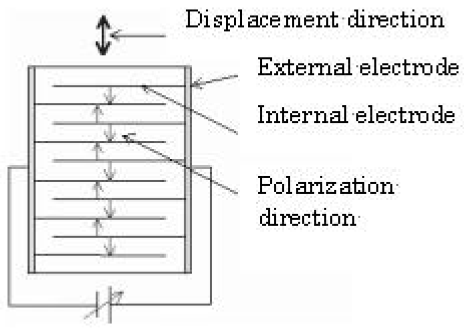

Fig. 8(a): Build up type of piezoelectric actuator.

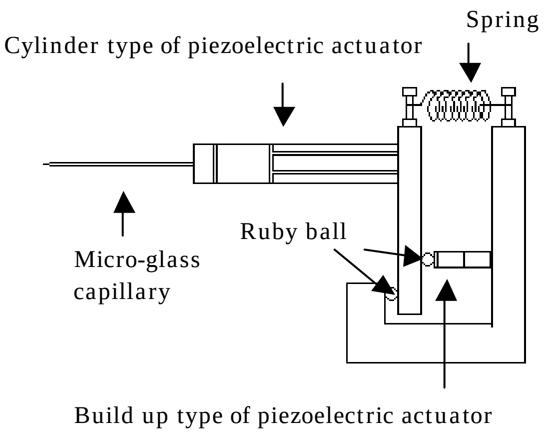

Fig. 8(b): Constitution diagram of scanning probe.

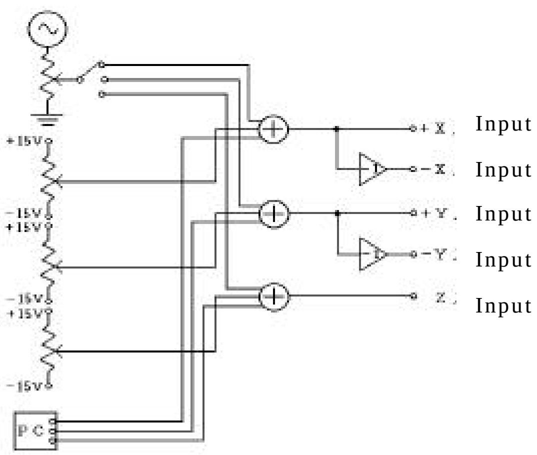

Fig. 9: The circuit of the probe controller.

To measure the local impedance (Figs. 1 & 2), sinusoidal mechanical movement was generated in the probe with sinusoidally oscillating input voltage (Fig. 9). In the system, the input voltages are controlled with the oscillator, with DC voltage between -15 V and +15 V, and with the personal computer. These voltages govern the corresponding movement (at +X, -X, +Y, -Y, Z, (Fig. 7(b))) of the probe.

Experimental Procedure

When both of two electrodes were dipped in the saline solution, power spectra of output voltage were measured to detect the spectrum of vibration of electrode.

The manufactured system was evaluated in the experiment with endothelial cells. The endothelial cells were exfoliated from the descending aorta of a rat, and cultured on the hydrophilic modified surface of silicon rubber [7]. One of the electrodes was stuck into the cell under the microscope, and vibrated at the frequency of 100 Hz. Another electrode was dipped into the culture medium, and fixed in the close vicinity of the cell. The input alternating signal was 100 mV with the frequency of 150 Hz. The output signal was amplified with feedback resistance (*R* in Fig. 4) of 3 mega-ohm and the frequencies were analyzed.

3. RESULT

Fig. 10 shows power spectra of output voltage with the saline solution, when the frequency of input alternating voltage is 100 Hz. The spectrum position moves from 10 Hz to 30 Hz, as the frequency of vibration is altered. The spectrum at 60 Hz corresponds to noise from electric source.

Fig. 11 shows power spectra of the output voltage, when the frequency of input alternating voltage is 150 Hz. The electrode was vibrated at 100 Hz. The spectra at 60 Hz correspond to noise from the electric source. The spectrum at 100 Hz appears in Figs. 11(b) & (c) with vibration of electrode.

The local impedance was calculated from the spectra at 100 Hz and the amplification constant of 20 (Table 1). The order of the impedance was 0.1 kilo-ohm. The local impedance in the endothelial cell was lower than that of medium.

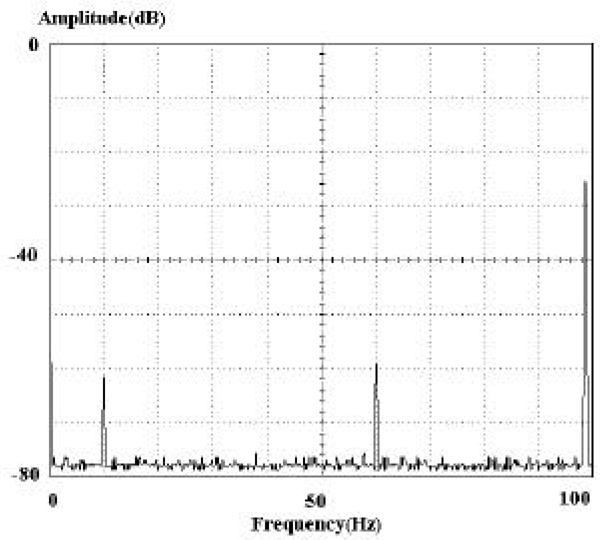

Fig. 10(a): Power spectra of the output voltage with saline solution. The electrode was vibrated at 10 Hz. The input voltage was oscillated at 100 Hz. The spectra at 10 Hz, 60 Hz and 100 Hz correspond to *A Vdx*, noise from the electric source and input signal, respectively.

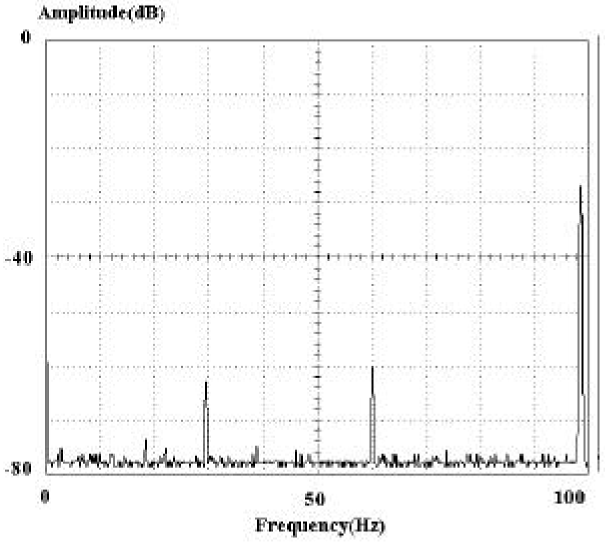

Fig. 10(b): Power spectra of the output voltage with saline solution. The electrode was vibrated at 30 Hz. The input voltage was oscillated at 100 Hz. The spectra at 30 Hz, 60 Hz and 100 Hz correspond to *A Vdx*, noise from the electric source and input signal, respectively.

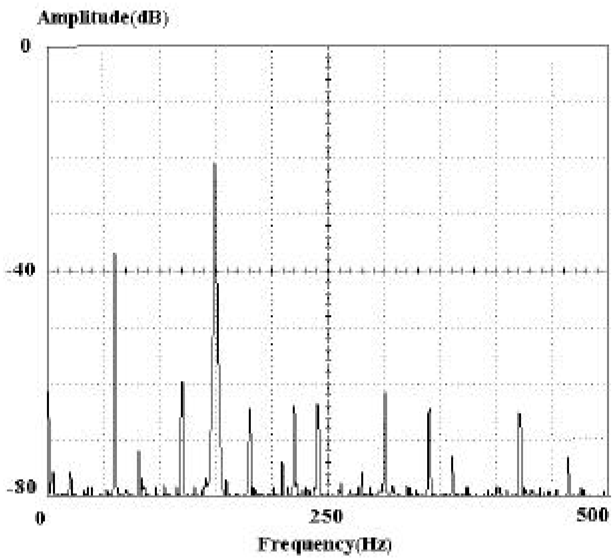

Fig. 11(a): Power spectra of the output voltage with the medium. The electrode was not vibrated. The input voltage was oscillated at 150 Hz. The spectra at 60 Hz and 150 Hz correspond to noise from the electric source and input signal, respectively.

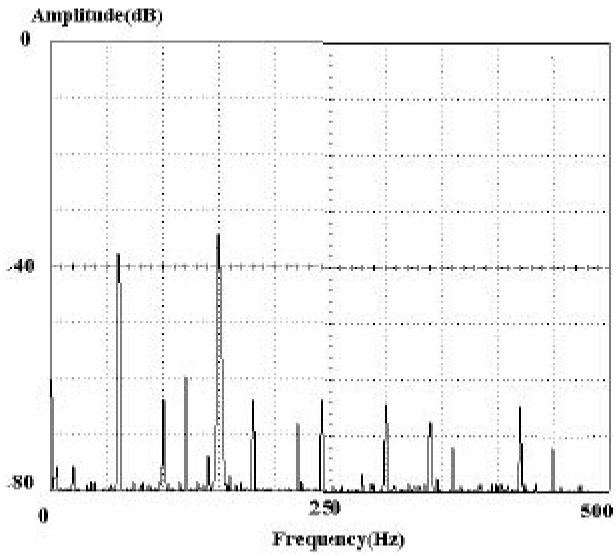

Fig. 11(b): Power spectra of the output voltage with endothelial cell. The electrode was vibrated at 100 Hz. The input voltage was oscillated at 150 Hz. The spectra at 60 Hz, 100 Hz and 150 Hz correspond to noise from the electric source, *A Vdx* and input signal, respectively.

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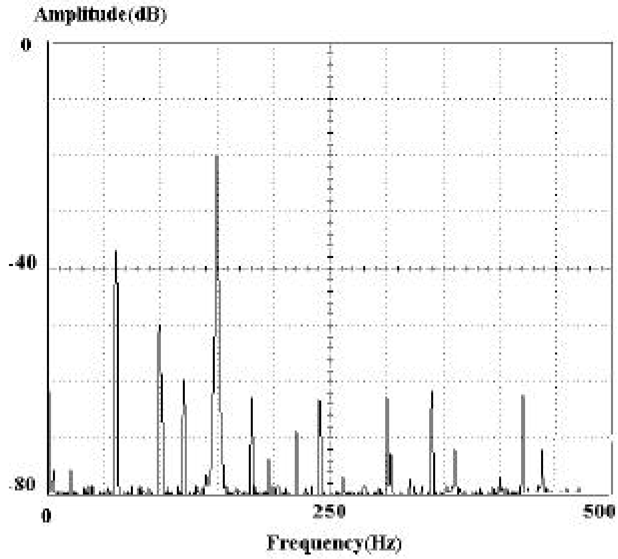

Fig. 11(c): Power spectra of the output voltage with the medium. The electrode was vibrated at 100 Hz. The input voltage was oscillated at 150 Hz. The spectra at 60 Hz, 100 Hz and 150 Hz correspond to noise from the electric source, *A Vdx* and input signal, respectively.

Table 1: The local impedance

Vibrated in	D (dB)	Z_{dx} (kilo-ohm)
Endothelial cell	64.0	0.095
Medium	50.0	0.47

4. DISCSSION AND CONCLUSION

Our measurement system can present some advantage of measuring the impedance. It is not necessary to stick more than two electrodes into the cell to measure local impedance, which might destroy the membrane of the cell. The variation of impedance with vibration is independent of impedance of the other part of the electric circuit in the measurement system, which might add artifacts to the signals.

In the present experiment, the calculation formulae did not include both capacitance and inductance, because only minor phase shift was measured between electric current and voltage.

Different from rigid glass plate, the elasticity of silicone rubber base was useful to avoid destruction of the tip of the electrode while the electrode was stuck into a cell [7].

The present study shows that the proposed system with vibrating electrode makes it possible to measure local electric impedance of gel.

5. ACKNOWLEDGMENT

This work was supported in part by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

6. REFERENCES

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