

Design of Surface Electrode for Measurement of Electric Impedance of Arrangement of Cells Oriented on Micro Striped Pattern

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ABSTRACT

The surface electrode has been designed for measurement of the electric impedance of biological cells oriented on the micro striped pattern *in vitro*. Several parallel lines of micro rectangular grooves (1 μm depth, 3 μm width, and 3 μm interval) have been made between a pair of the surface titanium electrodes at the surface of the scaffold of glass by the photolithography technique. Two types of arrangements of lines are designed at the micro ridges: parallel, and perpendicular to the electric line of force. Variation has also been made on the shape of surface electrodes (thickness; 0.2 μm): I-shape and T-shape. Two kinds of cells were alternatively cultured on the micro-patterned scaffold: C2C12 (mouse myoblast cell line), and 3T3-L1 (mouse fat precursor cells). The electric impedance between electrodes was measured for 24 hours with the sinusoidal electric waves (frequency, 1 kHz < f < 1 MHz; amplitude, $V = \pm 0.05$ V). The experiment shows that the impedance is higher at the arrangement of myoblasts oriented in perpendicular position than that in parallel position to the electric line of force, and that the impedance increases with the number of adhered cells.

Keywords: Biomedical Engineering, Electric Impedance, Micro-machining, Surface Electrode, C2C12, 3T3-L1 and Orientation.

1. INTRODUCTION

In previous studies the orientation of cells in the tissue was tried to be controlled by several methods. The orientation of cells in the tissue also was tried to be measured by several methods. The electric impedance is one of the method to measure the orientation of cells in the tissue [1-13]. The electric impedance of the biological tissue was measured in the previous study to distinguish the kinds of tissue [11]. The biological membrane has capacitance, and biological tissue has frequency characteristic. The electric impedance depends on the orientation of cells.

The surface morphology might govern the orientation of cells. Several kinds of the surface micro morphology were applied to the scaffold in the previous studies [14]. The photolithography technique enables making the micro morphology on the surface

of the scaffold for the cell culture *in vitro*. The micro stripe pattern on the scaffold plate is one of the effective methods to make orientation of cells [14]. In the present study, the surface electrode has been designed for measurement of the electric impedance of the group of biological cells oriented on the micro striped pattern *in vitro*.

2. METHODS

Scaffold

A scaffold of solid surface, which has both micro patterns and surface electrodes, has been designed to measure electric signals of oriented cells. Several parallel lines of micro grooves have been made in the square area of 1 mm \times 1 mm at the surface of the scaffold. The depth (H), the width (W), and the interval (I) of the rectangular grooves are 1 μm , 3 μm , and 3 μm , respectively. Two types of arrangements of lines are designed at the micro ridges: parallel, and perpendicular to the electric line of force (Fig. 1).

Three types of electrodes has been designed: T-shape, I-shape, and asymmetric shape (Fig. 2). In T-shape, the width (B_1) of the electrode is 0.1 mm, the length (L_1) of each electrode is 1 mm, and the distance between electrodes (d_1) is 1.2 mm (Fig. 2a). In I-shape, the width (B_2) of the electrode is 0.1 mm, the length of (L_2) each electrode is 0.1 mm, and the distance between electrodes (d_2) is 1.4 mm (Fig. 2a).

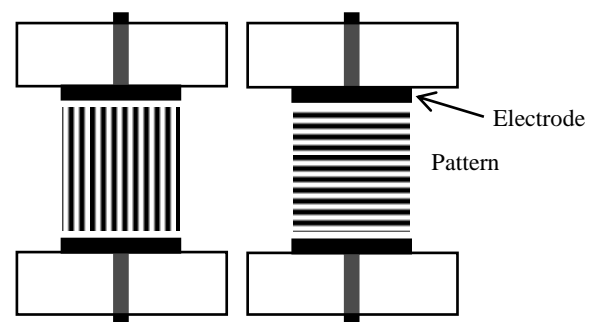


Fig. 1: Micro pattern between electrodes: parallel (left), perpendicular (right).

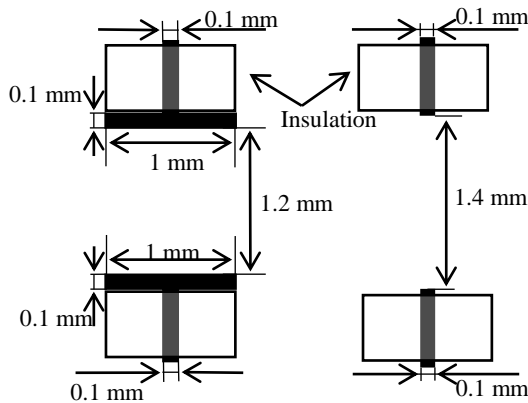


Fig. 2a: Surface electrode of T-shape (left) and I-shape (right).

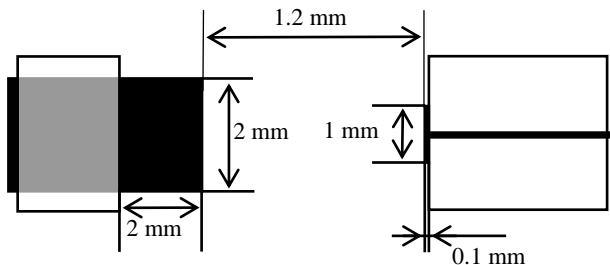


Fig. 2b: Dimension of asymmetric surface electrodes.

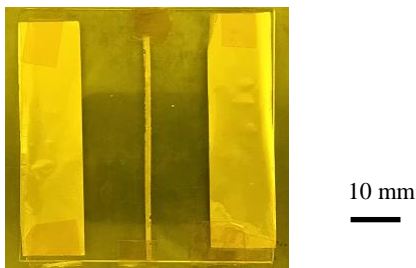


Fig. 3: Photomask for SU-8: dimension 50 mm (left to right).

In the asymmetric shape, the distance between electrodes (d_3) is 1.2 mm. At the left electrode, the width (B_3) of the electrode is 2 mm, and the length of (L_3) the electrode is 2 mm. At the right electrode, the width (B_4) of the electrode is 0.1 mm, and the length of (L_4) the electrode is 1 mm (Fig. 2b).

The borosilicate glass (Tempax) disk was used for the base of the scaffold through micromachining process. The diameter and the thickness of the disk are 50 mm and 1.1 mm, respectively. To remove micro particles on the surface of the glass, the oxygen (0.1 Pa, 30 cm³/min) plasma ashing was applied to the surface of the glass by a reactive ion etching system (FA-1, Samco Inc., Kyoto, Japan).

Electrode with Micro Pattern

After machining the micro striped grooves [1], Titanium of 0.2 μm thickness was deposited on the surface of the glass in the electron beam vapor deposition apparatus (JBS-Z0501EVC JEOL Ltd., Tokyo, Japan).

After plasma ashing, HMDS (hexamethyldisilazane: Tokyo Chemical Industry Co., Ltd., Tokyo) was coated at 3000 rpm for thirty seconds with a spin coater to improve the affinity between

the glass and the photo-resist material. The positive photoresist material of OFPR-800 (Tokyo Ohka Kogyo Co., Ltd., Tokyo, Japan) was coated at 3000 rpm for thirty seconds with the spin coater. The photoresist was baked at the hotplate at 373 K for ninety seconds.

The photomask of the shape of the electrodes (Fig. 2) was mounted on the surface of OFPR-800, and the photoresist was exposed to the UV light through the mask in the mask aligner (M-1S, Mikasa Co. Ltd., Japan). The photoresist was baked in the oven (DX401, Yamato Scientific Co., Ltd) at 373 K for three minutes. The photoresist was developed with tetra-methyl-ammonium hydroxide (NMD-3, Tokyo Ohka Kogyo Co., Ltd., Kawasaki, Japan) for one minute. The disk was rinsed with the ultrapure water, and dried by the spin-dryer (SF-250, Japan Create Co., Ltd., Tokorozawa, Japan). The titanium was etched with the plasma gas using a reactive ion etching system (RIE-10NR, Samuco Inc., Kyoto, Japan). For etching, the gas of SF₆ (30 cm³/min at 1013 hPa) with Ar (30 cm³/min at 1013 hPa) was applied at 100 W for nine minutes.

To insulate the residual area of the surface electrode, the titanium was covered with the coating of the negative photoresist material (SU-8: Micro Chem Corp., MA, USA). SU-8 was coated with the spin coater (at 3000 rpm for 20 s). The photoresist was baked in the oven at 373 K for three minutes. The coating area was exposed to the UV light through the mask (Fig. 3) in the mask aligner. The photoresist was baked in the oven at 393 K for five minutes. The photoresist was developed with SU-8 Developer (Micro Chem Corp., MA, USA). The surface was rinsed with IPA (2-propanol, Wako Pure Chemical Industries, Ltd.) and the ultrapure water.

PDMS Wall

The wall of culture dish is made of the donut-ring of Polydimethylsiloxane (PDMS) (Fig. 4a). After the borosilicate glass (Tempax) disk (35 mm diameter) was enclosed with a peripheral wall of polyimide tape, PDMS (Sylgard 184 Silicone Elastomer Base, Dow Corning Corp., MI, USA) was poured into the dish with the curing agent (Sylgard 184 Silicone Elastomer Curing Agent, Dow Corning Corp., MI, USA). The volume ratio of PDMS to curing agent is ten to one. After degassing, PDMS was baked at 373 K for one hour in an oven. The baked PDMS was machined by the punch to make the donut-ring.

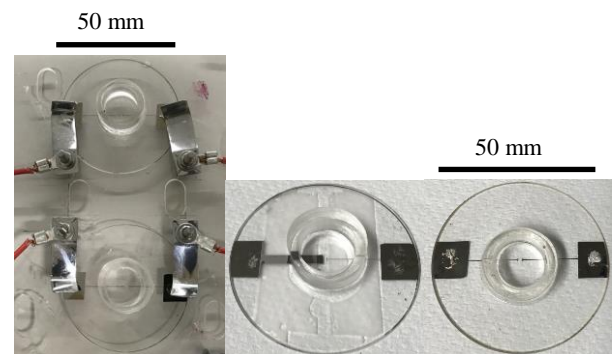


Fig. 4a: A pair of devices fixed on plate to be placed on stage of microscope (left), asymmetry (middle), T-shape (right).

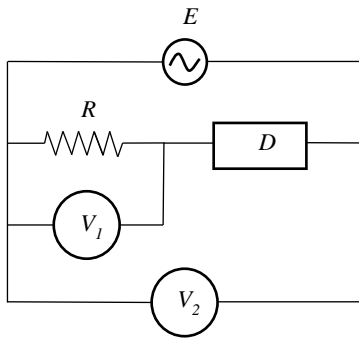


Fig. 4b: Electric circuit.

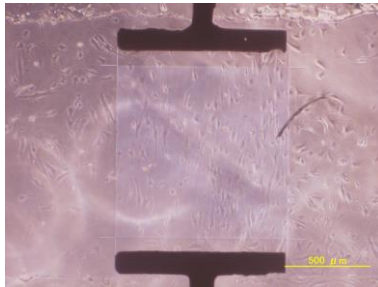


Fig. 5: Cells on micro pattern (parallel) between electrodes.

The donut-ring of PDMS was adhered on the disk with the surface electrodes with extra paste of PDMS. The lead wire was connected to the surface electrode with the crimp style terminals and with the conductive paste (Dotite, Fujikura Kasei Co., Ltd., Tokyo) (Fig. 4a). A pair of devices enables simultaneous tests of two kinds of the setting of the devices.

Cell Culture

Two kinds of cells were used in the experiment: C2C12 (Mouse myoblast cell line originated with cross-striated muscle of C3H mouse; passage between fifth and tenth), and 3T3-L1 (mouse fat precursor cells, a cell line derived from cells of mouse 3T3; passage between third and sixth). D-MEM (Dulbecco's Modified Eagle Medium) containing 10% FBS (Fetal Bovine Serum) and 1% penicillin/ streptomycin was used for the medium. After the oxygen plasma ashing by a reactive ion etching system (FA-1), cells were seeded on the scaffold with the density of 5×10^3 cells/cm² (or 5×10^4 cells/cm²). Cells were cultured in the incubator at 310 K with 5% CO₂. Cells were observed with an inverted phase-contrast microscope (IX71, Olympus Co., Ltd., Tokyo) for 24 hours.

Electric Measurement

The sinusoidal electric waves (frequency, $1 \text{ kHz} < f < 1 \text{ MHz}$; amplitude, $V = \pm 0.05 \text{ V}$) were generated with an electric stimulator (WS8102, Toyo Corporation, Japan; 1910 Function Synthesizer, NF Corporation, Japan). The stimulator (*E*) was connected to the electrodes, and the sinusoidal electric waves were introduced to the medium (*D*) of cells. An electric resistance (*R*) of 10 kΩ is serially inserted between the electrode and the stimulator (Fig. 4b). The electric voltages (*V*₁, *V*₂) were monitored by an oscilloscope during measurement of impedance of the medium. *V*₁ and *V*₂ are the voltages between the terminals of the resistance, and of the electric stimulator, respectively. The impedance of the specimen (*Z*) is calculated by the voltage (*V*) between electrodes divided by

electric current (*I*) (Eq. 1). The voltage is calculated by the difference between *V*₂ and *V*₁. The electric current (*I*) is calculated *V*₁ divided by *R*.

$$Z = V / I \quad (1)$$

3. RESULTS

Fig 5 exemplifies cells on the micro pattern (parallel) between electrodes. Most of cells make orientation along the longitudinal direction of the micro striped pattern. Figs. 6-11 show the relationship between the impedance between electrodes and the frequency of the sinusoidal electric voltage load. The impedance is the lowest at 0.1 MHz.

Fig. 6a shows the impedance of medium without cells between three kinds of electrodes related to frequency: long dashed line (I-shape), dotted line (T-shape), solid line (asymmetry). The impedance is the highest between the electrodes of I-shape, and the lowest between the electrodes of asymmetric shape. Fig. 6b shows the impedance between three kinds of electrodes immediately after seeding of cells (C2C12). Fig. 6c shows the impedance between three kinds of electrodes at 24 hours after seeding of cells (C2C12). The impedance tends to increase and the difference between three kinds of electrodes tends to decrease. Fig. 7 shows the impedance of medium with C2C12 on two kinds of micro pattern between T-shape electrodes. The impedance with the perpendicular micro stripe pattern is higher than that with the parallel one.

Fig. 8a shows the impedance between T-shape electrodes immediately after seeding of 3T3-L1 at 5×10^3 cell/cm². Fig. 8b shows the impedance between T-shape electrodes at 24 hours after seeding of 3T3-L1. The impedance with the parallel micro stripe pattern is higher than that with the perpendicular one at 24 hours. Fig. 9a shows the impedance between T-shape electrodes immediately after seeding of 3T3-L1 at 5×10^4 cell/cm². Fig. 9b shows the impedance between T-shape electrodes at 24 hours after seeding of 3T3-L1. The impedance is higher at 5×10^4 cell/cm² than at 5×10^3 cell/cm². The impedance with the parallel micro stripe pattern is higher than that with the perpendicular one.

Fig. 10a shows the impedance between T-shape electrodes immediately after seeding of C2C12 at 5×10^3 cell/cm². Fig. 10b shows the impedance between T-shape electrodes at 24 hours after seeding of C2C12. The impedance with the perpendicular micro stripe pattern tends to be higher than that with the parallel one at 24 hours. Fig. 11a shows the impedance between T-shape electrodes immediately after seeding of C2C12 at 5×10^4 cell/cm². Fig. 11b shows the impedance between T-shape electrodes at 24 hours after seeding of C2C12. The impedance is higher at 5×10^4 cell/cm² than at 5×10^3 cell/cm². The impedance with the perpendicular micro stripe pattern is higher than that with the parallel one.

4. DISCUSSION

The impedance increases with the distance between electrodes. The impedance also increases with decrease of the cross sectional area of electric flow path between electrodes. The results of the present experiments of three kinds of electrodes are consistent to the law of physics.

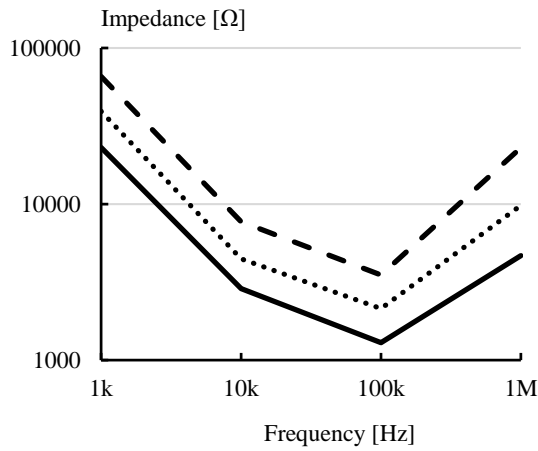


Fig. 6a: Impedance of medium between three kinds of electrodes without cells related to frequency: long dashed line (I-shape), dotted line (T-shape), solid line (asymmetry).

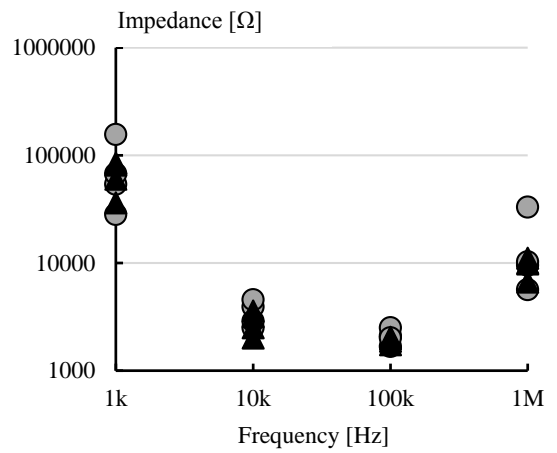


Fig. 7: Impedance of medium with C2C12 on two kinds of micro pattern at 24 h between T-shape electrodes related to frequency: circle (perpendicular), triangle (parallel).

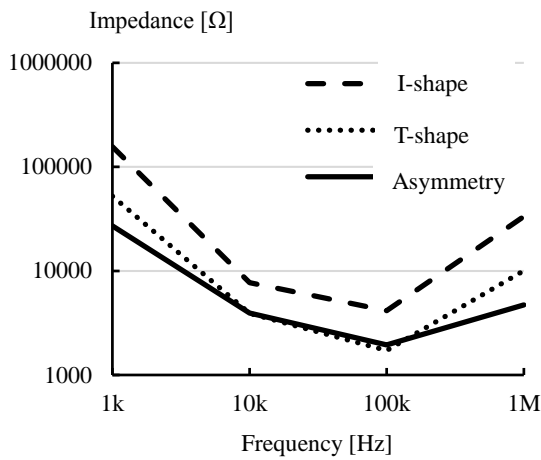


Fig. 6b: Impedance of medium with C2C12 at 0 h between three kinds of electrodes related to frequency: long dashed line (I-shape), dotted line (T-shape), solid line (asymmetry).

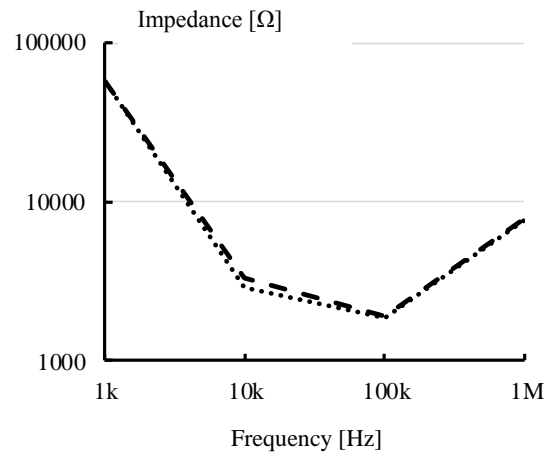


Fig. 8a: Impedance of 3T3-L1 (5×10^3 cell/cm²) at 0 h on two kinds of micro pattern between T-shape electrodes related to frequency: long dashed line (parallel), dotted line (perpendicular) in Figs. 8-11.

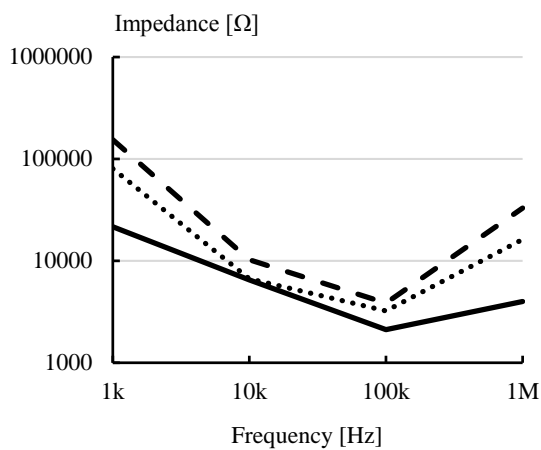


Fig. 6c: Impedance of medium with C2C12 at 24 h between three kinds of electrodes related to frequency: long dashed line (I-shape), dotted line (T-shape), solid line (asymmetry).

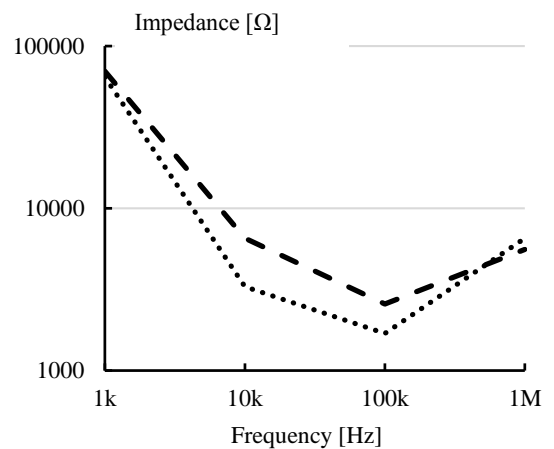


Fig. 8b: Impedance of 3T3-L1 (5×10^3 cell/cm²) at 24 h on two kinds of micro pattern between T-shape electrodes related to frequency.

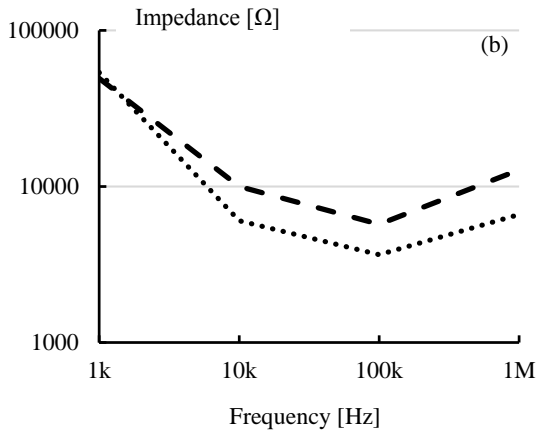
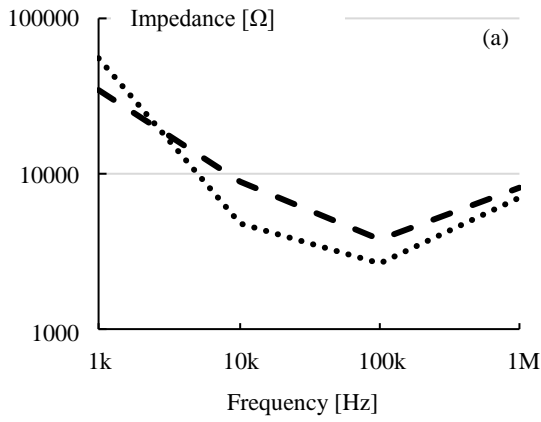


Fig. 9: Impedance of 3T3-L1 (5×10^4 cell/cm²) at 0 h (upper) 24 h (lower) on two kinds of micro pattern between T-shape electrodes related to frequency.

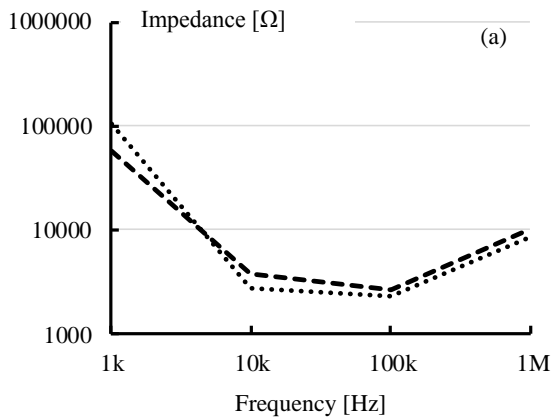


Fig. 10a: Impedance of C2C12 (5×10^3 cell/cm²) at 0 h on two kinds of micro pattern between T-shape electrodes related to frequency.

In the previous study, impedance was measured at the frequency between 1 Hz and 1 MHz. The range of the frequency between 10 kHz and 100 kHz is selected for comparison of impedance of the colony of cells in the present study, because impedance depended on the orientation of cells in the range of the frequency in the previous study [1].

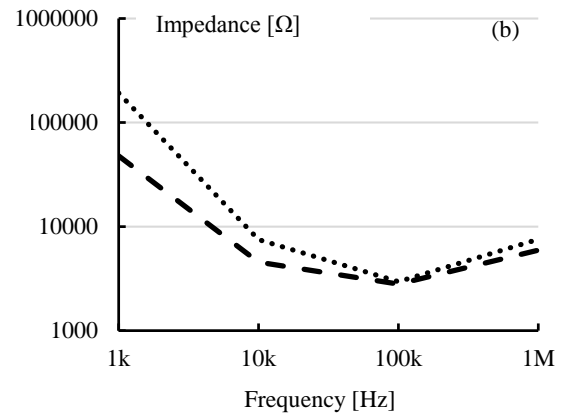


Fig. 10b: Impedance of C2C12 (5×10^3 cell/cm²) at 24 h between T-shape electrodes related to frequency:

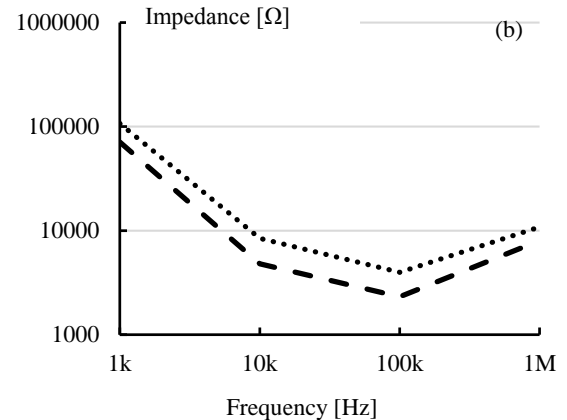
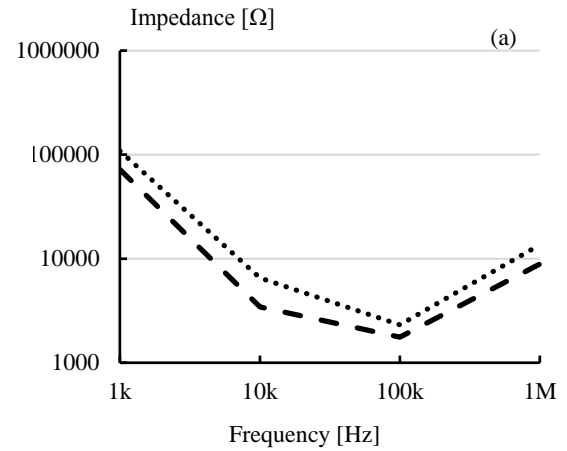


Fig. 11: Impedance of C2C12 (5×10^4 cell/cm²) at 0 h (upper) 24 h (lower) on two kinds of micro pattern between T-shape electrodes related to frequency.

The number of cells of C2C12 was not the same as that of 3T3-L1 in the present study. As the number of cells adhered on the scaffold between the electrodes increases, the impedance between the electrodes might increase. The mean size of 3T3-L1 is smaller than that of C2C12, which might affect the value of impedance. C2C12 is differentiated into myotubes to make fusion between cells. The fusion might decrease the impedance between electrodes, especially at the parallel

orientation of cells. The sinusoidal electric impedance signal between 10 kHz and 0.1 MHz is sensitive to the condition of the colony of cells, which might be related to the structure of each cell. To control the orientation of cultured cells *in vitro*, the photolithography technique is effective to make optimal size of micro morphology on the surface of the scaffold [1, 14]. In the present study, the micro patterned scaffold has been successfully manufactured between the surface titanium electrodes on the glass by the photolithography technique. Myoblasts have been able to be cultured on the micro patterned scaffold. Orientation of myoblasts along the micro grooves has been able to be observed by an inverted phase-contrast microscope. To detect the local electric impedance, several preparations of electrodes were designed in the previous studies [2, 11, 15]. Those electrodes can be used for the electric stimulations on the biological cells [2, 10]. The surface electrode of the present study can also be used for the electric stimulation on the cultured cells. The response of biological cells depends on the direction of electric signal. The electric stimulation causes magnetic stimulation, simultaneously. The impedance includes capacitance component, which related to the membranes of the cell [1].

5. CONCLUSION

The surface electrode has been designed for measurement of the electric impedance of the group of biological cells oriented on the micro striped pattern *in vitro*. Several parallel lines of micro rectangular grooves (0.001 mm depth, 0.003 mm width, and 0.003 mm interval) have been made between a pair of the surface titanium electrodes (I-shape and T-shape) at the surface of the scaffold of glass by the photolithography technique. Two kinds of cells were alternatively cultured on the micro-patterned scaffold: C2C12 (mouse myoblast cell line), and 3T3-L1 (mouse fat precursor cells). The electric impedance between electrodes was measured for 24 hours with the sinusoidal electric waves (frequency, $1 \text{ kHz} < f < 1 \text{ MHz}$; amplitude, $V = \pm 0.05 \text{ V}$). The experiment shows that the impedance is higher at the arrangement of myoblasts oriented in perpendicular position than that in parallel position to the electric line of force, and that the impedance increases as the number of adhered cells. The designed surface electrodes can detect the arrangement of the orientation of cells.

6. ACKNOWLEDGMENT

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