Cell Behavior around Surface-Electrode with Electric Pulses

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ABSTRACT

Behavior of biological cells around the surface-electrode with electric pulse has been observed *in vitro*. A thin film (thickness of 300 nm) of titanium coated on the glass was used for the surface-electrode. Variation has been made on the tip of the pairs of surface electrodes: rectangular and triangular. The distance between electrodes is 0.1 mm, or 0.3 mm. Two kinds of cells were seeded around the surface electrodes: C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse), or Hepa1-6 (mouse hepatoma cell line of C57L mouse). After 2 minutes from seeding, electric pulses (period 0.00001 s, amplitude 10 V (0.0005 A), duration 0.000005 s) were applied to the film. The microscopic observation on the experimental system shows movement of cells between electrodes depends on the shape of tips.

Keywords: Biomedical Engineering, Dielectrophoresis, Titanium, Surface Electrode, C2C12, Hepa1-6, and Electric Pulse.

1. INTRODUCTION

Several methods have been applied to control the movement of biological cell suspended in the medium *in vitro*: micro slit [1, 2], flow [3, 4], gravitational field [5], electric field [6], magnetic field [7], Van der Waals force, affinity of surface, and pressure.

These methods might contribute to several applications of manipulation of cells: arrangement of cells to make a tissue [8], sorting of cells, and measurement of cells.

Movement of a charged particle depends on the electric field. The effect is applied to the electrophoresis device.

When a particle is subjected to a non-uniform electric field, a force is exerted even on a non-charged particle, because the polarization generates in the particle. The phenomenon is called dielectrophoresis, which depends on the several parameters: the electrical property of the particle, shape and size of the particle, the electrical property of the medium, and frequency of the electric field [9].

The behavior of the cell depends on the micro morphology of the surface of the scaffold [10, 11]. The electric pulses affect to the behavior of the cell [12, 13]. The cell can be cultured on the surface of the electrode [14, 15].

In the present study, behavior of biological cells around the surface-electrode with electric pulse has been observed *in vitro*.

2. METHODS

Surface Electrode

The titanium film was used for the surface electrode. Titanium was coated on the borosilicate glass (Tempax) disk (50 mm diameter, 1mm thickness). The thickness of coating is 300 nm. The width of the coating is 4 mm. The pattern of the coating is controlled by the film type of mask.

To make variation on non-uniformity of the electric field, variation has been made on the tip of the cathode of surface electrodes: rectangle and triangle. The distance between electrodes is 0.3 mm (Fig. 1A&B), or 0.1 mm (Fig. 1C).

Photomask for Electrode

The pattern of the coating is controlled by the film type of mask (Fig. 2). A carbon disk (0.5 mm of thickness) was used for the mask for the deposition of titanium surface electrode. Three kinds of hole was machined at the mask by the ultrashort pulse laser (IFRIT, Cyber Laser Inc., Tokyo, Japan): rectangles of 5 mm × 20 mm (A); a rectangle and a pentagon (B); a rectangle and a closer thin pentagon (C). The distances between surface electrodes are 0.3 mm at A and B, and 0.1 mm at C.

Deposition of Titanium

Before the deposition of titanium, the surface of the glass was hydrophilized by the oxygen (>0.1 Pa) plasma ashing for ten minutes at 100 W by RIE (FA-1, Samco International, Kyoto, Japan). Titanium was deposited on the surface of the borosilicate glass (Tempax) disk (50 mm diameter, 1.1 mm thickness) in the electron beam vapor deposition apparatus (JBS-Z0501EVC JEOL Ltd., Tokyo, Japan). The thickness of deposition of titanium film is 300 nm.

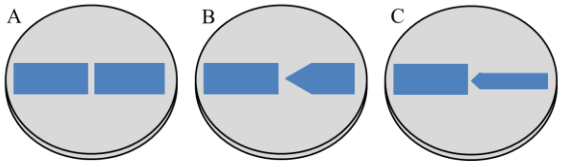

Fig. 1: Three kinds of surface electrode on glass.

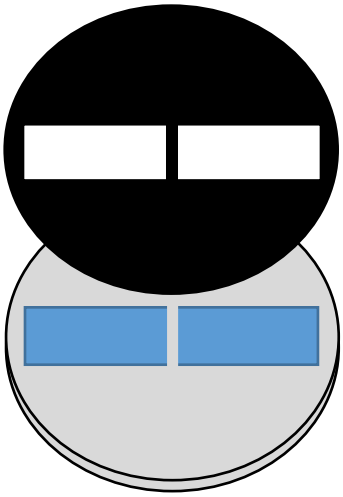

Fig. 2: Photomask (upper) for surface electrode (lower).

Photomask for Cover of PDMS

The borosilicate glass (Tempax) disk was used for the base of the photomask. After hydrophilization by the oxygen plasma ashing for ten minutes at 100 W by RIE, the negative photoresist material of high viscosity (SU-8 10: Micro Chem Corp., MA, USA) was coated on the disk with the spin coater. The photoresist was baked in the oven. The pattern was drawn on the mask (in the area of 1 mm × 1 mm) with a laser drawing system (DDB-201K-KH, Neoark Corporation, Hachioji, Japan): the diameter of the circle is 0.03 mm, and the distance between centers of the circle is 0.1 mm. The photoresist was baked in the oven at 368 K for three minutes. The photoresist was developed with Remover PG (MicroChem Corp., MA, USA) for twenty seconds, and rinsed with the ultrapure water.

Surface Electrode Covered with Micro-pattern of SU-8 10

The surface electrode of titanium covered with SU-8 10 film with micro holes (0.03 mm diameter) was made by the following process (Fig. 3).

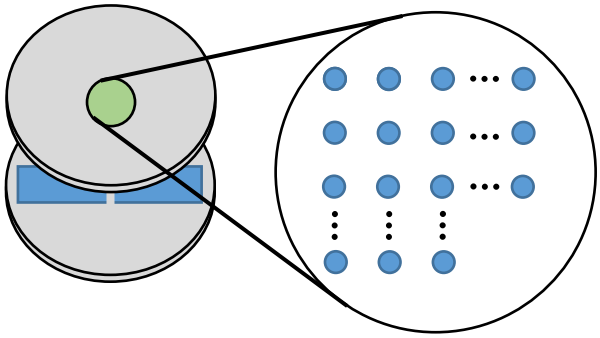

Fig. 3: Surface electrode covered with SU-8 with micro-holes.

After cleaning and hydrophilization, the negative photoresist material (SU-8 10) was coated over the titanium surface electrodes on the glass at 2000 rpm for one minute with the spin coater. The photoresist was baked in the oven at 368 K for seven minutes. The photomask was mounted on the surface of SU-8 10, and the photoresist was exposed to the UV light through the mask in the mask aligner (M-1S, Mikasa Co. Ltd., Japan) at 10 V for 30 s. The photoresist was baked in the oven at 368 K for three minutes. The photoresist was developed with Remover PG for twenty seconds. The glass surface with the micro pattern was rinsed with acetone, ethanol, and ultrapure water, and dried by the spin-dryer.

PDMS Ring

The wall of culture dish was made of the donut-ring of Polydimethylsiloxane (PDMS) (Fig. 4). PDMS (Sylgard 184 Silicone Elastomer Base, Dow Corning Corp., MI, USA) was mixed 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 poured on the glass disk. After degassing again, PDMS was baked at 373 K for one hour in an oven (DX401, Yamato Scientific Co., Ltd). The baked PDMS was machined by the punch to make the donut-ring. The outer diameter, the inner diameter and the thickness of the ring are 32 mm, 10 mm and 5 mm, respectively.

The donut-ring of PDMS was adhered on the disk with the surface electrodes with extra past of PDMS. The lead wire was connected to the surface electrode with crimp style terminals.

Electric Pulses

The electric pulses (period 0.00001 s, amplitude 10 V, duration 0.000005 s) were generated with an electric stimulator (1910 FUNCTION SYNTHESIZER, NF Corporation, Japan). The stimulator was connected to the titanium film, and the pulses were introduced to the medium of cells. An electric resistance of 1000 ohm is serially inserted between the electrode and the stimulator. The electric signal (V) is monitored by an oscilloscope during electric pulse application between the titanium surface electrodes. V is the voltage between the terminals of the resistance.

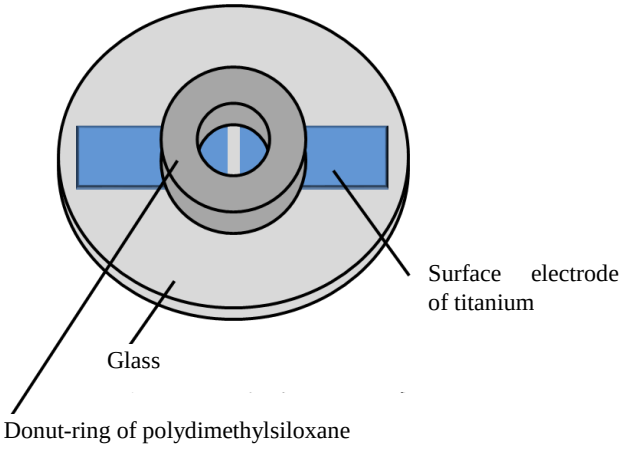

Fig. 4: PDMS rings are attached on surface electrodes.

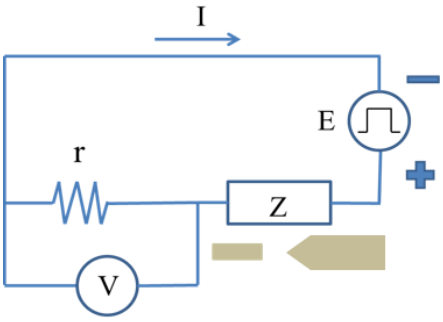

Fig. 5: Electric circuit during introduction of electric pulses (*E*) to scaffold (*Z*) of cells.

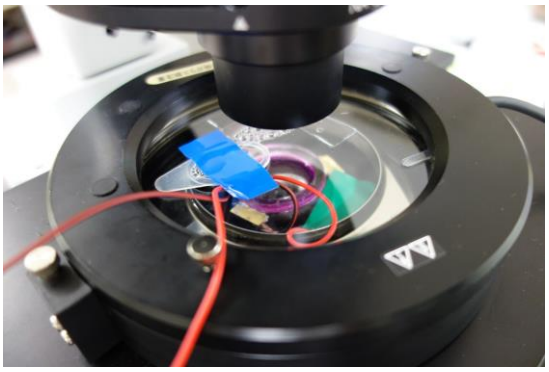

Fig. 5: Observation of cells between electrodes with an inverted phase-contrast microscope.

A Crimp terminal was used to make connection between the surface electrode and the lead wire.

Cell Culture

Two kinds of cells were used in the test: C2C12 (Mouse myoblast cell line originated with cross-striated muscle of C3H mouse), and Hepa1-6 (mouse hepatoma cell line of C57L mouse). D-MEM (Dulbecco's Modified Eagle Medium) containing 10% FBS (Fetal Bovine Serum) and 1% penicillin/ streptomycin was used for the medium. The cells were suspended in the medium and poured on the surface electrode. The cells were rest for two minutes, before stimulation by electric pulses.

Behavior of cells was observed with an inverted phase-contrast microscope (IX71, Olympus Co., Ltd., Tokyo).

3. RESULTS

Figs. 6-9 exemplify cells during the experiment. The movement of cells is able to be microscopically observed through the transparent scaffold between the surface electrodes. The cells are not able to be observed by the optical microscope on the other hand, through the surface electrode of titanium: the black part in Figs. 6-9.

A micro bubble has been generated by electrolysis adjacent to the electrode. While the electric pulses applied to the electrode, the bubble vibrates synchronous with the electric pulse.

Most of cells adhere to the scaffold of the plate in two minutes. Between the electrodes with 0.3 mm distance, a few cells moved to electrode (anode). Any movement of cells was observed between electrodes with 0.1 mm distance.

The electrical measurement at the electric circuit shows that the conductance of the film is 0.002 S. Cells show vibration synchronous to the electric pulse, which is applied between the insulated electrodes with SU-8 10 coating. The focus on the cell adhered on the surface is shifted, which shows exfoliation of the cell.

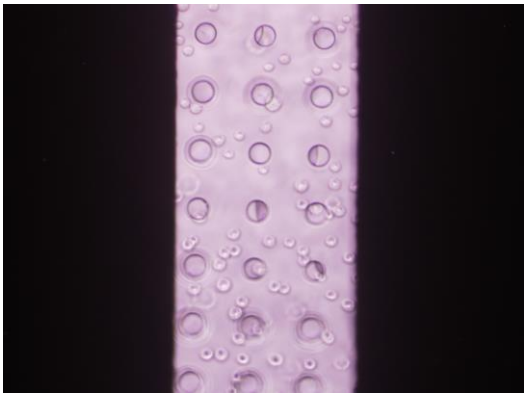

Fig. 6a: C2C12 between electrodes A immediately after electric stimulation.

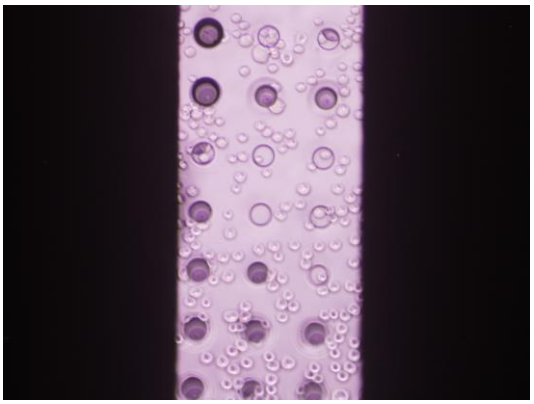

Fig. 6b: C2C12 between electrodes A at 210 s.

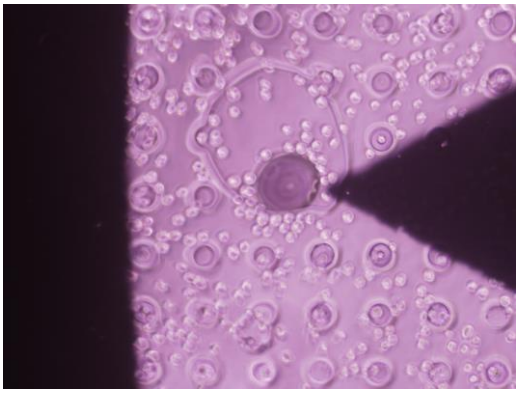

Fig. 7a: C2C12 between electrodes B immediately after stimulation.

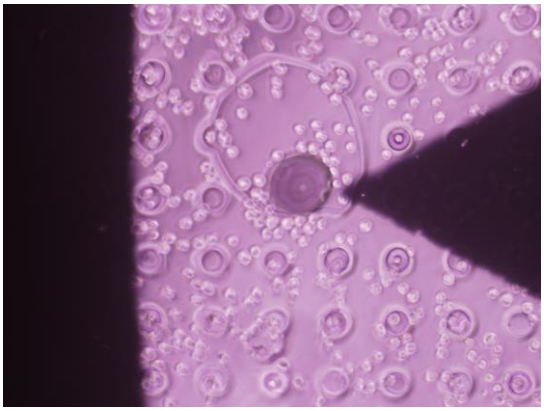

Fig. 7b: C2C12 between electrodes B at 210 s.

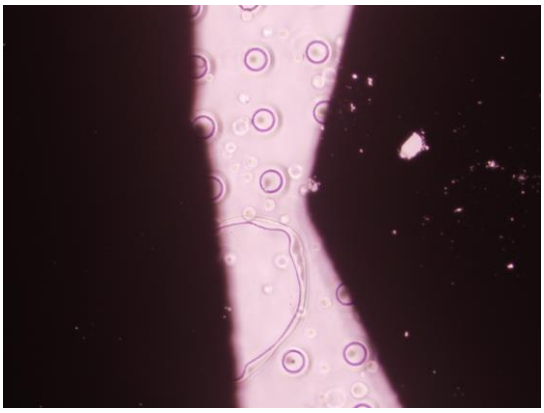

Fig. 8a: C2C12 between electrodes C, immediately after stimulation.

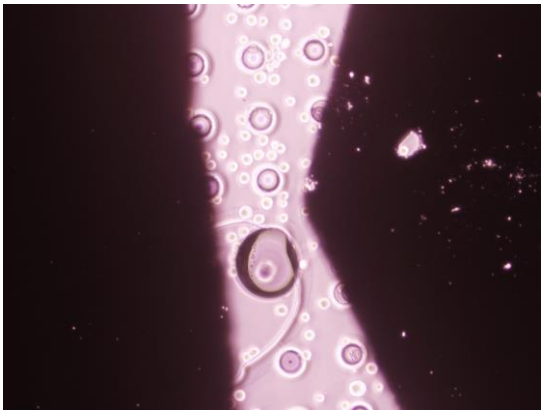

Fig. 8b: C2C12 between electrodes C at 210 s.

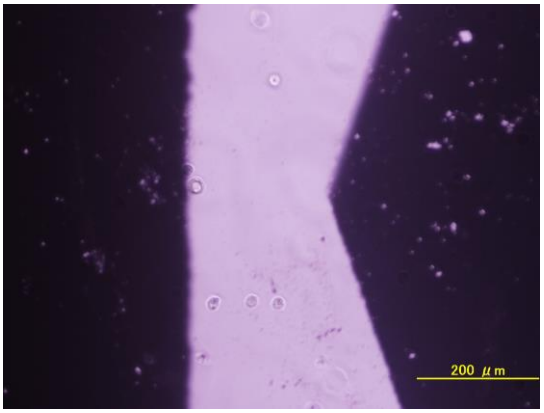

Fig. 9a: Hepa1-6 between electrodes C immediately after electric stimulation.

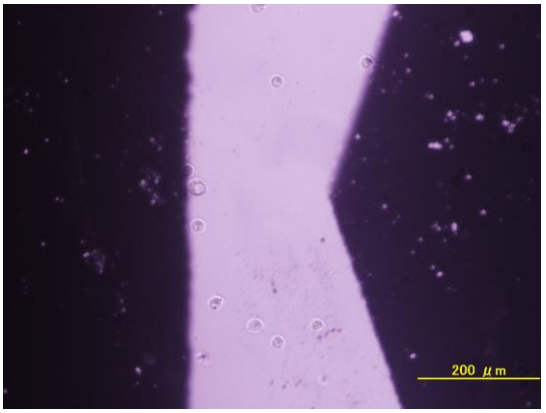

Fig. 9b: Hepa1-6 between electrodes C at 270 s

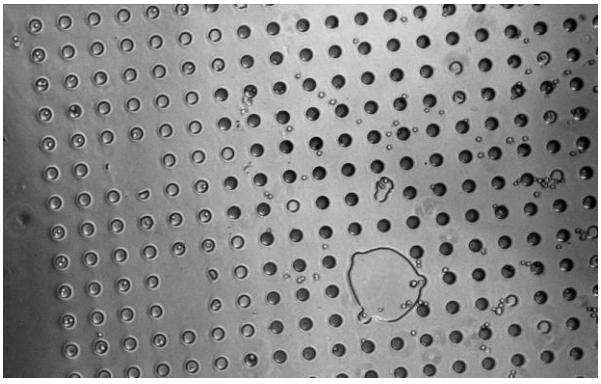

Fig. 10: Hepa1-6, 10 min

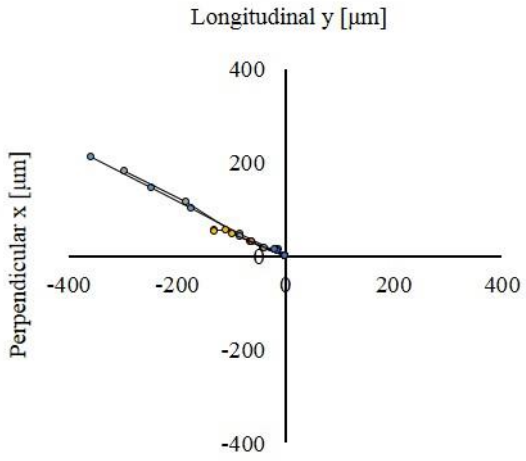

Fig. 11a: ITO, Hepa1-6 without stimulation.

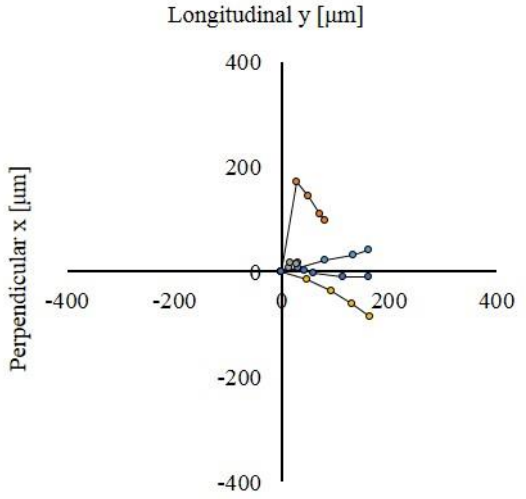

Fig. 11b: ITO, Hepa1-6 with stimulation.

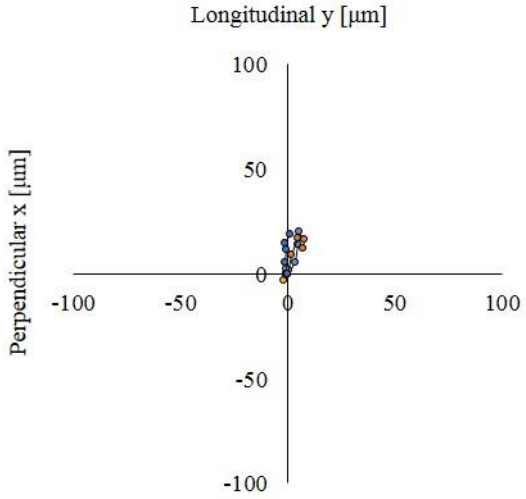

Fig. 11c: ITO, C2C12 without stimulation.

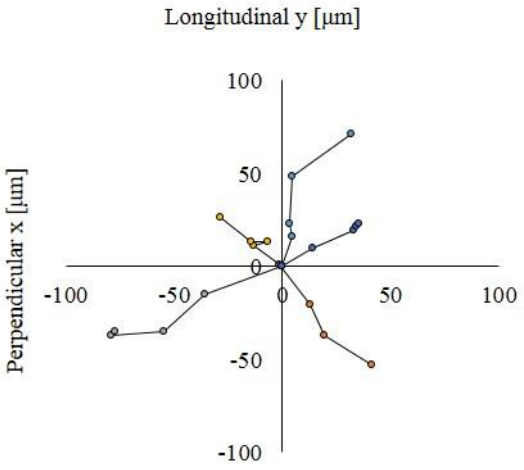

Fig. 11d: ITO, electrode, C2C12 with stimulation.

Fig. 11 shows the movement in the x-y plane of five cells traced every minute at the time-lapse image. The origin corresponds to the starting point of each cell. The cells move together to the same direction as flow of the medium without electric pulses (Fig. 11a & c). The cells, on the other hand, change the direction to move, during application of electric pulses (Fig. 11 b & d).

4. DISCUSSION

To maximize the effect of dielectrophoresis, the values (10 V, and 100 kHz (period of 0.00001 s)) are selected for the amplitude and frequency of the rectangular wave as the maximum value at the instrument (the electric stimulator) in the present study [9].

When the film electrode of titanium is covered with SU-8, the cells show the vibrating movement in the medium. The cells, on the other hand, do not show the vibrating movement in the medium near the film of electrode without SU-8. The electric voltage signal is modified from the rectangular wave, when the film of electrode is dipped in the medium without the cover of SU-8.

Although the contact area between medium and electrode was limited by the film of SU-8 with micro holes, the film is effective to protect the titanium film on the glass from exfoliation during electric stimulation. The behavior of cells is able to be observed through transparent film. The holes play the role of marker, which is convenient to trace the movement of cells.

The electric resistance (R) of the titanium is calculated by Eq. 1.

$$R = (\rho\, l) / A \qquad (1)$$

In Eq. 1, A is cross sectional area [m^2], ρ is resistivity [Ω m], and l is length [m].

$l = 0.04$ [m]
$A = 0.004 \times (300 \times 10^{-9})$ [m^2]
$\rho = 55 \times 10^{-8}$ [Ω・m]

The electric resistance is calculated as 18 Ω.

The electric pulses applied to the medium are confirmed by the synchronized vibration of cells and bubbles in the present study. Two minutes is enough for cells to adhere on the scaffold of plate.

Titanium is one of the materials which has been implanted *in vivo*. In the previous study, myoblast was cultured on the micro coil spring of titanium [13].

In the previous study, dielectrophoresis has been tried to apply on the biological cell manipulation technology [9]. The micro grooves were used for trapping of flowing cells in the previous study [11]. The movement of cells also depends on the flow of the medium. Cells do not fall into the micro holes at film during the observation in the present study.

The movement of cells between surface electrodes depends on the morphology of surface electrodes (the angle of the tip), which relates to non-uniformity of electric field. Dielectrophoresis of cell depends on asymmetric figure of electrodes. The higher slope of electric field with non-uniformity might be necessary to move cells around the electrode.

The effect of the surface of the scaffold on cell culture was studied in the previous studies [10]. Several micro-fabrication processes have been designed to control adhesion of biological cells *in vitro* [14].

5. CONCLUSION

Behavior of biological cells around the surface-electrode with electric pulse has been observed *in vitro*. A thin film (thickness of 300 nm) of titanium coated on the glass was used for the surface-electrode. Variation has been made on the tip of the pairs of surface electrodes: rectangular and triangular. The distance between electrodes is 0.1 mm, or 0.3 mm. C2C12 moves to the anode at the potential difference of 10 V between asymmetric electrodes with short distance.

6. ACKNOWLEDGMENT

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