Myoblast Behavior around Surface Electrodes in Flow Channel

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ABSTRACT

The behavior of biological cells around the surface electrode in the flow channel has been observed *in vitro*. The surface electrodes of titanium (thickness of 200 nm) are made along the edges of the flow channel by the photolithography technique. At the one of the edge, variations are made at the angle of the tip of each surface electrode: 0.26, 0.52, 0.79, or 1.0 rad at triangle or rectangle. The other edge is the reference electrode. One of three modes of cyclic electric stimulations (0.01 ms period) was applied between the surface electrodes: sinusoidal, pulse, or rectangular. Two kinds of cells were used in the test: porcine red blood cells, and C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse). The suspension of cells was introduced to the flow channel, and the flow rate was controlled by the syringe pump. The experiment shows the following results. The movement of myoblasts changes by the electric stimulation through the surface electrodes during the flow along the channel. The movement depends on the tip angle of the electrode.

Keywords: Biomedical Engineering, Dielectrophoresis, Titanium, Surface Electrode and C2C12.

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 [6-9], magnetic field [10, 11], 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 [12-14], sorting of cells [1, 15, 16], and measurement of cells.

Movement of a charged particle depends on the electric field. The effect is applied to the electrophoresis device [17]. 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 [18-25]. Electrophoresis is a phenomenon, in which a particle is moved by the Coulomb force between the charge of the particle and the electric field. Dielectrophoresis, on the other hand, is a phenomenon, in which a particle moves due to the interaction between the charge induced in a neutral particle placed in a non-uniform electric field and the electric field. The force of dielectrophoresis is calculated by the equivalent dipole moment method.

$$F = \pi \varepsilon_m r^3 Re\,[K(\omega)] \quad \nabla E^2 \tag{1}$$

In Eq. 1, r is the radius of the particle, ε_m is the dielectric constant of the surrounding medium, and E is the maximum value of the electric field. $Re\,[K(\omega)]$ represents the real part of the function expressed by the following equation.

$$K(\omega) = (\varepsilon_p^* - \varepsilon_m^*) / (\varepsilon_p^* + 2\,\varepsilon_m^*) \tag{2}$$

In Eq. 2, ε_p^* is the complex dielectric constant of the particle, ε_m^* is the complex dielectric constant of the solution.

In the present study, the behavior of biological cells around the surface electrode in the flow channel has been observed *in vitro*.

2. METHODS

Surface Electrode

The titanium film was used for the surface electrode. Titanium was coated on the glass plate (76 mm × 26 mm × 1 mm, Matsunami Glass Ind., Ltd., Japan). The thickness of coating is 200 nm.

At the one of the edge, five tips of electrodes are placed: four triangles and one rectangle (Fig. 1). These tips are arranged with the interval of 1 mm. To make variation on non-uniformity of the electric field, variations are made at the tip angle of the triangle shape of each surface electrode: 0.26, 0.52, 0.79, or 1.0 rad. The distance between electrodes (d) is 0.3 mm, or 1 mm.

Deposition of Titanium

Before the deposition of titanium, the surface of the glass plate was hydrophilized by the oxygen (30 cm^3/min, 0.1 Pa) plasma ashing for five minutes at 100 W by the reactive ion etching system (FA-1, Samco International, Kyoto, Japan). Titanium was deposited on the surface of the glass plate in the electron beam vapor deposition apparatus (3.1×10^{-4} Pa, 0.5 nm/s, JBS-Z0501EVC JEOL Ltd., Tokyo, Japan).

Photomask A

The titanium coated glass plate was hydrophilized by the oxygen (30 cm^3/min, 0.1 Pa) plasma ashing for five minutes at 100 W by the reactive ion etching system (FA-1). To improve affinity between titanium and photoresist material, HMDS (hexamethyldisilazane: Tokyo Chemical Industry Co., Ltd., Tokyo) was coated on the glass plate at 3000 rpm for 30 s with a spin coater (Fig. 2). The positive photoresist material of OFPR-800LB (Tokyo Ohka Kogyo Co., Ltd, Tokyo, Japan) was coated on the HMDS with the spin coater (at 500 rpm for 5 s, at 5000 rpm for 30 s, and at 7000 rpm for 0.2 s). The photoresist was baked in the oven in two processes: at 338 K for one minute, and at 368 K for three minutes. The pattern of the electrode was drawn on the mask with a laser drawing system (DDB-201K-KH, Neoark Corporation, Hachioji, Japan). To control the dimension of the pattern on the photomask with the laser drawing system, the parameters were selected as follows: the voltage of 3.35 V, the velocity of 0.151 mm/s, and the acceleration of 0.35 mm/s^2. The photoresist was developed with tetra-methyl-ammonium hydroxide (NMD-3, Tokyo Ohka Kogyo Co., Ltd., Kawasaki, Japan) for three minutes, rinsed with the distilled water, and dried by the spin-dryer (SF-250, Japan Create Co., Ltd., Tokorozawa, Japan). The titanium coating plate was etched with the plasma gas by the reactive ion etching system (FA-1). For etching, the gas of CF_4 (30 cm^3/min at 1013 hPa) was applied at 100 W at 0.1 Pa for ten minutes. OFPR-800LB was removed by acetone.

Photomask B

The titanium coated glass plate was hydrophilized by the oxygen (30 cm^3/min, 0.1 Pa) plasma ashing for five minutes at 100 W by the reactive ion etching system (FA-1). To improve affinity between titanium and photoresist material, HMDS (hexamethyldisilazane: Tokyo Chemical Industry Co., Ltd., Tokyo) was coated on the titanium at 3000 rpm for 30 s with a spin coater (Fig. 3). The negative photoresist material of low viscosity (SU-8 2: Micro Chem Corp., MA, USA) was coated on the HMDS with the spin coater (at 500 rpm for 5 s, at 5000 rpm for 30 s, and at 6000 rpm for 0.2 s). The photoresist was baked in the oven at 368 K for five minutes. The "photomask A" was mounted on the surface of SU-8 2, and the photoresist was exposed to the UV light through the mask in the mask aligner (M-1S, Mikasa Co. Ltd., Japan) at 15 mW/cm^2 for 20 s. The photoresist was baked in the oven at 368 K for five minutes. The photoresist was developed with SU-8 Developer (Micro Chem Corp., MA, USA) for five minutes. The glass surface with the micro pattern was rinsed with IPA (2-propanol, Wako Pure Chemical Industries, Ltd.) for one minute, and pure water for one minute. The surface was dried by the spin-dryer: at 300 rpm for 30 s with the pure water, and at 1100 rpm for 30 s with N_2 gas. The titanium coating plate was etched with the plasma gas by the reactive ion etching system (FA-1). For etching, the gas of CF_4 (30 cm^3/min at 1013 hPa) was applied at 100 W at 0.1 Pa for ten minutes.

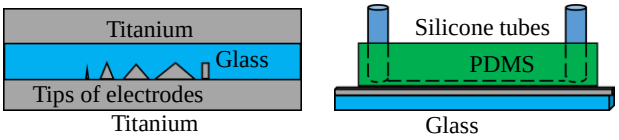


Fig. 1: Plan view: five kinds of tips at surface electrodes on glass (left). Front view: flow channel (right).

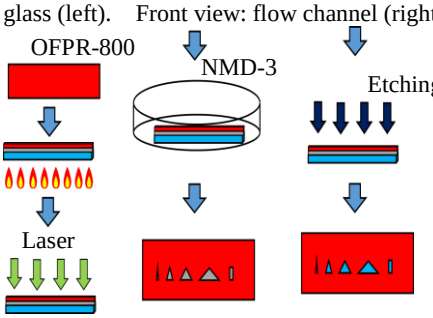


Fig. 2: Photolithography process for photomask A.

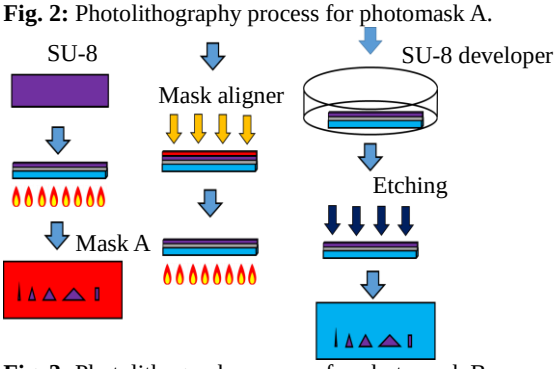


Fig. 3: Photolithography process for photomask B.

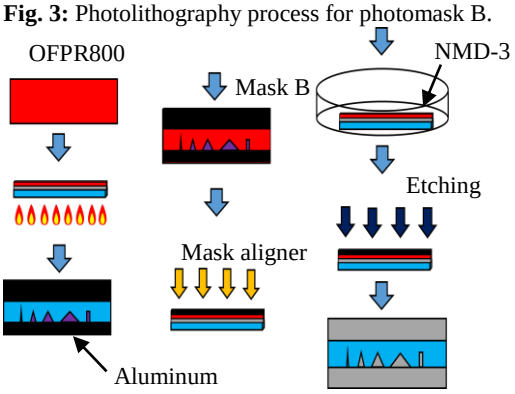


Fig. 4: Photolithography process for surface electrodes.

Surface Electrode

To improve affinity between titanium and photoresist material, HMDS was coated on the titanium at 3000 rpm for 30 s with the spin coater (Fig. 4). OFPR-800LB was coated on the HMDS with the spin coater (at 5000 rpm for 30 s). The photoresist was baked in the oven in two processes: at 338 K for one minute, and at 368 K for three minutes. The surrounding area of the "photomask B" was covered with the film of aluminum to protect from exposure to the UV light. The "photomask B" was mounted on the surface of OFPR-800LB, and the photoresist was exposed to the UV light through the "photomask B" in the mask aligner at 15 mW/cm^2 for 20 s. The photoresist was developed with tetra-methyl -ammonium hydroxide for three minutes, rinsed with the pure water, and dried by the spin-dryer: at 300 rpm for 30 s with the pure water, and at 1100 rpm for 30 s with N_2 gas. The titanium coating plate was etched with the plasma gas by the reactive ion etching system (FA-1). For etching, the gas of CF_4 (30 cm^3/min at 1013 hPa) was applied at 100 W at 0.1 Pa for ten minutes.

Flow Channel

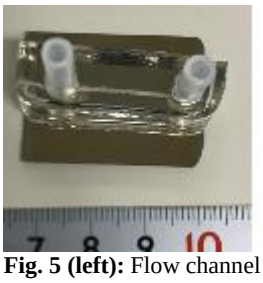
The wall of flow channel was made of Polydimethylsiloxane (PDMS). The polyimide tape (0.055 mm thickness, 1 mm width) was pasted at the center of the glass plate to make groove (0.055 mm depth) for the flow channel. After the slide glass plate was enclosed with a peripheral wall of polyimide tape, PDMS (Sylgard 184 Silicone Elastomer Base, Dow Corning Corp., MI, USA) was poured 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 plate enclosed with a peripheral wall of polyimide tape. After degassing again, PDMS was baked at 333 K for one hour in an oven (DX401, Yamato Scientific Co., Ltd). The baked plate of PDMS (3 mm thickness) was exfoliated from the slide glass plate. Two holes with the interval of 20 mm were punched by a punching tool (trepan MK405, Kai Industries Co., Ltd., Gifu, Japan) to make the inlet and the outlet. The silicone tube of 5 mm outer diameter was inserted in to the hole. The upper plate of PDMS was stuck on the lower plate of the glass with the surface electrodes to make the flow channel. A rectangular parallelepiped channel of 25 mm length × 1 mm width × 0.05 mm height is formed between upper and lower plates. The two plates were stuck together with extra past of PDMS and baked at 333 K for one hour in the oven (Fig. 5).

Electric Stimulation

The electric stimulations (E) were generated with an electric stimulator (WS8102, Toyo Corporation, Japan) or (1910 Function Synthesizer, NF Corporation, Japan) (Fig. 6). The stimulator was connected to the titanium film electrode, and the electric signal was introduced to the medium of cells (Z). An electric resistance (R) of 1 kΩ (or 51 Ω) is serially inserted between the electrode and the stimulator. The electric signal (V) is monitored by an oscilloscope during electric stimulation application between the titanium surface electrodes. V is the voltage between the terminals of the resistance (Fig. 6). One of three modes of cyclic (0.01 ms period) electric stimulations was applied between the surface electrodes: sinusoidal (1 kΩ resistance), pulse (0.0002 ms pulse width, 1 kΩ resistance), and rectangular (±10 V, 51 Ω resistance).

Cell

Two kinds of cells were used in the test: porcine red blood cells, and C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse). The porcine blood was diluted by the saline solution. D-MEM (Dulbecco's Modified Eagle Medium) containing 10% FBS (Fetal Bovine Serum) and 1% penicillin/ streptomycin was used for the medium for C2C12.



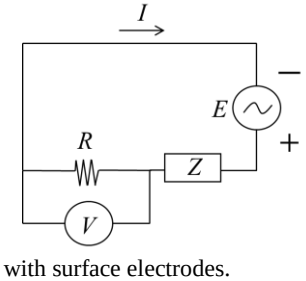


Fig. 5 (left): Flow channel with surface electrodes.
Fig. 6 (right): Electric circuit: electric stimulation (E) applied to suspension of cells (Z): V, voltage between terminals of resistance R: I, current.

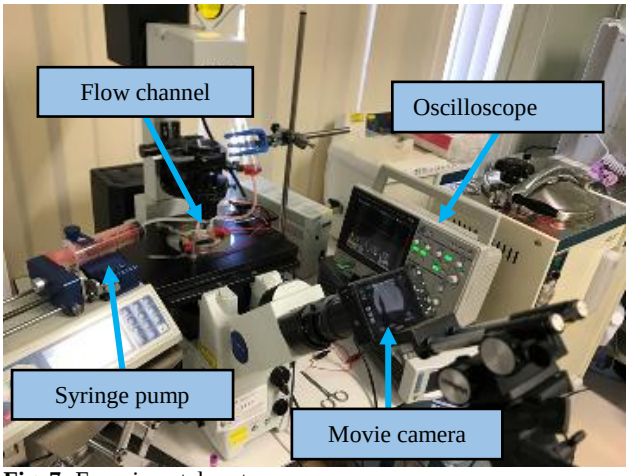


Fig. 7: Experimental system.

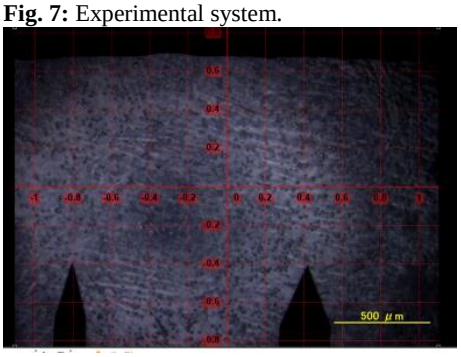


Fig. 8: Coordinates for tracing of cell.

The cells were suspended in the medium and injected into the channel with the surface electrodes. Behavior of cells was observed with an inverted phase-contrast microscope (IX71, Olympus Co., Ltd., Tokyo), and recorded by the video camera (Fig.7), while the flow rate (1 mm^3/hour < Q <10 mm^3/hour) of the suspension was controlled by the syringe pump. The movement of each cell was analyzed by Kinovea at the microscopic image. The dimension was calibrated by the interval between the electrodes in the image (Fig. 8).

3. RESULTS

Fig. 9 exemplifies the red blood cell near the electrode of 0.52 rad. The cell moves with the flow of the media from right to left at the velocity of 0.05 mm/s. During the sinusoidal electric stimulation, the movement of each cell is parallel to the x axis: y coordinate is constant (Fig. 10). Fig. 11 shows the tracings of the voltage between terminals of the resistance of 1 kΩ. The sinusoidal electric current (I) of ±5 mA is calculated by the voltage of ±5 V between terminals of the resistance of 1 kΩ. Fig. 12 exemplifies C2C12 near the electrode of 1 rad. The movement is parallel to the x axis: y coordinate is constant (Fig. 13). The cell moves forward with the flow of the media from right to left at first, then stopped, and moves backward against the flow of the media (see x coordinate of the tracings). Fig. 14 shows the tracings of the voltage between terminals of the resistance of 1 kΩ. The amplitude of the electric pulse current of 0.01 A is calculated by the amplitude of the electric pulse voltage of 10 V between terminals of the resistance of 1 kΩ. Fig. 15 exemplifies C2C12 near the electrode of 0.26 rad. The movement is parallel to the x axis: y coordinate is constant (Fig. 16). The cell moves forward with the flow of the media

from right to left at first, then stopped for several seconds (see *x* coordinate of the tracings). Fig. 17 exemplifies C2C12 near the electrode of 1 rad. The movement is parallel to the *x* axis: *y* coordinate is constant (Fig. 18). The cell moves forward with the flow of the media from right to left at first, then stopped for few seconds (see *x* coordinate of the tracings). Fig. 19 shows the tracings of the voltage between terminals of the resistance of 51 Ω. The amplitude of the electric rectangular current of ±0.04 A is calculated by the amplitude of the electric rectangular voltage of ± 2 V between terminals of the resistance of 51 Ω.

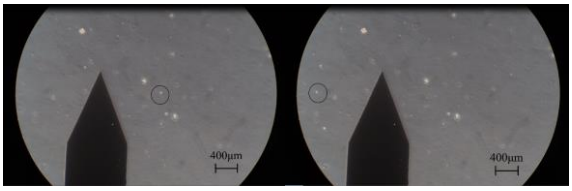


Fig. 9: Red blood cell (marked in circle) near electrode of 0.52 rad: flow from right to left: after 10 s (right): *d* = 1 mm.

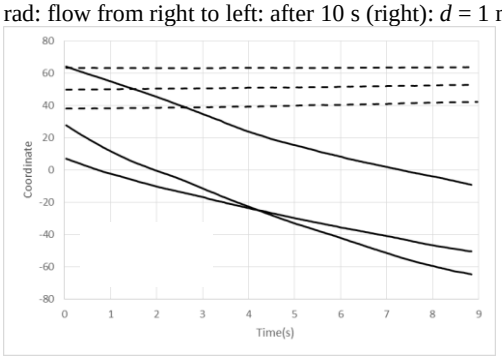


Fig. 10: Each movement of three red blood cells: *x* coordinate (solid line) and *y* coordinate (dotted line).

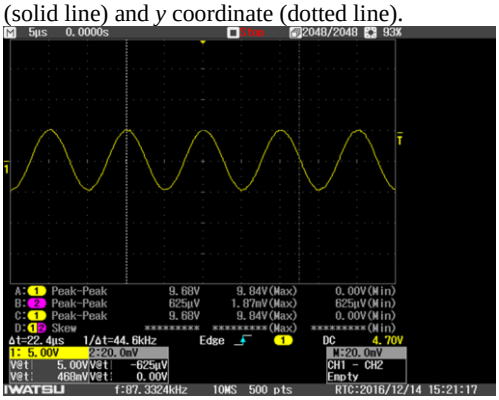


Fig. 11: Tracings of sinusoidal *V* between terminals of 1 kΩ resistance.

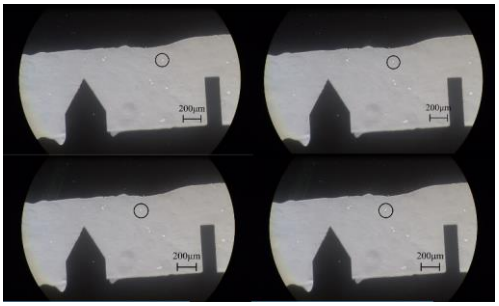


Fig. 12: C2C12 (marked in circle) near electrode of 1 rad: flow from right to left: every five seconds: upper left, upper right, lower left, lower right, in sequence: *d* = 0.3 mm.

Fig. 20 exemplifies C2C12 between electrodes of 0.26 rad and 0.52 rad. The cell moves closer to the electrode of 0.26 rad. Fig. 21 exemplifies C2C12 between electrodes of 1 rad and 1.6 rad. The cell moves closer to the electrode of 1 rad. A micro bubble has been generated by electrolysis at the tip of the electrode during the electric stimulation of rectangular current of ±0.04 A (Fig. 21).

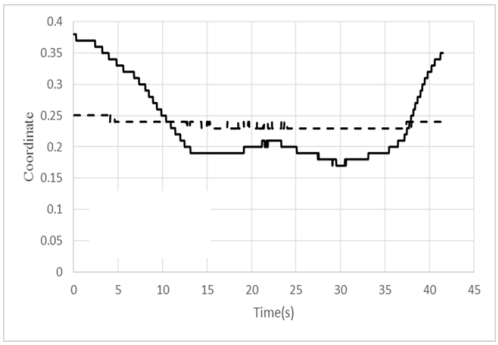


Fig. 13: Movement of C2C12: *x* coordinate (solid line) and *y* coordinate (dotted line).

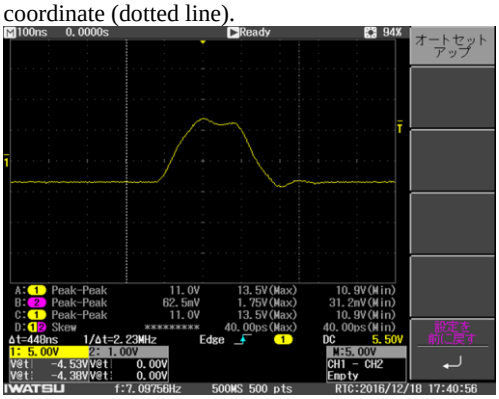


Fig. 14: Tracings of pulse *V* between terminals of 1 kΩ resistance: 0.0002 ms pulse width.

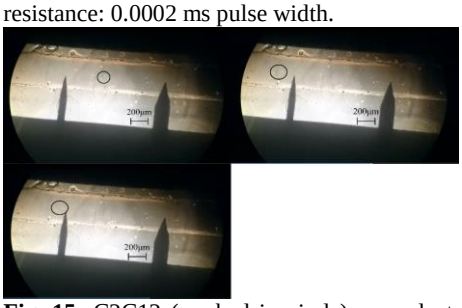


Fig. 15: C2C12 (marked in circle) near electrode of 0.26 rad: flow from right to left: every five seconds: upper left, upper right, lower left, in sequence: *d* = 0.3 mm.

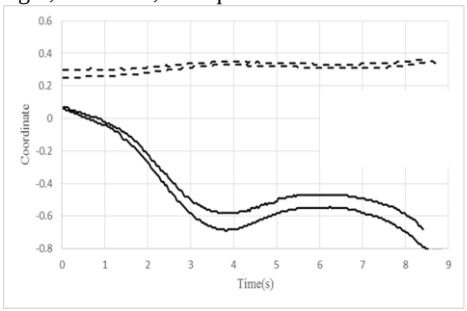


Fig. 16: Each movement of two cells of C2C12: *x* coordinate (solid line) and *y* coordinate (dotted line).

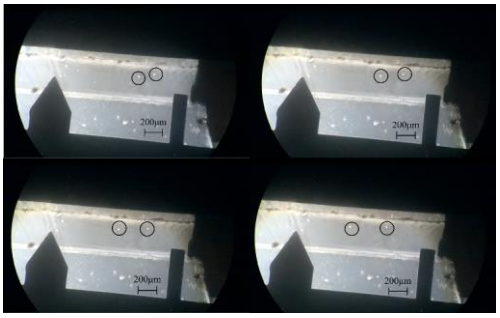


Fig. 17: C2C12 (marked in circles) between electrodes of 1 rad and rectangle: flow from right to left: every five seconds: upper left, upper right, lower left, lower right, in sequence: d = 0.3 mm.

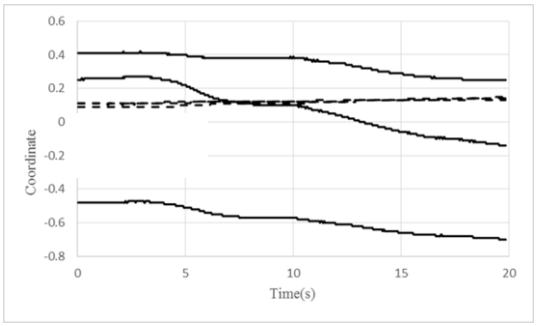


Fig. 18: Each movement of three cells of C2C12: x coordinate (solid line) and y coordinate (dotted line).

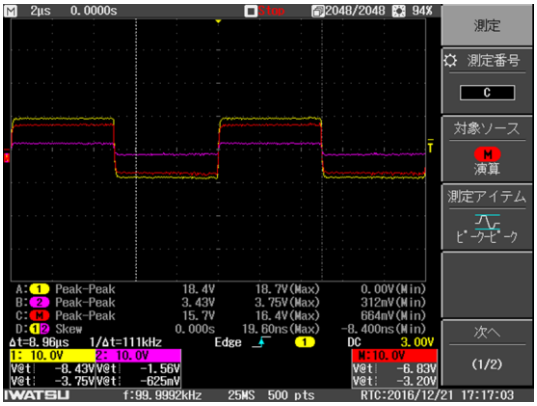


Fig. 19: Tracings of V (2ch. purple) between terminals of 51 Ω resistance: rectangular, E = ±10 V (1ch. yellow).

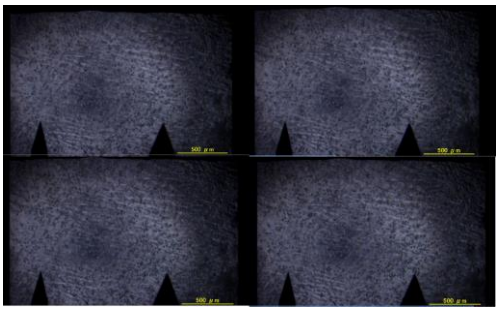


Fig. 20: C2C12 between electrodes of 0.26 rad and 0.52 rad: flow from right to left: every five seconds: upper left, upper right, lower left, lower right, in sequence: d = 1 mm.

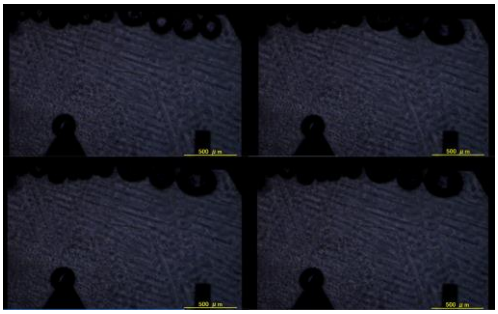


Fig. 21: C2C12 between electrodes of 1 rad and rectangle: flow from right to left: every five seconds: upper left, upper right, lower left, lower right, in sequence: d = 1 mm.

4. DISCUSSION

In the previous studies, myoblasts were cultured and differentiated into myotubes on the micro coil spring of titanium [14]. To generate the stronger contractive force at the myotube, the electric pulse field might have a potential to accelerate the selective adhesion of myoblast on the micro coil spring. The cells move together to the same direction as the flow of the medium without electric stimulation. The cells, on the other hand, change the direction to move, during application of electric stimulation in the present study.

The higher voltage or current may cause electrolysis at the tip of the electrode. The amplitude of the electric stimulation has been limited within the threshold value to prevent electrolysis in the present study. Figs. 9-21 show the movement in the x-y plane. The movement of the direction perpendicular to the flow (z) is very small, because the height of the channel is 0.055 mm. 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 smaller angle of the tip of the electrode might make non-uniform electric fields, which accelerate dielectrophoresis. The irregular movement of cells occurs close to the electrode of the smaller tip angle in the present study. The behavior of the cell depends on the micro morphology of the surface of the scaffold [26]. The cell can be cultured on the surface of the electrode [6]. The electric resistance of the titanium electrode is estimated to 2 Ω by the resistivity of 55×10^{-8} Ω m. In the previous study, dielectrophoresis has been tried to apply on the biological cell manipulation technology [18-25]. The micro grooves were used for trapping of flowing cells in the previous study [16].

5. CONCLUSION

The behavior of biological cells around the surface electrode in the flow channel has been observed *in vitro*. The surface electrodes (the tip of electrode: 0.26, 0.52, 0.79, or 1.0 rad at triangle or rectangle.) of titanium are made along the edges of the flow channel by the photolithography technique. Sinusoidal, pulse, or rectangular cyclic electric stimulations (0.01 ms period) was applied between the surface electrodes. Porcine red blood cells, or C2C12 (mouse myoblast cell line)

were used. The experiment shows that the movement of myoblasts changes by the electric stimulation through the surface electrodes during the flow along the channel, and that the movement depends on the tip angle of the electrode.

6. ACKNOWLEDGMENT

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