Design of Flow Channel for Cell Sorter by Dielectrophoresis with Photolithography Technique

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ABSTRACT

The flow channel for cell sorter *in vitro* has been designed by dielectrophoresis with photolithography technique. A pair of asymmetric surface electrodes of titanium (thickness of 200 nm) were incorporated just before the branching flow channel: a triangular electrode with the tip angle of 0.35 rad, and a rectangular electrode of the flat edge as reference. The cyclic alternating electric current of the square wave (between 0.25 μs and 0.3 μs of periods) was introduced between the surface electrodes. The suspension of C2C12 (mouse myoblast cell line) was injected into the flow channel, and the flow rate was controlled by the pressure head between the inlet and the outlet. Experimental results of the pilot test show that the absolute value of the amplitude of the acceleration by the electric field, which is perpendicular to the flow direction, increases with the radius of the cell.

Keywords: Biomedical Engineering, Cell Sorting, Dielectrophoresis, Surface Electrode and C2C12.

1. INTRODUCTION

The movement of a biological cell suspended in the medium is governed by several factors: movement of the medium, gravity [1], electric force [2], Van der Waals force, and affinity of surface.

Various methods have been applied to control the movement of cells *in vitro*: the flow [3], the shear field [4, 5], the filter, the slit [6–8], the magnetic field [9], the gravitational field [10, 11], laser [12], and the electric field [13]. These methods might contribute to several applications of manipulation of cells [14, 15]: detection of targeted cells [16–18], sorting of cells [19–22], arrangement of cells to make a tissue, and measurement of the character of cells [23].

Movement of a charged particle depends on the electric field. The effect is applied to the electrophoresis device [24]. When a particle is subjected to a non-uniform electric field, a force acts even on a non-charged particle, because the polarization generates in the particle. The phenomenon is called dielectrophoresis [10]. The force of dielectrophoresis depends on the several parameters: the electrical property of the particle, shape and size of the particle [25], the electrical property of the medium, and the electric field (the amplitude and the frequency) [26].

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 [27], on the other hand, is a phenomenon, in which a particle moves due to the interaction between the electric field and the charge induced in a neutral particle, when the particle is placed in a non-uniform electric field.

With the aid of the micromachining technique [28], many kinds of micro systems were designed. In some systems, dielectrophoresis was applied to sort biological cells floating in the medium. In the present study, the flow channel for cell sorter *in vitro* has been designed by dielectrophoresis with photolithography technique.

2. METHODS

Lower Plate
The titanium film was used for the surface electrode. Titanium was coated on the glass plate. The thickness of coating is 200 nm. The tip angle of the triangle shape of the one of the surface electrodes is 0.35 rad (Fig. 1). The other reference electrode has a flat edge. The distance between electrodes is 0.1 mm. The shortest connecting line between electrodes is vertical to the flow direction. The micro flow channel with surface electrodes was manufactured by the photolithography technique.

The pattern of the electrode with the flow channel was drawn on the mask with a laser drawing system (DDB-201K-KH, Neoark Corporation, Hachioji, Japan). The thickness of the layer (SU-8 100) on the surface electrode was measured by the stylus (with 3×10^{-5} N) of the contact profilometer (Dektak XT-E, Bruker Corporation).

Upper Plate
The upper plate of polydimethylsiloxane has holes with the diameter of 5 mm for the inlet and the outlet of the suspension. The upper plate was exposed to the oxygen gas in the reactive ion etching system (FA-1), and coated with

Aminopropyltriethoxysilane. After ten minutes, the upper plate was adhered on the lower plate under the pressure of 0.5 N/m^2 by sandwiching between plates of poly-methyl-methacrylate (Fig. 2). A rectangular parallelepiped channel of 18 mm length × 0.5 mm width × 0.035 mm height is formed between upper and lower plates. The flow channel is placed on the stage of the inverted phase-contrast microscope.

Electric Stimulation

The electric stimulation of the alternating rectangular cyclic wave (0.25 μs < period (T) < 0.3 μs; −15 V < amplitude (Ea) < +15 V) was generated with an electric stimulator. The stimulator was connected to the titanium film electrode, and the electric stimulation was applied to the medium flow. An electric resistance (R) of 2 kΩ is serially inserted between the electrode and the stimulator to measure the electric current (I < ±7.5 mA).

Cell

C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse) was used in the test. D-MEM (Dulbecco's Modified Eagle Medium) containing 10% FBS (Fetal Bovine Serum) and 1% penicillin/ streptomycin was used for the medium.

Before the flow test, the inner surface of the flow channel was hydrophilized by the oxygen plasma ashing. The bovine serum albumin solution was prefilled in the flow channel, and was incubated for thirty minutes at 310 K in the incubator.

Before the flow test, the cells were exfoliated from the plate of the culture dish with trypsin including EDTA (ethylenediaminetetraacetic acid), and were suspended in the medium. The suspension of cells was poured at the inlet of the flow channel. The flow was made by the pressure difference between the inlet and the outlet, which was kept by the gravitational level of the medium (< 5 mm).

Each cell passing between the electrodes was observed by the inverted phase-contrast microscope, and recorded by the video camera, which is set at the eyepiece of the microscope. The contour of each cell was traced, and the projected two-dimensional area (S) at the image of each cell was calculated using "Image J". The equivalent radius (r) was calculated by Eq. 1.

$$S = \pi r^2 \quad (1)$$

The movement of the cell was analyzed by "Kinovea (Ver. 8.23, Commons Attribution)" at the video images: 30 frames per second. To trace the movement of the cell, the coordinates are defined as that in Fig. 1. The main flow direction of the medium is defined as x. The perpendicular direction from the reference electrode to the tip of the triangular electrode is defined as y. The origin is adjusted at the tip of the triangular electrode. The components (v_x, and v_y) of velocity were calculated at the tracings of each cell. The components (α_x, and α_y) of acceleration of the velocity was calculated at the velocity tracings of each cell.

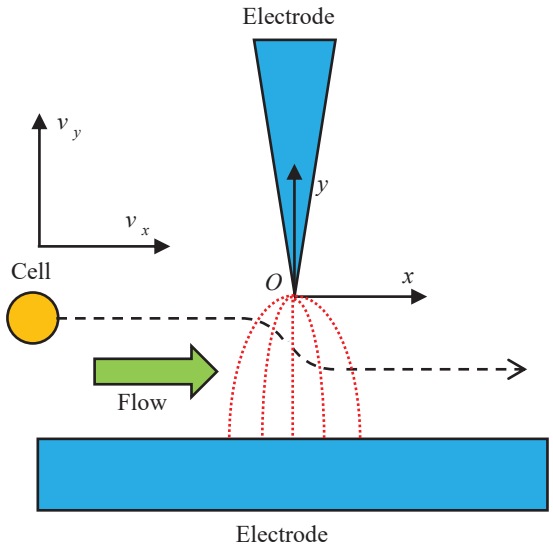

Fig. 1: Two-dimensional movement of cell flowing near tip of electrode: position (x, y), and velocity (v_x, v_y) of cell.

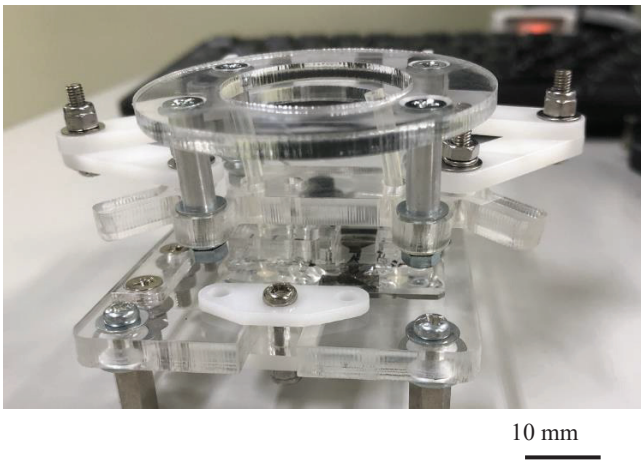

Fig. 2: Upper and lower plates sandwiched between plates of poly-methyl- methacrylate.

Photomask A for Branched Flow Channel

The branched flow channel for dielectrophoretic sorting of cells has been designed (Fig. 3). Before the deposition of titanium, the surface of the glass plate was hydrophilized by the oxygen plasma ashing by the reactive ion etching system. The positive photoresist material of OFPR-800LB-20cP (Tokyo Ohka Kogyo Co., Ltd, Tokyo, Japan) was coated on the titanium with the spin coater. The photoresist was baked at the hotplate.

The pattern of the electrode with the flow channel was drawn on the mask with a laser drawing system (DDB-201K-KH, Neoark Corporation, Hachioji, Japan). The photoresist was developed with tetra-methyl-ammonium hydroxide (NMD-3, Tokyo Ohka Kogyo Co., Ltd., Kawasaki, Japan), rinsed with the distilled water, and dried.

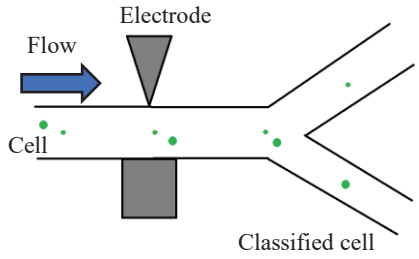

Fig. 3: Dielectrophoretic classification of cells by branched flow channel.

The titanium coated plate was etched with the plasma gas using RIE-10NR (Samco International, Kyoto, Japan). For etching, the gas of SF_6 (50 cm^3/min at 1013 hPa) with Ar (50 cm^3/min at 1013 hPa) was applied at 75 W at 4 Pa for seven minutes.

3. RESULTS

Fig. 4 exemplifies the microscopic image, which shows cells flowing near the tip of the triangular electrode. The movie shows the sifted movement of the flowing cell passing adjacent to the tip of the electrode by dielectrophoresis.

Fig. 5 shows the relationship among parameters displayed by three-dimensional graph (RINEARN Graph 3D ver. 5.6): maximum acceleration (α_{ymax}), radius (r), and initial position (y_0). The period of rectangular cyclic wave is 0.3 μs in Fig. 5a, and 0.25 μs in Fig. 5b. The absolute value of the maximum amplitude of the acceleration (α_{ymax}) of the velocity of each cell during passing over the tip of the electrode is displayed in Fig. 5. The absolute value of the maximum amplitude of the acceleration (α_{ymax}) is high, when the cell flows along the streamline ($y_0 = 0$) through the tip of the electrode. The absolute value of the maximum amplitude of the acceleration (α_{ymax}) by the electric field, which is perpendicular to the flow direction, increases with the radius of the cell (r).

Fig. 6 shows the design of the flow channel. A cell flows from left to right. After passing between electrodes, the cells are classified at the bifurcation into two branches. The number in Fig. 6 shows dimension with the unit of millimeter. Fig. 7 shows the manufactured photomask for the flow channel with bifurcation. The branch pattern was successfully drawn on the mask.

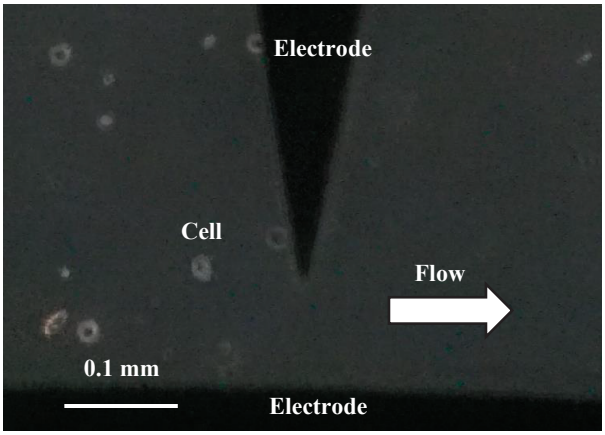

Fig. 4: Cell is passing adjacent to tip of electrode.

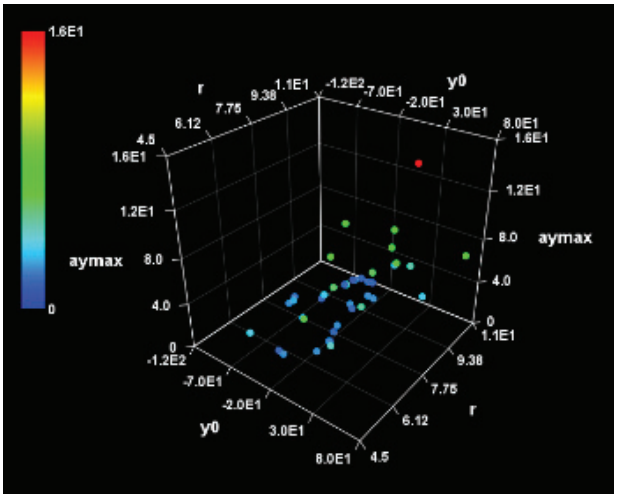

Fig. 5a: Relationship among maximum acceleration (α_{ymax}), radius (r) , and initial position (y_0): period of 0.3 μs, amplitude of ±15 V.

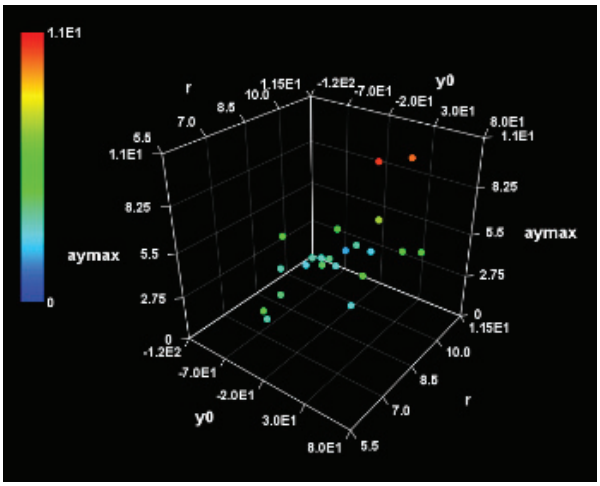

Fig. 5b: Relationship among maximum acceleration (α_{ymax}), radius (r) , and initial position (y_0): period of 0.25 μs, amplitude of ±15 V.

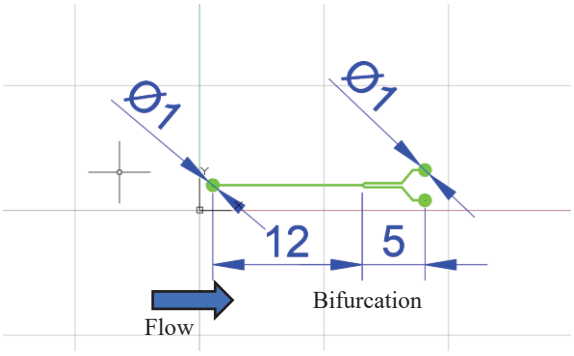

Fig. 6: Design of branched flow channel: unit mm.

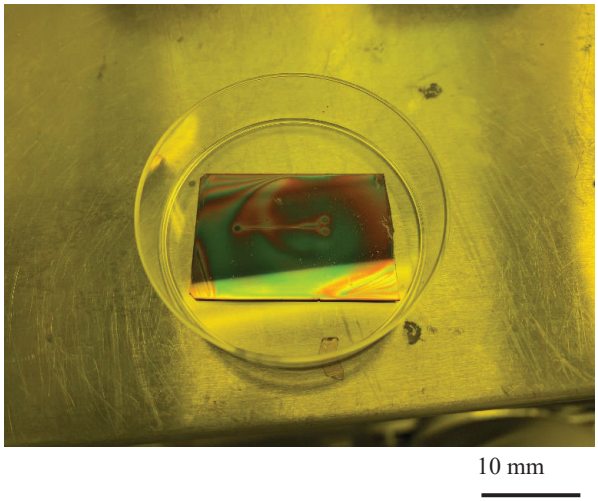

Fig. 7: Photomask for branched flow channel in dish.

4. DISCUSSION

Dielectrophoresis was applied to the cell sorting system as minimally invasive method in the previous studies [13, 29–33]. The shift distance [17, 27] was very small (< 0.1 mm) in the previous experiment. If the lateral shift movement of the cell adjacent to the tip of the electrode is enlarged at the downstream by the inertial movement, the method can be applied to the cell sorting. The lateral shift can be amplified by accumulation with the multi-electrodes system on the flow channel [24, 34]. Spiral Microchannel was used for sorting in the previous study [35]. Ratchet Microchannels were also applied to sorting device [36]. The relationship between the streamline of the cell and the position of the side wall of the flow channel should be considered for the continuous movement of the cell at the downstream. To apply the present system for cell sorting, both the initial position and the velocity [37] should be controlled before the non-uniform electric field in the flow channel.

In the present study, the dielectrophoretic movement of the cell has been tried to be observed by the optical microscope. The couple of electrodes arrangement with the shorter distance each other has not been designed, because the electrodes in the present study are not optically transparent [2]. The shift depends on the passing route position of the cell relative to the position of the tip of the electrode. The dielectrophoretic effect is highest adjacent to the tip of the electrode [38–40].

The movement of cells between surface electrodes depends on the topography of surface electrodes (the angle of the tip) [38–40], which relates to non-uniformity of the electric field. The higher slope of electric field with non-uniformity is necessary to enlarge the movement of cells around the electrode. In the previous study, variation of electrodes was used for sorting of cells [41].

In principle, dielectrophoretic effect increases with the diameter of the particle [26]. The present experiment shows that the acceleration of the cell along the electric field (perpendicular to the flow direction) increases with the radius of the cell (Fig. 5).

The rectangular fluctuating voltage between electrodes [15, 35, 42] higher than ±15 V enlarged the lateral shift of the flowing cell. The amplitude of the electric stimulation has been limited within the threshold value to prevent electrolysis [43] in the present study.

Fig. 5 shows the two-dimensional movement of each cell in the *x-y* plane projected to the microscopic video image. The real movement of each cell is three dimensional. The movement of the perpendicular direction to *x-y* plane is very small, because the height (0.035 mm) of the flow channel is small compared with the width (0.5 mm) of the flow channel in the present experiment. The position of the streamline of the target cell was tried to be controlled before passing through the electric field area in the previous study [22].

In the previous studies, dielectrophoresis was tried to be applied to the biological cell manipulation technology [14, 44]. It was used to measure the mechanical properties of biological cells [45]. The micro grooves, on the other hand, were used for trapping of flowing cells to distinguish mechanical properties of flowing cells in the previous study [11]. The cell sorting technology would be applied to tissue engineering [46] (e.g., bio-actuator made from myoblast [47, 48]). The cell sorting technology would also be applied to diagnostics in medicine [49, 50]. The technology was also applied to yeast cells [51–53]. Another microflow system was applied for cell sorting [54], and cell salvage [55].

5. CONCLUSION

The flow channel for cell sorter *in vitro* has been designed by dielectrophoresis with photolithography technique. Experimental results of the pilot test show that the absolute value of the amplitude of the acceleration by the electric field, which is perpendicular to the flow direction, increases with the radius of the cell. As application of the results of the pilot test, the branched flow channel has been designed for cell sorting.

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