Sorting of Cells with Flow Channel: Movement of Flowing Myoblast Cell at Oblique Micro Grooves

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ABSTRACT

The movement of a flowing cell near the oblique micro groove on the bottom surface in the micro flow channel has been measured to sort biological cells *in vitro*. The micro groove of the rectangular shape (4.5 μm depth, and 0.2 mm length) has been fabricated on the polydimethylsiloxane (PDMS) disk by the photolithography technique. The angle between the flow direction and the longitudinal axis of the groove is 45 degree. Variation has been made on the width (0.03 mm < w < 0.05 mm) of the groove. A rectangular flow channel (0.05 mm height × 1 mm width × 25 mm length) has been constructed between two transparent PDMS disks. C2C12 (mouse myoblast cell line) was used in the test. A flow velocity (0.04 mm/s < v_x < 0.80 mm/s) of the suspension of cells was controlled by the pressure difference between the inlet and the outlet. The shifted distance of each cell along the oblique groove depends on the diameter of the cell. In the present device, the malnourished cell with the different density can be distinguished by the shifted distance according to the velocity of the cell.

Keywords: Biomedical Engineering, C2C12, Micro Groove, Sorting, and Polydimethylsiloxane.

1. INTRODUCTION

Several methods were proposed for the sorting of biological cells *in vitro* [1-12]. The micro fluidic systems were used in some methods [4-12]. In these systems, variety of properties of cells were picked up for the sorting. In the present study, physical properties have been picked up: diameter, and specific gravity.

The technology of sorting of cells can be applied to regenerative medicine to select the target cells. It also can be applied to diagnostics to handle the target cell [13].

To capture the target cell, several kinds of morphology were designed in the micro fluidic systems: electrodes [10], micro slits [14, 15], micro holes [16, 17], or micro grooves [18, 19]. The photolithography technique enables manufacturing the micro-morphology [20].

In the present study, the movement of the single cell flowing over the micro groove, which is manufactured by the photolithography technique, has been analyzed *in vitro*.

2. METHODS

Micro Grooves
For trapping cells, several micro grooves of the rectangular shapes (4.5 μm depth, and 0.2 mm length) have been fabricated on the surface of the polydimethylsiloxane (PDMS) plate with the photolithography technique. Several grooves are arranged on the same wall for the bottom of the micro flow channel. The angle between the flow direction and the longitudinal axis of the groove is 45 degree. At the groove arrangement from upstream to downstream, variation has been made on the width (w) of the groove: 0.03 mm, 0.04 mm, and 0.05 mm. The angle between the longitudinal direction of the groove and the flow direction is 45 degrees.

Photomask for Lower Plate
The slide glass (Matsunami Glass Ind., Ltd., Japan) plate (38 mm length, 26 mm width, and 1.0 mm thickness) was used for the base of the photomask. Before the deposition of titanium, the surface of the glass plate was cleaned 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 with 200 nm thickness in the spattering system (back pressure < 9×10^{-4} Pa, RF100W, 0.5 Pa, 8 min, L-210S-FH Canon Anelva Corporation, Kawasaki, Japan).

To improve affinity to photoresist material, HMDS (hexamethyldisilazane: Tokyo Chemical Industry Co., Ltd., Tokyo) was coated on the glass plate at 3000 rpm for 30 s with the spin coater (1H-DX2, Mikasa Co. Ltd., Tokyo). The positive photoresist material of OFPR-800LB (Tokyo Ohka Kogyo Co., Ltd, Tokyo, Japan) was coated on the titanium with the spin coater (at 2500 rpm for 30 s). The photoresist was baked in the oven (DX401, Yamato Scientific Co., Ltd) at 368 K for three minutes.

The pattern for the groove (Fig. 1) 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 mold with the laser drawing system, the parameters were selected as follows: the voltage of 3.26 V, the velocity of 0.137 mm/s, the acceleration of 0.5 mm/s^2, and the focus offset at +3.17.

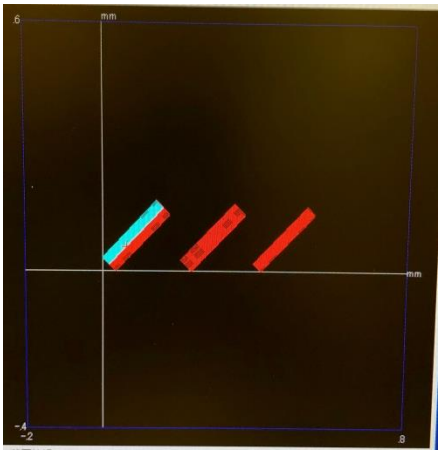

Fig. 1: Micro patterns for the groove at drawing.

The photoresist was baked in the oven (DX401) at 368 K for five minutes. The photoresist was developed with tetra-methyl-ammonium hydroxide (NMD-3, Tokyo Ohka Kogyo Co., Ltd., Kawasaki, Japan) for five minutes, rinsed with the distilled water (300 rpm, 30 s), and dried by the spin-dryer (1100 rpm, 30 s, with N_2 gas, SF-250, Japan Create Co., Ltd., Tokorozawa, Japan).

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 100 W at 4 Pa for six minutes. The residual OFPR-800LB was removed by acetone dipped for one minute. The plate was dipped in the distilled water in one minute, after it is dipped in ethanol for one minute. The plate was dried by the spin-dryer: 300 rpm for 30 s with the distilled water, and 1100 rpm for 30 s with N_2 gas.

Lower Plate

The slide glass (Matsunami) plate (38 mm length, 26 mm width, and 1.0 mm thickness) was used for the base of the mold. 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).

The epoxy based negative photo-resist material (SU8-5: Micro Chem Corp., MA, USA) was coated on the glass at 2700 rpm for 120 s with a spin coater. The photoresist was baked in the oven (DX401) at 338 K for three minutes, and at 368 K for seven minutes successively.

The photoresist was exposed to the UV light through the photomask in the mask aligner (M-1S, Mikasa Co. Ltd., Japan) at 15 mW/cm^2 for 15 s. The photoresist was baked in the oven at 363 K for seven minutes. The photo-resist was developed with SU8 developer (Nippon Kayaku Co., Ltd, Tokyo, Japan) for five minutes to make micro rectangular ridges, where the laser beam was radiated.

The glass surface with the micro pattern was rinsed with IPA (2-propanol, Wako Pure Chemical Industries, Ltd.) for one minute. The plate was dried by the spin-dryer: 300 rpm for 30 s with the distilled water, and 1100 rpm for 30 s with N_2 gas.

After the 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) on the plate. The volume ratio of PDMS to curing agent is ten to one. After degassing, PDMS was baked at 368 K for twenty minutes in the oven.

Upper Plate

The polyimide tape (0.055 mm thickness, 1 mm width, and 25 mm length) was pasted at the center of the poly-methyl-methacrylate plate for the mold.

After the plate was set in the mold of poly-methyl-methacrylate (Fig. 2), PDMS was poured with the curing agent on the plate. The volume ratio of PDMS to curing agent is ten to one. After degassing, PDMS was baked at 368 K for twenty minutes in the oven (DX401). Two holes (diameter of 5 mm) with the interval of 20 mm were made for the inlet and the outlet (Fig. 3).

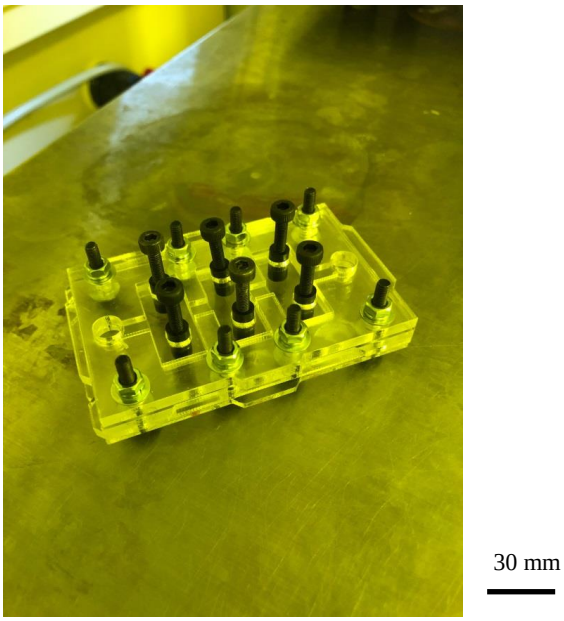

Fig. 2: Mold for upper plate.

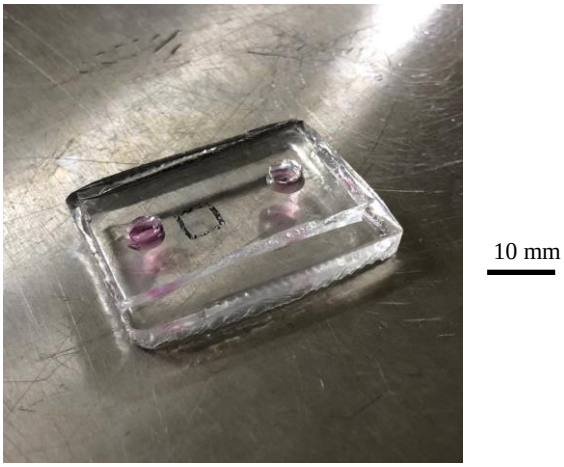

Fig. 3: Upper plate.

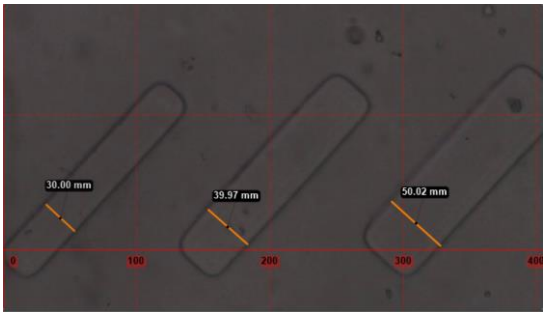

Fig. 4: Dimension of width of each groove. 0.05 mm

Flow System

Both the upper and the lower plates were exposed to the oxygen gas (0.1 Pa, 30 cm^3/min) in the reactive ion etching system (FA-1) (oxygen plasma ashing, 50 W, for thirty seconds). Immediately after ashing, the upper disk adheres (plasma bonding) to the lower disk to make the flow path (0.055 mm height × 1 mm width × 25 mm length) between them. The dimension of the width of each groove was measured on the microscopic image (Fig. 4). The flow channel is placed on the stage of the inverted phase-contrast microscope (IX71, Olympus Co., Ltd., Tokyo) (Fig. 5).

Flow Test

C2C12 (passage < 10, mouse myoblast cell line originated with cross-striated muscle of C3H mouse) was used in the test.

Cells were cultured with the D-MEM (Dulbecco's Modified Eagle's Medium) containing 10% FBS and 1% of Antibiotic-Antimycotic (penicillin, streptomycin and amphotericin B, Life Technologies) in the incubator for one week.

For comparison with normal cells (group A), malnourished cells (group B) were prepared. In the group B, cells were kept in the cryopreservation solution (the serum type Cellbanker1 (including dimethylsuloxide: Nippon Zenyaku Kogyo Co., Ltd, Koriyama, Japan)) in the incubator (at 310 K with 5% of CO_2) for one week without medium change after cryopreservation.

The inner surface of the flow channel was hydrophilized by the oxygen (30 cm^3/min, 0.1 Pa) plasma ashing for one minute at 100 W by the reactive ion etching system (FA-1), and prefilled with the bovine serum albumin solution for thirty minutes at 310 K.

Before the flow test, the cells were exfoliated from the plate of the culture dish with trypsin, and suspended in the D-MEM (Dulbecco's Modified Eagle's Medium). A part of the suspension of cells (4000 cells/cm^3, 0.06 cm^3) was poured at the inlet of the flow channel. The flow occurs by the pressure difference between the inlet and the outlet. The inlet hole (the depth of 3 mm and the diameter of 5 mm) makes the pressure head.

The residual part of the suspension of cells were continuously cultured in the medium for 30 minutes to confirm the vitality of cells by adhesion to the scaffold. The specific gravity of the cell was measured in comparison with the density of the phthalic acid ester solution between 1.05 g/cm^3 and 1.08 g/cm^3 by centrifuge.

Fig. 5: Experimental system.

Each cell rolling over the micro grooves was observed by the microscope, and recorded by the camera (DSC-RX100M4, Sony Corporation, Japan), which is set at the eyepiece of the microscope. The movement of each cell was analyzed by "Kinovea" at the video images (Fig. 6a): 30 frames per second. At the images, the contour of each cell was traced with "Image J", and approximated to the circle to calculate the diameter (D).

The velocity of each cell (v_x) immediately before the groove was traced on the component parallel to the direction of the main flow (Fig. 6b). The shifted distance of each cell along the groove was traced on the component (y) perpendicular to the main flow direction.

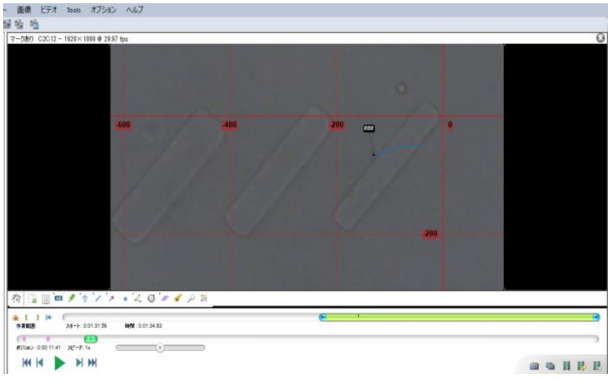

Fig. 6a: Tracings (blue line) of movement of cell passing over groove: flow from left to right. 0.1 mm

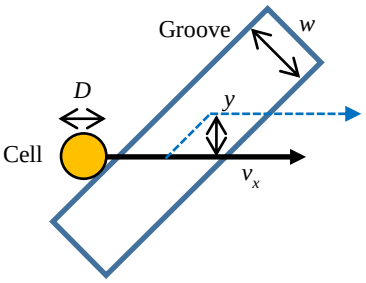

Fig. 6b: Parameter of movement of cell passing over groove.

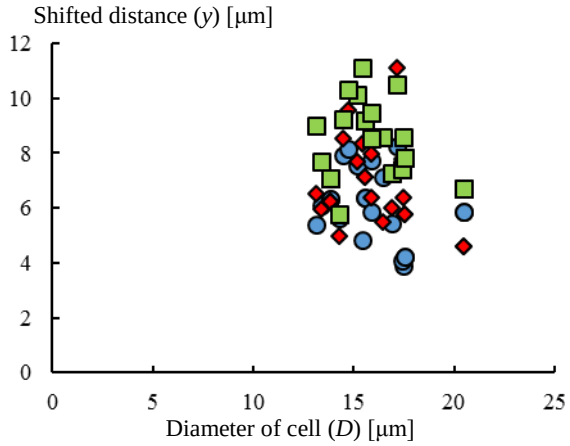

Fig. 7: Shifted distance (y) of C2C12 vs. diameter of cell (D): 0.03 mm (circle), 0.04 mm (triangle), and 0.05 mm (square).

3. RESULTS

The dimension of the width of each groove was confirmed at the microscopic image as the designed value (Fig. 4). The density of the normal C2C12 (group A) was 1.065 g/cm^3. The density of the malnourished C2C12 (group B) was lower than 1.06 g/cm^3. Unlike cells of group A, cells of group B did not adhere to the scaffold within 30 minutes in the incubator. The diameter of each cell was in the range between 13.1 μm and 21.7 μm. The velocity of each cell (v_x) immediately before the groove was in the range between 0.038 mm/s and 0.80 mm/s according to the pressure difference between the inlet and the outlet. The velocity decreases with the time, because the pressure head at the inlet decreases with time.

The shifted distance was in the range shorter than 11.1 μm. Data of the shifted distance (y) of C2C12 are collected in relation to the diameter of each cell (D) in Fig. 7. The shifted distance (y) at the groove (0.03 mm < w < 0.05 mm) scatters between 4 μm and 12 μm, when the diameter of each cell is in the range between 12 μm to 20 μm. The value of y tends to increase with the width of the groove.

Fig. 8 shows y in relation to D/w. The ratio of D/w is unity, when the diameter of the cell (D) is equal to the width of the groove (w). The shifted distance (y) tends to decrease with the increase of the ratio of D/w. When the diameter of the cell (D) is big compared with the width of the groove (w), the travel along the groove is shorten.

The results are divided into three graphs (Figs. 9-11), according to the velocity (v_x) of each cell: v_x < 0.1 mm/s, 0.1 mm/s < v_x < 0.2 mm/s, and 0.2 mm/s < v_x. At the low velocity, data scatters (Fig. 9). At the middle velocity, the tendency of decrease of the shifted distance (y) with the ratio of D/w is clearer around 0.4 of D/w (Fig. 10).

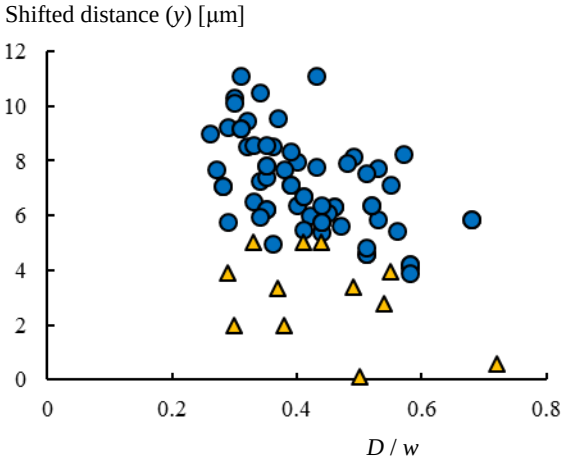

Fig. 8: Shifted distance (y) vs. D / w: normal cell (circle), and malnourished cell (triangle).

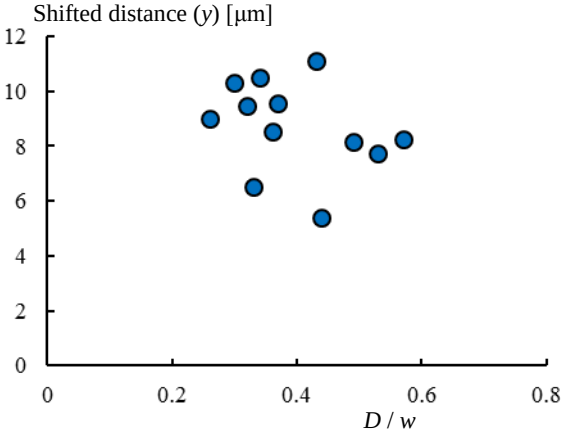

Fig. 9: Shifted distance (y) vs. D / w: v_x < 0.1 mm/s.

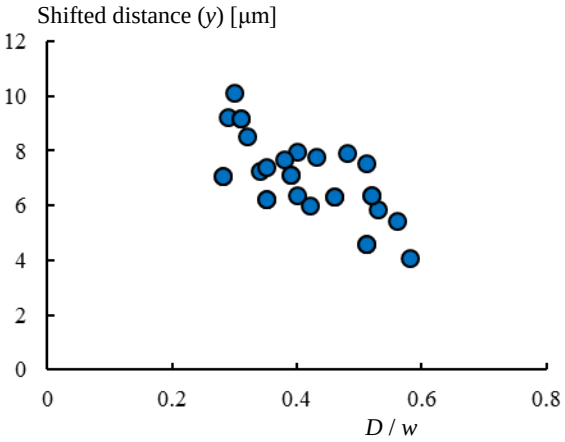

Fig. 10: Shifted distance (y) vs. D / w: 0.1 mm/s < v_x < 0.2 mm/s.

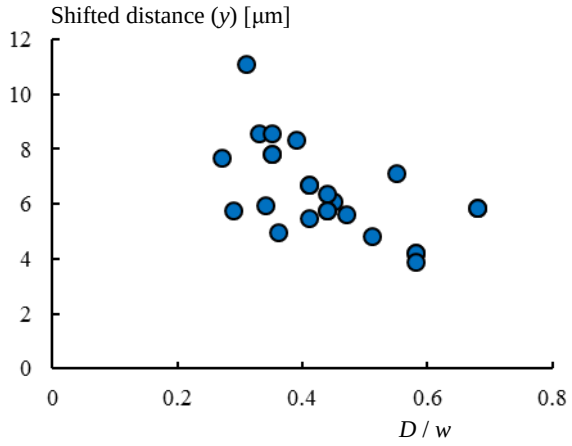

Fig. 11: Shifted distance (y) vs. D / w: 0.2 mm/s < v_x.

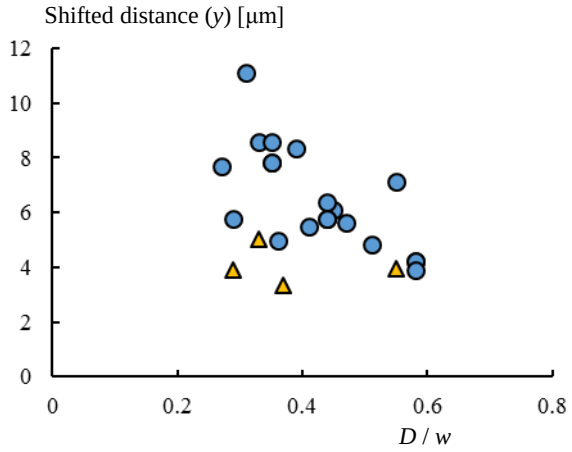

Fig. 12: Shifted distance (y) vs. D / w: 0.25 mm/s < v_x < 0.40 mm/s: normal cell (circle), and malnourished cell (triangle).

Data of malnourished cells are added in Fig. 8. The shifted distance (y) of the malnourished cell is shorter than that of the normal cell. The tendency is confirmed, when data are selected according to the range of the velocity (v_x) of each cell (Fig. 12).

4. DISCUSSION

Fluorescence technique was used in the previous sorting systems [1, 2]. Non-destructive cell sorting systems, on the other hand, were designed in the previous studies [3]. The label-free methods were designed with microfluidic systems [6, 12]. Some of them were designed to capture cancer cells [8, 13].

The rectangular grooves have been successfully manufactured on the wall of the micro fluid channel. The dimension of the grooves was confirmed by the laser microscope [18]. Several kinds of systems were designed for the cell sorting *in vitro* [1-19]. The micromachining technique has been applied to cell technology. The microfluidic system was applied to sort biological cells, and to trap biological cells [4-12]. The sorting technique might be applied to selection of cells for regenerative medicine and diagnostics of disease.

Several fluid flow systems were used in the previous studies. In the previous studies, cylindrical [16] and half cylindrical [17] holes were used for the trap of cells. The asymmetrical hole [17] might be suitable for trap than the symmetrical hole. The depths of the micro patterns were between 0.002 mm and 0.01 mm in the previous studies [16-20]. In the present study, the depth of the grooves is (4.5 μm, which is smaller than the diameter of the cells. The deeper hole may have advantage to trap every cell. At the shallower trap, on the other hand, it is not easy to tarp a cell. The trap of the appropriate dimension may distinguish cells. The duration of the trapped time of the cell might relate to interaction between the micro hole and the cell: affinity between the cell and the surface of the micro pattern, or deformability of the cell.

The results of the previous study show that the movement of cell travelling on the wall is modified by the oblique micro groove on the wall under the cell velocity lower than 1 mm/s [18]. The angle of 45 degrees between the longitudinal direction of the groove and the flow direction is effective to shift the streamline of the cell. The shift movement along the oblique groove depends on the several parameters: the diameter of cells, the width of the groove, the velocity of the cell, and the kinds of cells [18]. As the diameter of the cell decreases, the traveling length along the groove increases. The movement might be related not only to the diameter but also to the deformability of the cell.

The movement of flowing cell at the bottom surface of the flow channel might be related to the specific gravity of the cell: the density difference between the cell and the medium. The shifted distance of malnourished cells by the oblique groove is smaller than that of normal cells in the present study.

The cell density in the suspension is very low in this study to reduce the interaction between the cells. Cells can be sorted by the traveling length along the micro groove.

5. CONCLUSION

The movement of a flowing cell near the micro groove on the bottom surface in the micro flow channel has been measured to sort biological cells *in vitro*. The micro groove of the rectangular shape (4.5 μm depth, and 0.2 mm length) has been fabricated on the bottom surface of the flow channel of polydimethylsiloxane by the photolithography technique. The angle between the flow direction and the longitudinal axis of the groove is 45 degree. C2C12 (mouse myoblast cell line) was used in the test. The shifted distance of each cell along the oblique groove depends on the diameter of the cell. In the present device, the malnourished cell can be distinguished by the shifted distance according to the velocity of the cell around 0.3 mm/s.

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