Micro Groove for Trapping of Flowing Cell

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ABSTRACT

The micro groove has been designed to trap a biological cell, which flows through a micro channel *in vitro*. The micro groove of the rectangular shape (0.002 mm depth, 0.025 mm width and 0.2 mm length) has been fabricated on the surface of the polydimethylsiloxane (PDMS) disk with the photolithography technique. Variation has been made on the angle between the longitudinal direction of the groove and the flow direction: zero, 45, and 90 degrees. A rectangular flow channel (0.1 mm depth × 5 mm width × 30 mm length) has been constructed with a silicone film of 0.1 mm thick, which has been sandwiched by two transparent PDMS disks. Two types of biological cells were alternatively used in the test: C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse), or 3T3-L1 (mouse fat precursor cells). A constant flow (2.8×10^{-11} m^3/s) of the suspension of cells was introduced with a syringe pump. The behavior of cells moving over the micro groove was observed with an inverted phase contrast microscope. The results show that the cell is trapped with the micro grooves under the wall shear rate of 3 s^{-1} for a few seconds and that the trapped interval depends on the kind of cells.

Keywords: Biomedical Engineering, C2C12, 3T3-L1, Micro Flow Channel, Micro Groove and Polydimethylsiloxane.

1. INTRODUCTION

A biological cell adheres to the scaffold, and reveals several behaviors: migration, deformation, proliferation, and differentiation. The behavior of cells depends on the various environmental factors [1-5]. A flowing cell is captured to a wall of a flow path. Several cells adhere to the internal wall of the blood vessel, when the part of the wall has a defect. The morphology of the defect might govern the capture of cells. The capture might depend on the property of the cell.

The cancer cell transits from the original place to another place, and proliferates to make a tumor at another place. The transition occurs through the blood vessels and the lymph vessels. The cancer cells adhere to the inner wall of the vessels.

The photolithography technique enables manufacturing a micro-channel. The effect of the surface of the scaffold on cell culture has been studied in the previous studies [6-9]. Several micro-fabrication processes have been designed to control adhesion of biological cells *in vitro* [6-13], and to simulate the morphology of the microcirculation. The micro-fabrication technique has also been applied to design microfluidic systems *in vitro* [14-20]. Cells roll over on the surface of the wall in the shear flow, and make adhesion to the wall [10]. The surface was modified to capture flowing cells [10, 11, 21, 22]. The technique will also be applied to handle cells in diagnostics *in vitro* [23].

In the present study, a micro groove has been designed to trap a biological cell, which flows through a microchannel *in vitro*.

2. METHODS

Micro Traps

For trapping cells, several micro grooves of the rectangular shape (0.002 mm depth, 0.025 mm width, and 0.2 mm length) have been fabricated on the surface of the polydimethylsiloxane (PDMS) disk with the photolithography technique. Six grooves are arranged in two lines. The interval between grooves is 0.2 mm. Variation is made on the angle between the longitudinal direction of the groove and the flow direction: zero, 45, and 90 degrees (Fig. 1).

A silicon wafer (Type N, Matsuzaki Seisakusyo, Co., Ltd., Tokyo, Japan) was used for a surface mold for the disk. The diameter and the thickness of the wafer are 50 mm and 0.30 mm, respectively.

The photo-resist material of low viscosity (SU8-2: Micro Chem Corp., MA, USA) was coated on the wafer with 0.002 mm thick at 2000 rpm for 30 s with a spin coater. The photo-resist baked on the heated plate with two processes: at 338 K for 1 minute, before at 358 K for 3 minutes.

The pattern for grooves (Fig. 1) on the mold was drawn on the wafer 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 4 V, the velocity of 0.01 mm/s, the acceleration of 0.5 mm/s^2. The pattern was baked on the heated plate with two processes: at 338 K for 1 minute, before at 358 K for 3 minutes.

The photo-resist was developed with SU8 developer (Nippon Kayaku Co., Ltd, Tokyo, Japan) for six minutes to make micro rectangular ridges, where the laser beam was radiated. The wafer was rinsed with the isopropyl alcohol and the ultrapure water, before dried with the air gun.

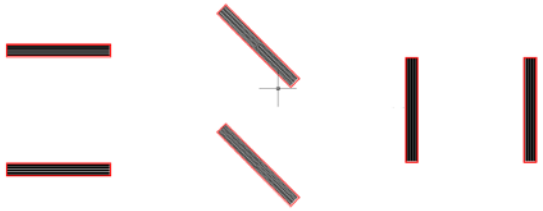


Fig. 1: Six grooves. Flow direction is vertical.

After development, the dimension of the micro rectangular ridges on the mold was measured with a laser microscope (VK-X200, Keyence Corporation, Osaka, Japan). The morphology along the longitudinal and transverse lines of each ridge was traced. The concave mold with micro patterns is used only for the lower disk of PDMS to make the micro grooves on the surface for trapping cells.

The surface of the wafer was coated with 0.001 mm thickness of Parylene (Specialty Coating Systems, Inc., IN, USA).

After the wafer was enclosed with a peripheral wall of polyimide, 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 wafer. The volume ratio of PDMS to curing agent is ten to one. After degassing, PDMS was baked at 383 K for one hour in an oven.

Flow System

A one-way flow system has been designed to control the wall shear rate at the wall of PDMS [4]. The system consists of a flow channel, a micro syringe pump, tubes and a microscope (Fig. 2). A silicone tube of 3 mm internal diameter and of 5 mm external diameter is used for the connector to the flow channel.

The flow channel consists of two transparent PDMS disks and a thin sheet of silicone rubber.

A thin sheet (0.1 mm thick) of silicone rubber, which has a rectangular void space of 5 mm × 30 mm, is sandwiched between two transparent PDMS plates (Fig. 3). The void space forms a channel of 30 mm length × 5 mm width × 0.1 mm depth. The three parts stick together with their surface affinity.

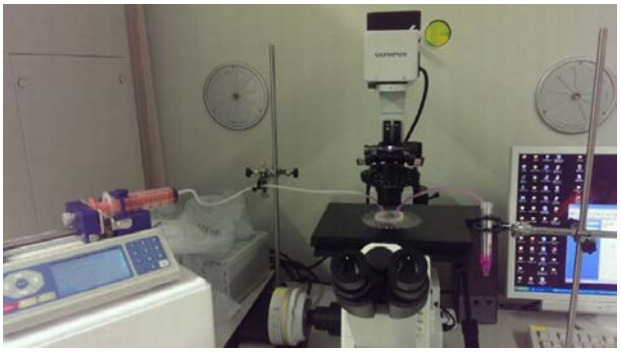


Fig. 2: Flow test system: flow chamber and microscope (middle), syringe pump (left).

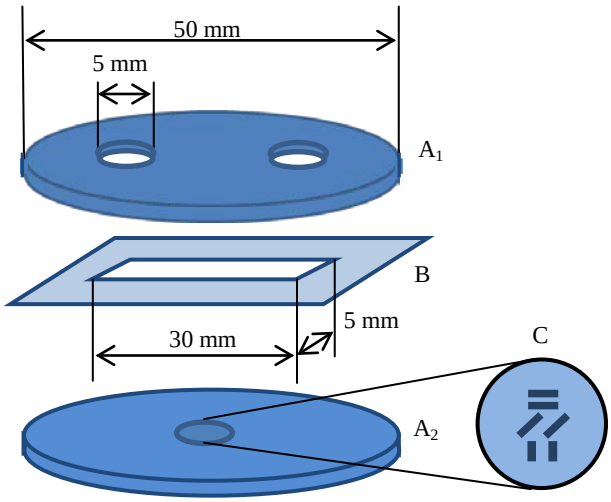


Fig. 3: The flow channel consists of two transparent polydimethylsiloxane (PDMS) disks (A_1, A_2) and a thin silicone rubber sheet (B). C is magnified pattern on A_2.

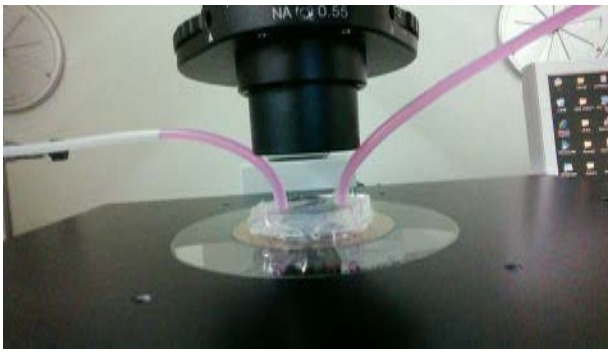


Fig. 4: The flow channel on the stage of microscope.

At the upper disk, two holes of 5 mm diameter (Fig. 4) are machined by a punching tool. The silicone tube is inserted to each hole of the upper PDMS disk. To seal the leak at the gap between elements, the liquid of PDMS was pasted on the junction of elements, and baked at 333 K for 90 minutes in an oven.

One of the tubes is connected to the syringe pump, and the other tube is connected to the reservoir of the suspension (Fig. 2). The chamber is placed on the inverted phase-contrast microscope (IX71, Olympus Co., Ltd., Tokyo).

Flow Test

Two types of biological cells were used in the test alternatively: C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse), or 3T3-L1 (mouse fat precursor cells).

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. Before the flow test, the cells were exfoliated from the plate of the culture dish with trypsin, and suspended in the D-MEM.

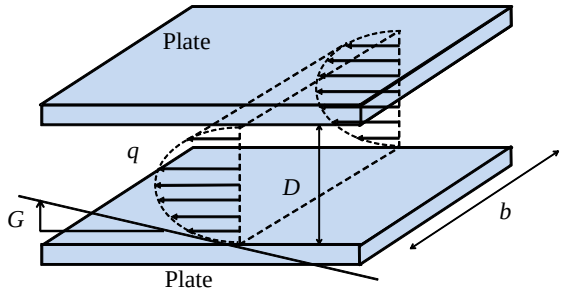


Fig. 5: Parabolic velocity profile between parallel plates.

The suspension of 4000 cells/cm^3 was introduced to the channel at the constant flow rate of 2.8 $\times 10^{-11}$ m^3/s with the micro syringe pump.

The flow path was carefully examined to avoid mixing of air bubbles, which might stir the medium in the flow channel. The behavior of cells moving over the grooves in the channel was observed with the microscope.

Shear Rate on Wall

The shear rate (G, [s^{-1}]) on the wall of the disk is calculated by Eq. 1, in which a parabolic velocity profile between parallel walls is hypothesized (Fig. 5).

$$G = 6\, q / (b\, D^2) \qquad (1)$$

In Eq. 1, q is the flow rate [m^3 s^{-1}], b is the width of the canal [m] and D is distance [m] between two parallel walls. In the present study, D is 0.1 mm, and b is 5 mm. The wall shear rate G is 3 s^{-1}, when the flow rate q is 2.8 $\times 10^{-11}$ m^3/s. The mean flow velocity (v) in the cross section of the channel is calculated by Eq. 2. In the present study, v is 0.00006 m/s.

$$v = q / (b\, D) \qquad (2)$$

3. RESULTS

The manufactured micro grooves observed by the inverted phase-contrast microscope are shown in Fig. 6. The laser measurement of surface morphology of the pattern (Fig. 7) shows the following dimension: the depth of the groove scatters between 0.00197 mm and 0.00206 mm, the width of the groove scatters between 0.026 mm and 0.028 mm, the length scatters between 0.196 mm and 0.198 mm.

The flow tests show the following results.

The moving cells over the grooves are able to be observed with the microscope in the flow test system. The diameter of the suspended cell is approximately 0.015 mm. The trapped time of the cell is measured compared with the movement of floating cell in the main flow.

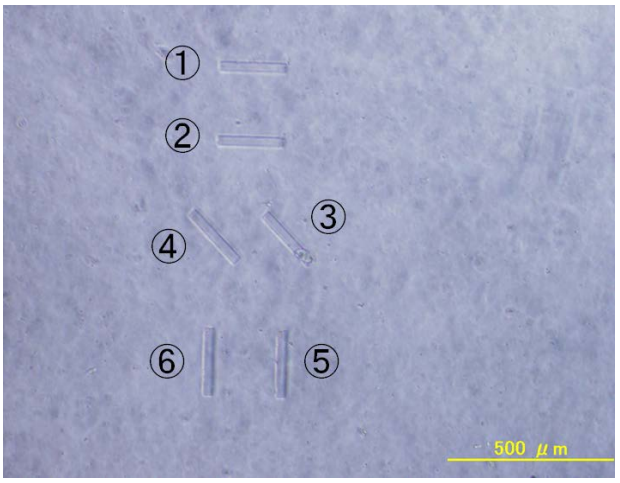


Fig. 6: Manufactured six grooves. Flow direction is horizontal.

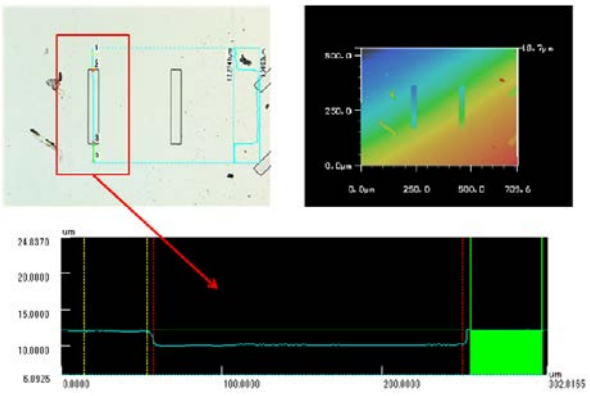


Fig. 7: Laser measurement of morphology of groove. Cross section (bottom) at longitudinal line A-B of groove.

In the case of C2C12, one of the cells is trapped in the groove of 45 degrees for four seconds in the flow rate of 2.8 $\times 10^{-11}$ m^3/s (Fig. 8). Another cell is trapped in the perpendicular groove for twenty-three seconds in the flow rate of 2.8 $\times 10^{-11}$ m^3/s (Fig. 9).

In the case of 3T3-L1, one of the cells moves along the fringe of the groove of zero degree for eight seconds in the flow rate of 2.8 $\times 10^{-11}$ m^3/s (Fig. 10). Another cell is trapped in the groove of 45 degrees for two seconds in the flow rate of 2.8 $\times 10^{-11}$ m^3/s (Fig. 11).

C2C12 tends to be trapped in the groove and hold still for longer time than 3T3-L1.

4. DISCUSSION

The photolithography technique has been applied to fabricate the micro channel. The microfluidic system has been applied to sort biological cells [17], and to trap biological cells [18, 21, 22]. The system also used to study local environment around the cultured cell [7]. The micro pattern of the surface has been applied to study the surface effect of adhesion of cells [6].

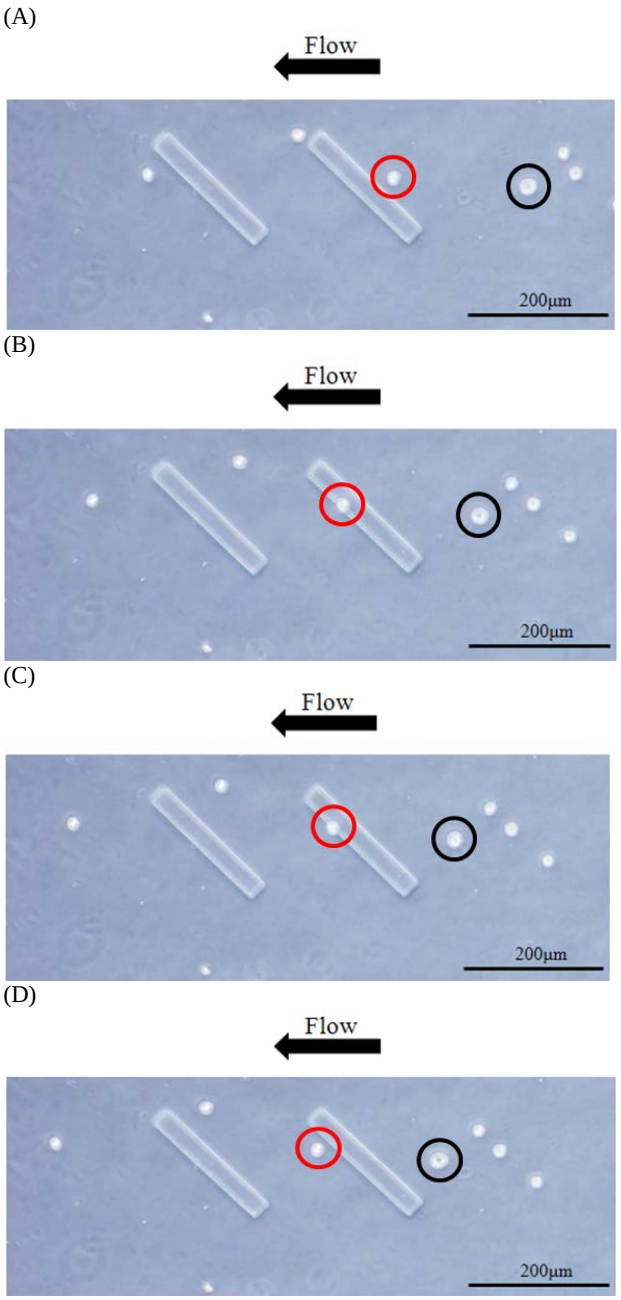
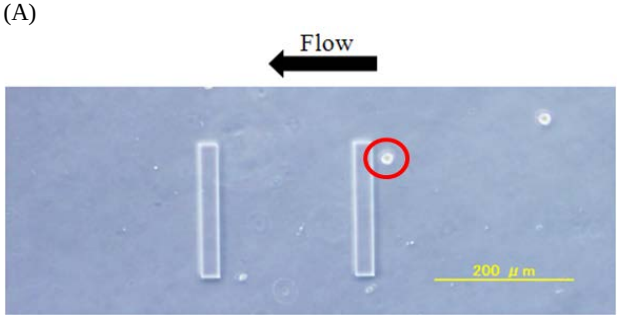


Fig. 8: C2C12 in red circle approaches to groove (A), is trapped in groove for 4 s (B, C), and leaves groove (D). Another C2C12 in black circle moves with main flow (2.8 $\times 10^{-11}$ m^3/s) from right to left.



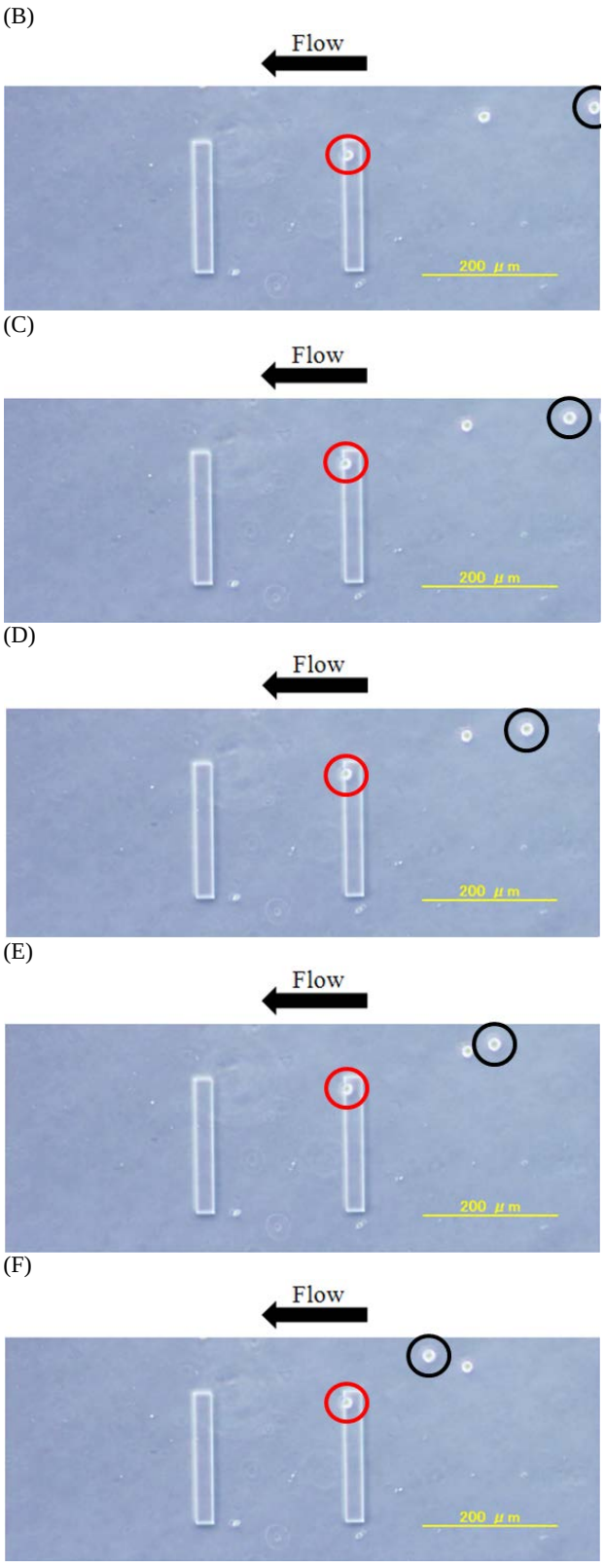


Fig. 9: C2C12 in red circle approaches to groove (A), and is trapped in groove (B-F) for 23 s. Another C2C12 in black circle moves with main flow (2.8 $\times 10^{-11}$ m^3/s) from right to left.

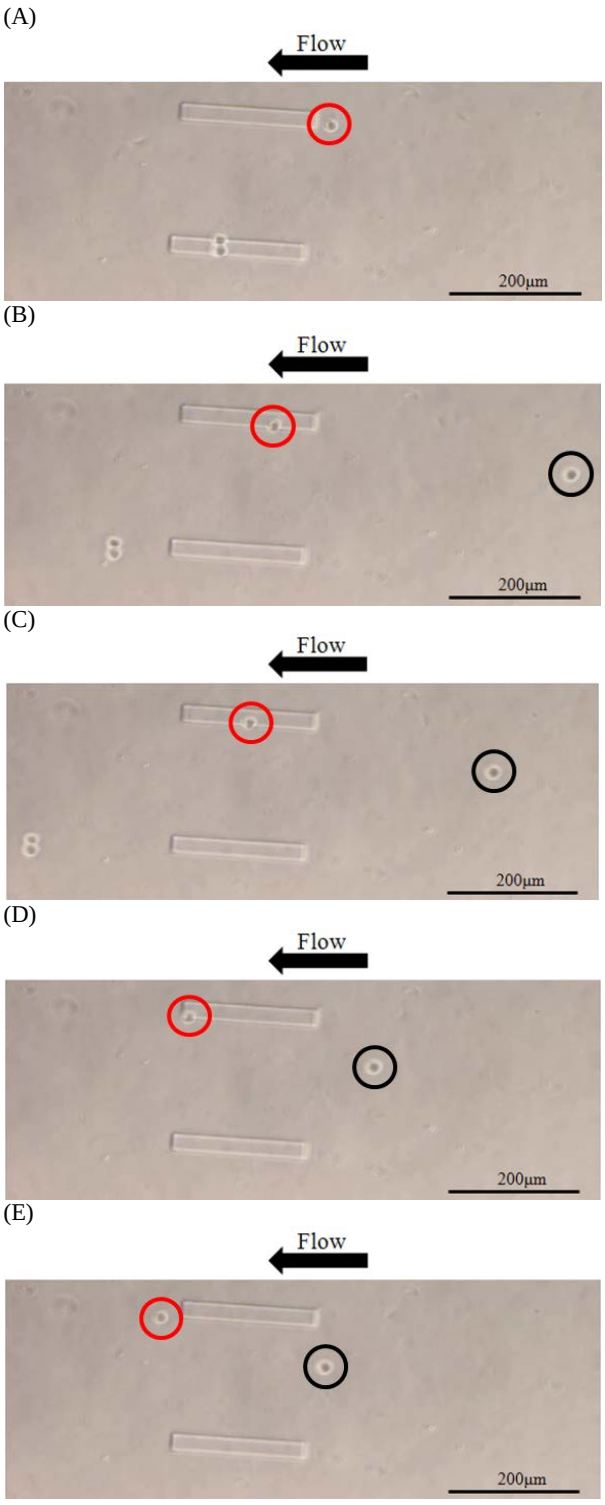


Fig. 10: 3T3-L1 in red circle approaches to groove (A), traces fringe of groove for 8 s (B-D), depart from groove (E). Another 3T3-L1 in black circle moves with main flow (2.8 $\times 10^{-11}$ m^3/s) from right to left.

The morphology of micro channel has simulated the lymph system in the circulatory system *in vivo*. In several studies, permeability has been tried to control in designing artificial vessels.

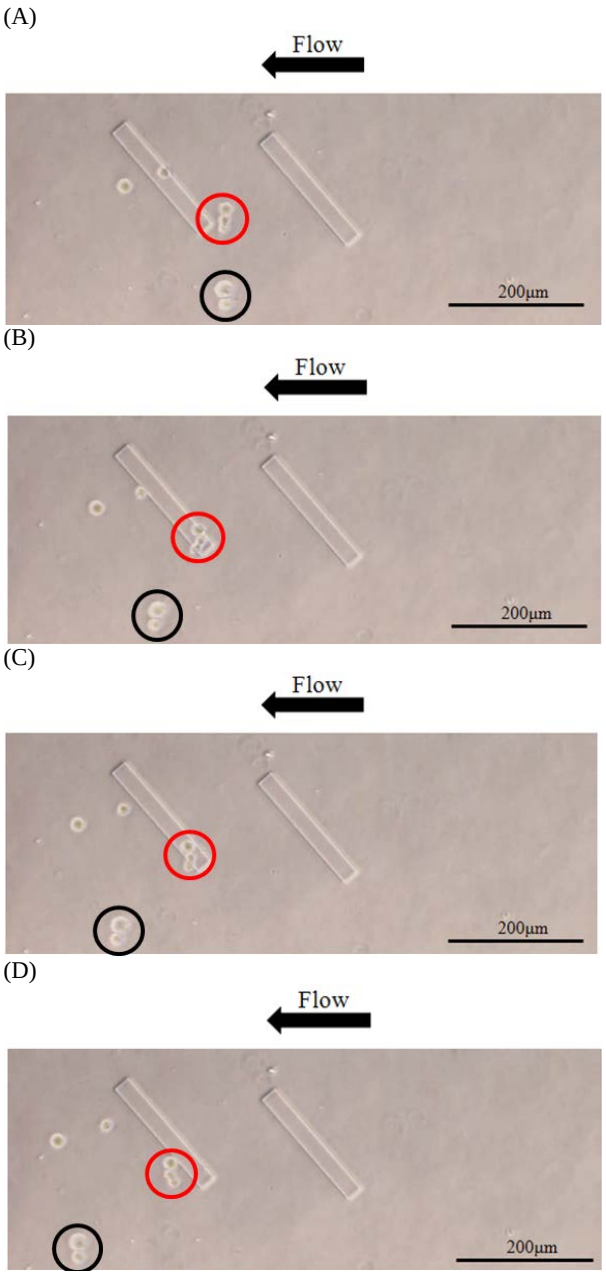


Fig. 11: 3T3-L1 in red circle approaches to groove (A), is trapped in groove for 2 s (B, C), leaves groove (D). Another 3T3-L1 in black circle moves with main flow (2.8 $\times 10^{-11}$ m^3/s) from right to left.

The experimental results might contribute to analyze adhesive mechanism of cancer cell during metastasis. The micro trap might simulate adhesive mechanism of flowing cells.

In the previous study, cylindrical [10] and half cylindrical [11] holes were used for trap of cells. The asymmetrical hole might be suitable for trap than the symmetrical hole. The depth of the micro holes was 0.01 mm in the previous study [10]. In the present study, the depth of the grooves of 0.002 mm is smaller than diameter of the cells. The deeper hole may have advantage to trap cells. The shallow trap, on the other hand, may distinguish cells. The duration of the trapped time of the

cell might relate to interaction between the micro hole and the cell: adhesiveness between the cell and the surface of the micro pattern, or deformability of the cell.

The property of the surface might govern the affinity between the surface and the cell. When the air trapped in the groove, every cell moves along the boundary of the groove. Behavior of biological cells might depend on several factors: magnetic field [1], electric field [2], or mechanical field [3-5].

5. CONCLUSION

Micro groove has been designed to trap a biological cell, which flows through a micro channel *in vitro*. The micro groove of a rectangular shape has been fabricated on the surface of the polydimethylsiloxane disk with the photolithography technique. Two types of biological cells were used in the test alternatively: C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse), or 3T3-L1 (mouse fat precursor cells). The results show that the cell is trapped with the micro grooves and that the trapped interval depends on the kind of cells.

6. ACKNOWLEDGMENT

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