Micro Groove for Trapping of Flowing Cell

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ABSTRACT

Micro grooves have been designed to trap a biological cell, which flows through a micro channel *in vitro*. Each micro groove of a 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, 0.79, or 1.57 rad. 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 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). A constant flow (2.8×10^{-11} m^3/s) of a suspension of cells was introduced with a syringe pump. The behavior of cells moving over the micro grooves 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 the cell depends on the various environmental factors [1-6]. 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.

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

A photolithography technique enables manufacturing a micro-channel. The effect of the surface of the scaffold on the cell culture has been reported in the previous studies [7-12]. Several micro-fabrication processes have been designed to control adhesion of biological cells *in vitro* [8-17], and to simulate morphology of microcirculation [18].

The micro-fabrication technique has also been applied to microfluidic systems *in vitro* [19-25]. Cells roll on the surface of the wall in the shear flow, and make adhesion to the wall [16]. The surface was modified to capture flowing cells [13, 14, 26, 27]. The technique will also be applied to handle cells in diagnostics *in vitro* [28].

In the present study, micro grooves have been designed to trap a biological cell, which flows through a micro channel *in vitro*.

2. METHODS

Micro Traps

For trapping cells, several micro grooves have been fabricated on the surface of the polydimethylsiloxane (PDMS) disk with the photolithography technique. Six grooves are arranged in two lines. Each groove has the rectangular shape: 0.002 mm depth, 0.025 mm width and 0.2 mm length. 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, 0.79, or 1.57 rad (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.

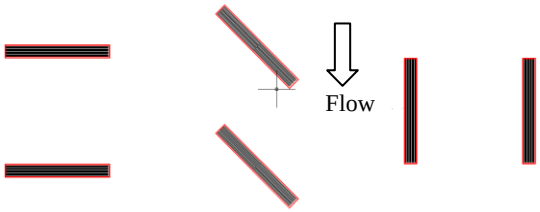


Fig. 1: Six grooves. The arrow shows flow direction.

The photo-resist was developed with SU8 developer (Nippon Kayaku Co., Ltd, Tokyo, Japan) for six minutes to make the 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.

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 surface morphology along the longitudinal and the transverse lines of each ridge was traced. The convex mold with the 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 curing agent was nine percent of PDMS. 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 shear rate at the wall of PDMS [4, 29]. 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 (B in Fig. 3), which has a rectangular void of 5 mm × 30 mm, is sandwiched between two transparent PDMS plates (A_1 and A_2 in Fig. 3). The void forms a channel of 30 mm length × 5 mm width × 0.1 mm depth. The three parts stick together with their surface affinity.

The lower PDMS disk, which has the micro grooves at the center of the upper surface (C in Fig. 3), was placed on the inner bottom of a culture dish of 50 mm diameter. At the upper disk, two holes of 5 mm diameter are machined by a punching tool. The silicone tube is inserted to each hole of the upper PDMS disk (Fig. 4). 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.

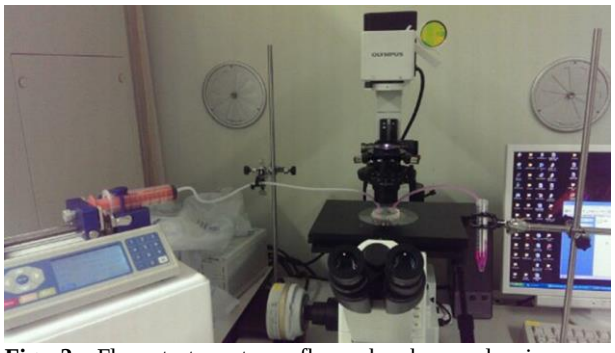


Fig. 2: Flow test system: flow chamber and microscope (middle), syringe pump (left).

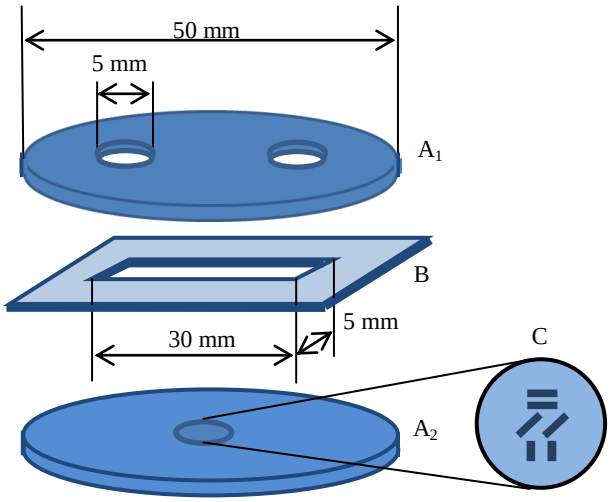


Fig. 3: Flow channel consists of two transparent polydimethylsiloxane (PDMS) disks A and a thin silicone rubber sheet B.

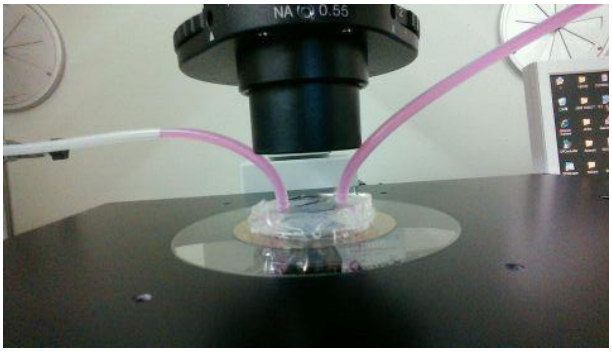


Fig. 4: Flow channel on the stage of microscope.

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% of 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.

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.

The purified water contact angles on PDMS were measured by the contact angle analyzer (Phoenix-300, Meiwafosis Co., Ltd., Tokyo, Japan) in the air.

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).

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

In Eq. 1, q is the flow rate [$m^3\ s^{-1}$], b is the width of the channel [m] and D is the 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 0.00006 m/s (Eq. 2).

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

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.

In the case of C2C12, one of the cells is trapped in the groove of 0.79 rad 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).

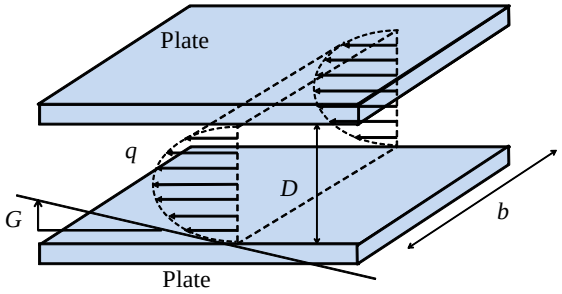


Fig. 5: Parabolic velocity profile between parallel plates. D is distance between two parallel walls, q is flow rate, b is width of channel and G is shear rate on the wall.

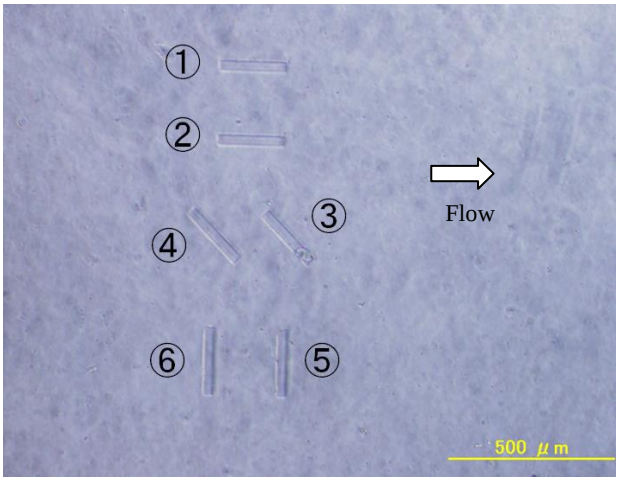


Fig. 6: Manufactured six grooves. Arrow shows flow direction.

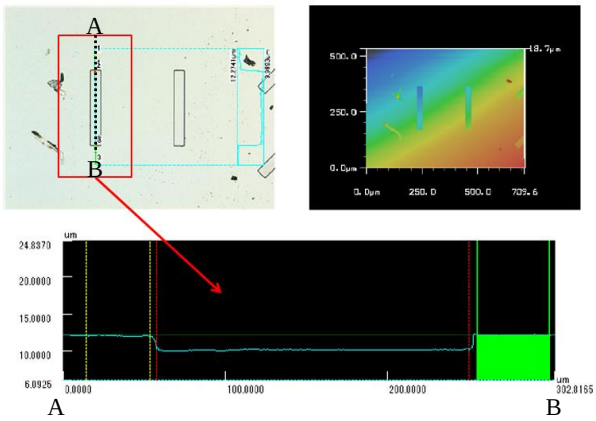


Fig. 7: Laser measurement of morphology of groove. Cross section (bottom) at longitudinal line A-B of groove.

In the case of 3T3-L1, one of the cells moves along the fringe of the groove of zero rad 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 0.79 rad for two seconds in the flow rate of 2.8 $\times 10^{-11}$ m^3/s (Fig. 11).

Fig. 12 exemplifies the distance between two cells of C2C12 along the main flow direction. The distance would be constant, if they move together at the same velocity. The distance decreases, while the front cell is trapped in the groove. Fig. 13 exemplifies velocity of C2C12, which is not trapped in the groove. The figure shows that the velocity of the rear C2C12 slightly decreases.

Fig. 14 exemplifies the distance between two cells of 3T3-L1 along the main flow direction. The distance increases, before the front cell is trapped in the groove. C2C12 tends to be trapped in the groove for longer time than 3T3-L1.

The time trapped in the micro groove is listed in the Table 1. C2C12 tends to be trapped in the micro groove for the longer period than 3T3-L1.

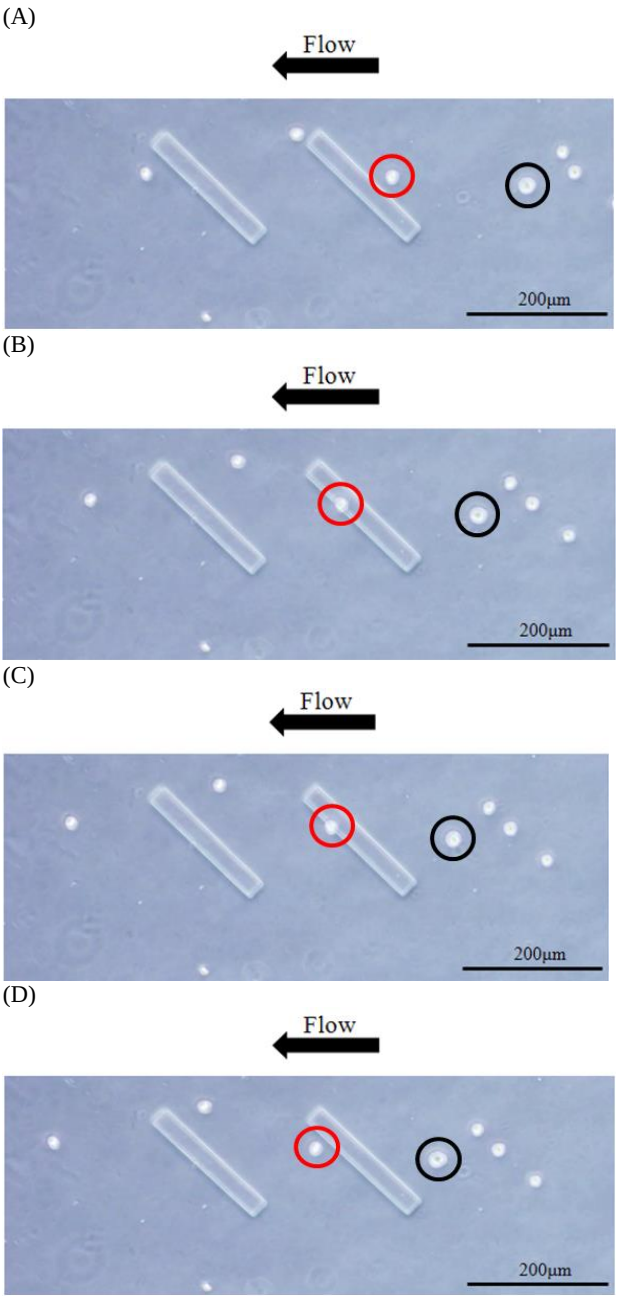
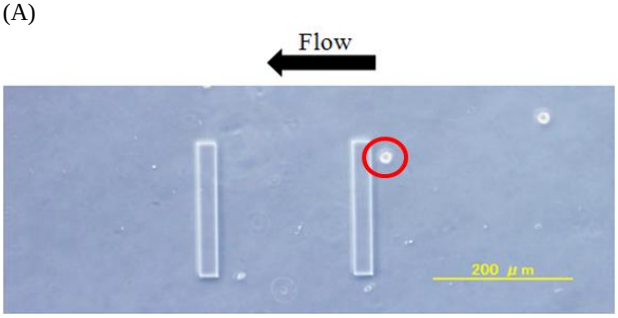


Fig. 8: C2C12 in red circle approaches to groove (A), is trapped in groove for 4 s (B, C), leaves groove (D). Another C2C12 in black circle moves with main flow (2.8×10^{-11} m^3/s) from right to left.



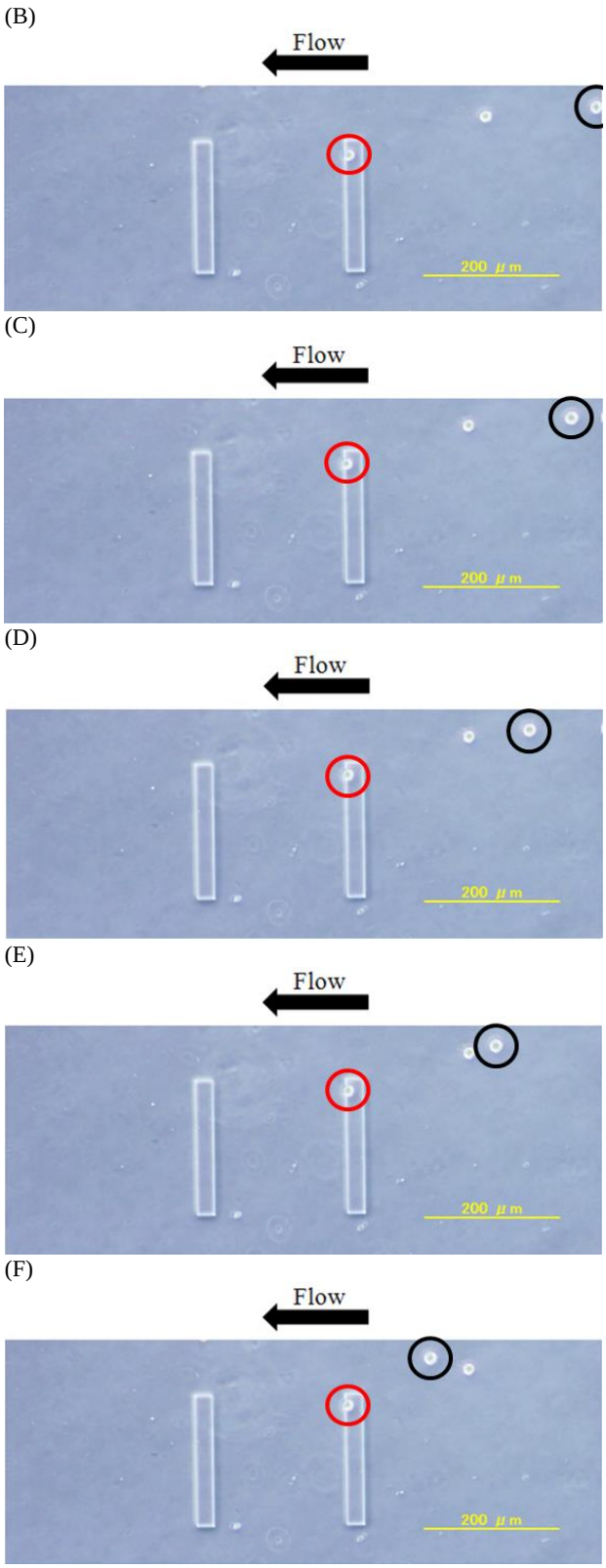


Fig. 9: C2C12 in red circle approaches to groove (A), is trapped in groove (B-F) for 23 s. Another C2C12 in black circle moves with main flow (2.8×10^{-11} m^3/s) from right to left.

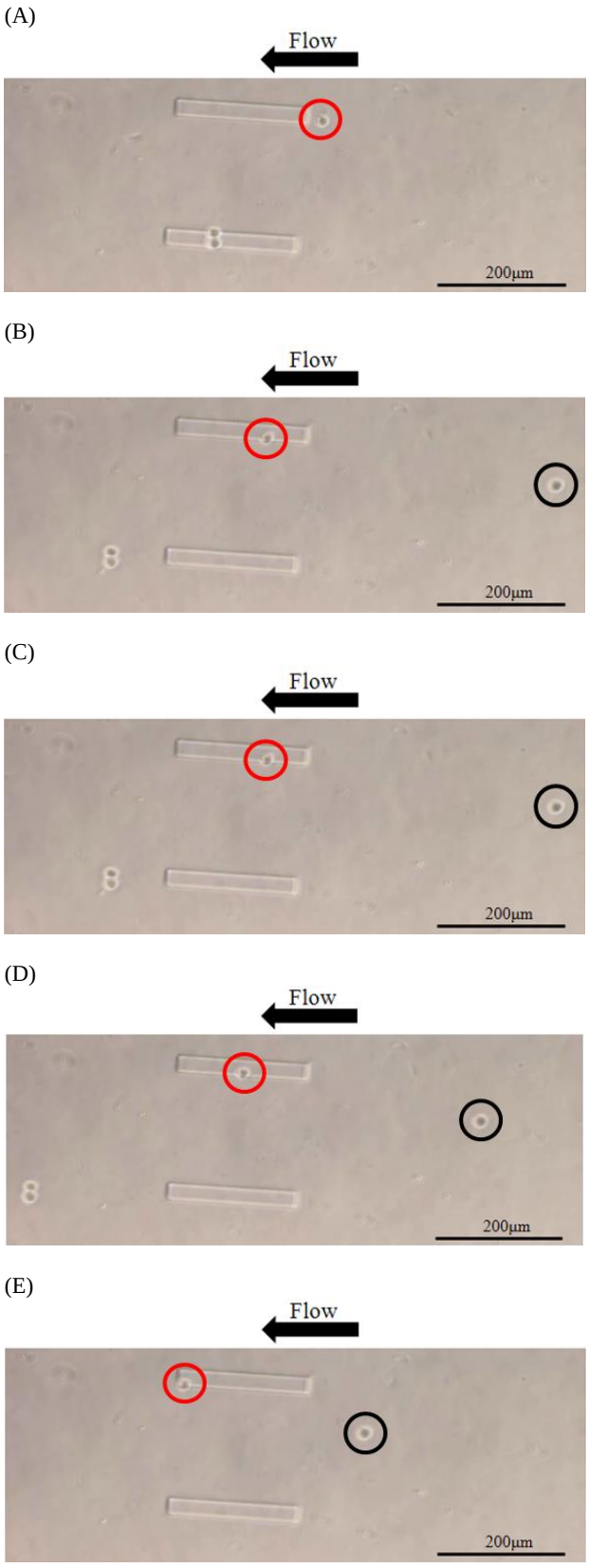


Fig. 10: 3T3-L1 in red circle approaches to groove (A), traces fringe of groove for 8 s (B-D), depart from groove (E). Another 3T3-L1 in black circle moves with main flow (2.8 $\times 10^{-11}$ m^3/s) from right to left.

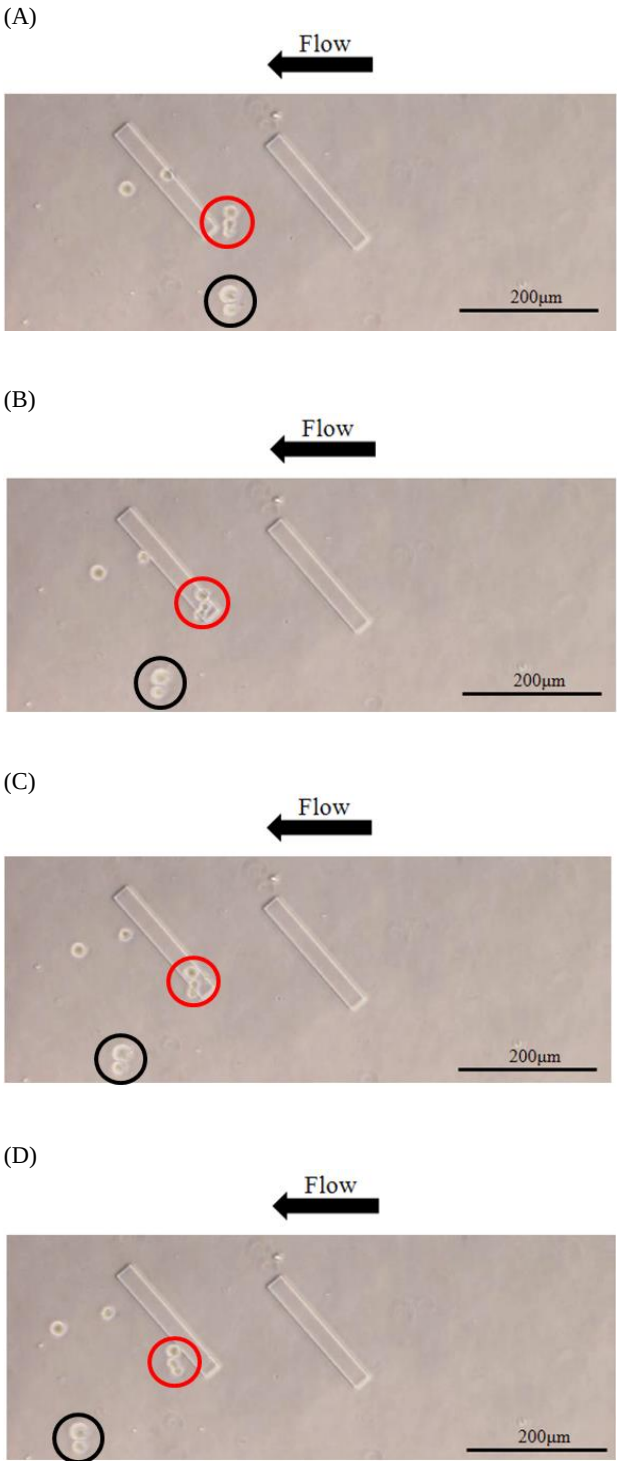


Fig. 11: 3T3-L1 in red circle approaches to groove (A), is trapped in groove for 2 s (B, C), leaves groove (D). Another 3T3-L1 in black circle moves with main flow (2.8 $\times 10^{-11}$ m^3/s) from right to left.

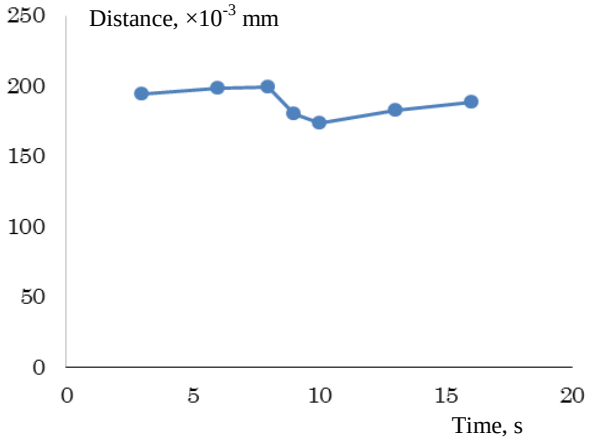


Fig. 12: Distance between two cells (C2C12) along the flow direction vs. time. A cell is trapped between 8 s and 10 s.

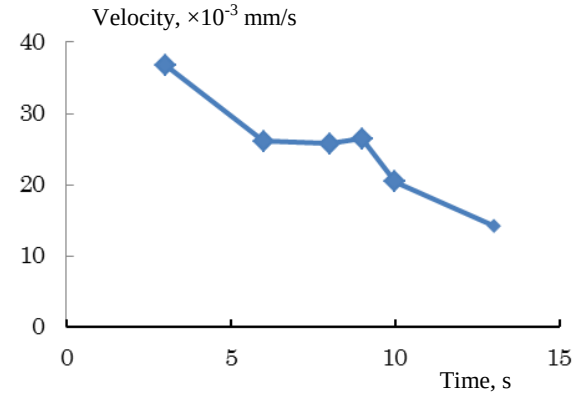


Fig. 13: Velocity of C2C12 vs. time.

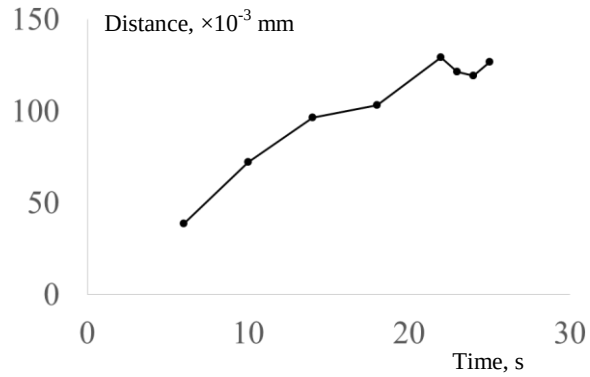


Fig. 14: Distance between two cells (3T3-L1) along the flow direction vs. time. A cell is trapped between 22 s and 24 s.

Table 1: Time trapped in the micro groove.

	0 rad	0.79 rad	1.57 rad
C2C12	none	4 s	23 s
3T3-L1	8 s	2 s	none

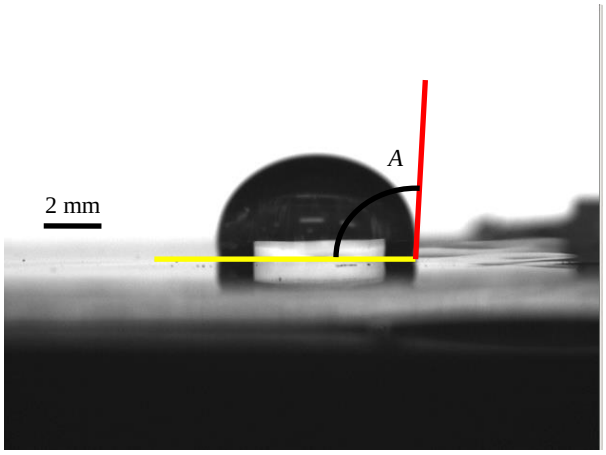


Fig. 15: Contact angle (*A*) of water on PDMS.

Fig. 15 shows the contact angle of 1.90 rad of the water on PDMS. The angle of 1.90 rad, which is larger than 1.57 rad, shows that the surface of PDMS is hydrophobic.

4. DISCUSSION

The photolithography technique has been applied to fabricate the micro channel. The microfluidic system has been applied to sort biological cells [22], and to trap biological cells [23, 26, 27]. The system also used to study local environment around the cultured cell [7, 10]. The micro pattern of the surface has been applied to study the surface effect of adhesion of cells [8, 9].

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. 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 [13] and half cylindrical [14] holes were used for the trap of cells. The asymmetrical hole might be more suitable for the trap than the symmetrical hole.

The depth of the micro holes was 0.01 mm in the previous study [13]. In the present study, the depth of the grooves is 0.002 mm, which is smaller than the diameter of the cells. The deeper hole may have an advantage to trap cells. The shallow trap, on the other hand, may distinguish cells. The duration of the trapping 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, roughness of the surface, or deformability of the cell.

The property of the surface might govern the affinity between the surface and the cell. The property of the outer surface of the membrane of the cell can be changed. The damaged cell might show different behavior on the micro groove. The damaged cell might stay in the groove long time.

When the air bubble is 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 [3-6]. The behavior of cells on the transparent conductive film can be observed by the microscope [30].

Deformability of the cell might govern the behavior of cell in the flow. Deformability can be measured during passing through the narrow slit [31]. The biochemical analysis has been tried at the local area in the cell [32].

The roughness of the surface of the bottom of the groove might be higher than that of the other surface of the flow path. The surface except the groove is made by the flat surface of the mold of the silicon wafer. The velocity of the cell might decrease, when the cell moves in the groove. The behavior is observed in the groove parallel to the main flow in the present study.

The velocity profile between the parallel walls is approximated to be parabolic in the present study, because the distance between two parallel walls (D = 0.1 mm) is very small compared with the width of the channel (b = 5 mm).

5. CONCLUSION

A 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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