Micro Trap for Flowing Cell

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ABSTRACT

A micro channel has been designed to trap flowing biological cells *in vitro*. Several micro traps of a cylindrical hole of 0.01 mm depth (diameter between 0.01 mm and 0.04 mm) have been fabricated on the surface of the polydimethylsiloxane disk with the photolithography technique. A rectangular flow channel (0.1 mm depth × 1 mm width × 20 mm length) has been made of the disk with a silicone film of 0.1 mm thick. Two types of biological cells were used in the test alternatively: L929 (fibroblast-like, mouse connective tissue, RCB1422, Riken Bio Resource Center, Tsukuba) or C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse). A constant flow of a suspension of cells was introduced with a syringe pump. The behavior of cells moving over the micro holes was observed with an inverted phase contrast microscope. The experimental results show that the cell is trapped with the micro hole under the wall shear rate less than 80 s^{-1}.

Keywords: Biomedical Engineering, C2C12, L929, Micro Trap and Polydimethylsiloxane.

1. INTRODUCTION

Recently, one per three persons dies according to cancer in Japan. A biological cell alters to a cancer cell by an internal or an external factor. The alteration is called "canceration". The cancer cell is out of the control system of the whole body. The cancer cell has several characters: morphological change, infinite proliferation, tumorigenic transformation, and metabasis. Metabasis often causes the cancer recurrence, which leads to patient's death. The tumor permeates through the lymph vessels. The cancer cell transits from the original place to another place, and proliferates to make another tumor. The transition occurs through the blood vessels and the lymph vessels. The cancer cells adhere to the endothelial cells, which cover the inner wall of the vessels. The lymph nodes are clinically ablated to avoid metastasis. Wastes and bacteria are transported through the lymph vessel and dissolved at the lymph node. Because the basement membrane of the lymph vessel is thin enough to take in things, the cancer cell easily metastasizes through the membrane.

The photolithography technique enables manufacturing a micro-channel [1, 2]. The effect of the surface of the scaffold on cell culture has been studied in the previous studies [3-6]. Several micro-fabrication processes have been designed to control adhesion of biological cells *in vitro* [4-6], and to simulate morphology of microcirculation [7]. The micro-fabrication technique has also been applied to design microfluidic systems *in vitro* [8-13]. The technique will also be applied to handle cells in diagnostics *in vitro*. In the present study, a micro channel has been designed to trap flowing biological cells *in vitro*.

2. METHODS

Micro Traps

Several micro traps (Fig. 1) of a cylindrical hole of 0.01 mm depth (diameter between 0.01 mm and 0.04 mm) have been fabricated on a polydimethylsiloxane (PDMS) disk with the photolithography technique (Fig. 2).

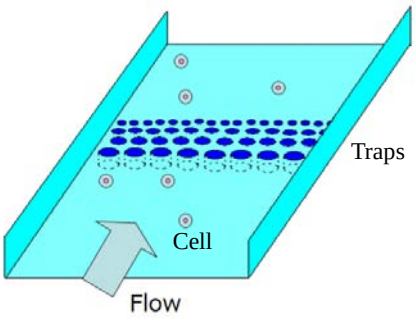

Fig. 1: Several micro traps in flow chamber.

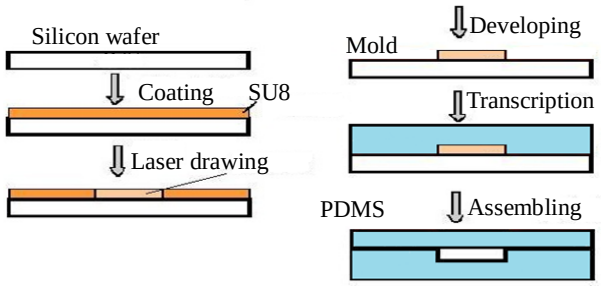

Fig. 2: Photolithography technique.

A silicon wafer (Type N, Matsuzaki Seisakusyo, Co., Ltd., Tokyo, Japan) is used for a surface mold for the disk (Fig. 3). The diameter and the thickness of the wafer are 50 mm and 0.30 mm, respectively.

The surface of the wafer is cleaned three times: with the isopropyl alcohol for ten minutes, with hydrogen peroxide solution for ten minutes, and ultrapure water for ten minutes. Then, the wafer was dried on the hot plate (PXW-4, Asahi-rika, Chiba, Japan) at 383 K for 10 minutes, and exposed to the oxygen gas in a reactive ion etching system (FA-1, Samco Inc., Kyoto) to be characterized as hydrophilic (oxygen plasma ashing).

The photo-resist material of SU8 10 (Micro Chem Corp., MA, USA) was coated on the wafer with 0.015 mm thick at 1000 rpm with a spin coater. The photo-resist was baked on the heated plate with two processes: at 338 K for 3 minutes, before at 368 K for 7 minutes.

The pattern of holes (Fig. 4) to make columns 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 0.1 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 368 K for 3 minutes.

The photo-resist was developed with SU8 developer (Nippon Kayaku Co., Ltd, Tokyo, Japan) for five minutes to make micro columns, where the laser beam was radiated. The wafer was rinsed with the distilled water, and dried on the heated plate. To decrease remaining stress and to increase the adhesiveness of the coating, the wafer was baked at 423 K for 5 minutes.
After development, the dimension of the micro columns on the mold was measured with a laser microscope (VK-9510, Keyence Corporation, Osaka, Japan). The convex mold with micro pattern is used only for the lower disk of PDMS to make the micro holes.

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

After the wafer is enclosed with a peripheral wall of polyimide, PDMS (Dow Corning Corp., MI, USA) is poured with the curing agent on the wafer. After degassing, PDMS is baked at 353 K for two hours in an oven (Fig. 5).

The diameter of two PDMS disks is 50 mm. The thicknesses of the upper and the lower disks are 10 mm and 2 mm, respectively. At the upper disk, two holes of 5 mm diameter (Fig. 6) are machined by a punching tool. The silicone tubes are stuck at the holes without an adhesive.

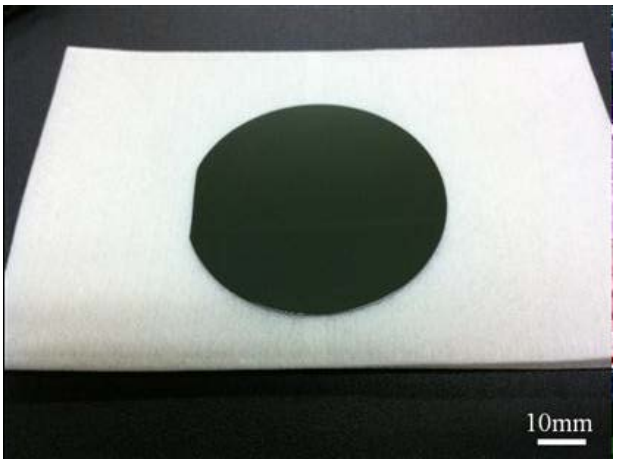

Fig. 3: Silicon wafer (diameter: 50 mm).

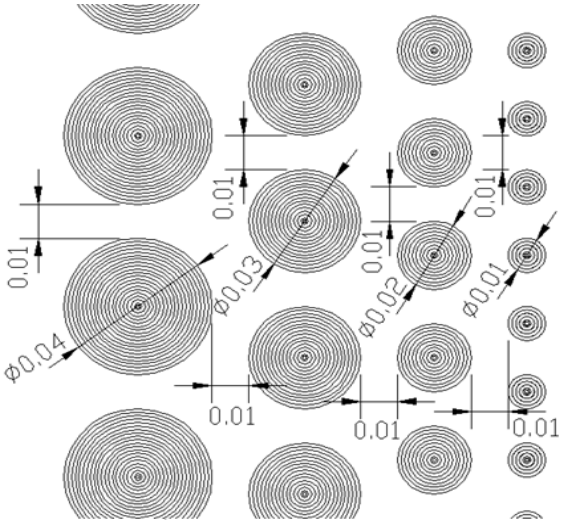

Fig. 4: Pattern of holes: dimension (mm).

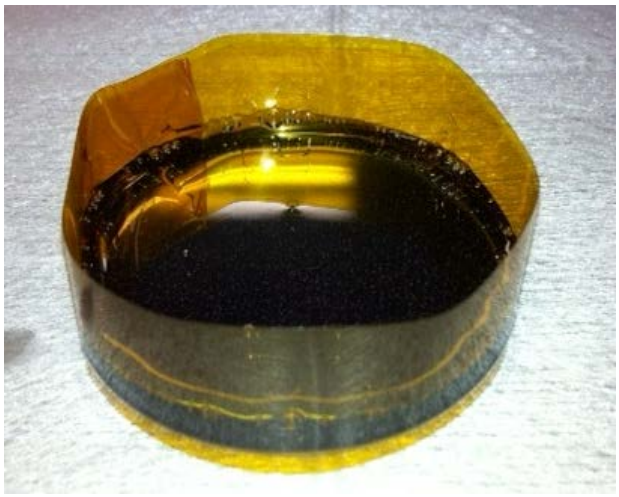

Fig. 5: PDMS baked on the mold.

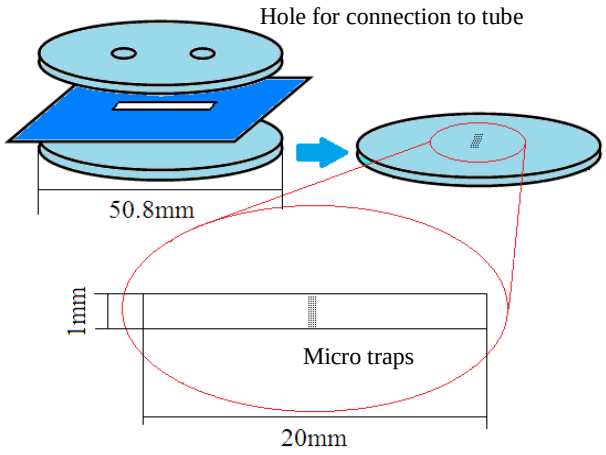

Fig. 6: The flow chamber consists of two transparent polydimethylsiloxane (PDMS) disks and a thin silicone rubber sheet.

Flow System

A one-way flow system is designed to control the wall shear rate at the disk of PDMS (Fig. 7) [14]. The system consists of a flow chamber, a micro syringe pump (International Scientific Instruments Supply Co., Ltd.), tubes and a microscope (Fig. 8). A plastic tube of 3 mm internal diameter and of 5 mm external diameter is used for the connector to the flow chamber. The flow chamber consists of two transparent polydimethylsiloxane (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 1 mm × 20 mm, is sandwiched between the PDMS plates (Fig. 6). The void space forms a channel of 20 mm length × 1 mm width × 0.1 mm depth. The three plates stick together with their surface affinity without adhesives. The inner surface of the chamber was exposed to the oxygen gas in a reactive ion etching system (FA-1, Samco Inc., Kyoto) to be characterized as hydrophilic (oxygen plasma ashing), before assembled.

Immediately after the characterization, the flow path of the chamber was rinsed with a saline solution, and the suspension of cells was introduced, successively.

One of the tubes is connected to the plastic syringe pump (Fig. 8). The room temperature was maintained at 25 degrees Celsius. 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: L929 (fibroblast-like, mouse connective tissue, RCB1422, Riken Bio Resource Center, Tsukuba) or C2C12 (Mouse myoblast cell line originated with cross-striated muscle of C3H mouse).

L929 was cultured on a dish with the E-MEM (Eagle's minimal essential medium) containing 10% FBS (fetal bovine serum) 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 E-MEM.

C2C12 was cultured on a dish with the D-MEM (Dulbecco's Modified Eagle Medium) containing 10% FBS 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.

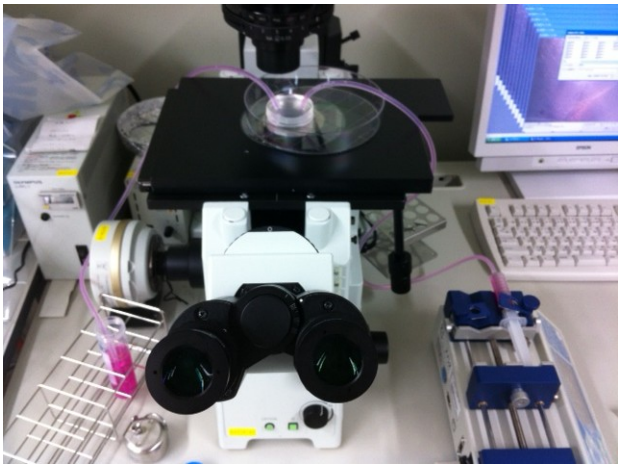

Fig. 7: Flow test system: flow chamber and microscope (middle), syringe pump (right).

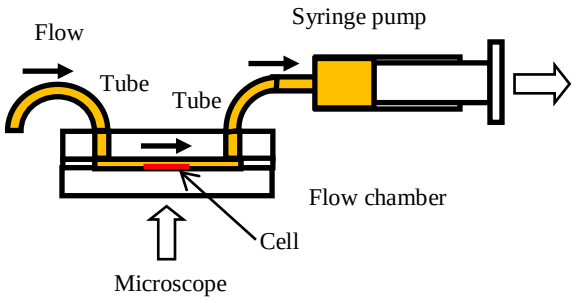

Fig. 8: Flow to syringe pump through flow chamber.

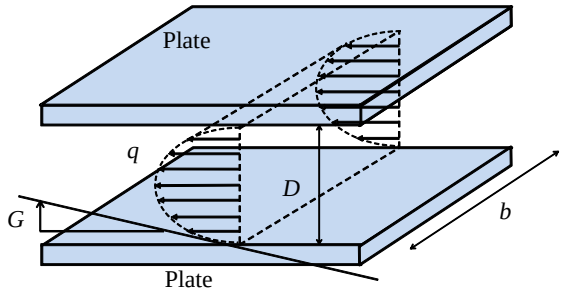

Fig. 9: Parabolic velocity profile between parallel plates.

The suspension was introduced to the chamber at the constant flow. Variation was made on the flow rate: 0.3 $\times 10^{-10}$ m^3/s, 0.7 $\times 10^{-10}$ m^3/s, 1.4 $\times 10^{-10}$ m^3/s and 2.8 $\times 10^{-10}$ m^3/s. Variation was made on the direction of the flow: from large holes to small holes, and vice versa.

The flow path was carefully examined to avoid mixing of air bubbles, which might stir the medium in the flow chamber and induce exfoliation of cells. The behavior of cells on the plate of the chamber 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 plates is hypothesized (Fig. 9).

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

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 1 mm (Fig. 9). The wall shear rate G varies from 16 to 160 per second, when the flow rate q varies from 0.3 $\times 10^{-10}$ m^3/s to 2.8 $\times 10^{-10}$ m^3/s.

3. RESULTS

The laser measurement of surface morphology of the mold shows that the diameter ranges from 0.01 mm to 0.04 mm, as the designed dimension. The laser measurement also shows that the mean height of micro columns is 0.01 mm (Fig. 10). The dimension of the height scatters around the mean value. Although the top surface of the column has asperity, the mold can be used to make micro holes on the surface of the disk of PDMS.

Figs. 11-14 exemplify L929 in the flow in the chronological order. In the figures, the circle shows interested cells, which flow to a hole. After the cells are trapped in the hole, one of the cells get out from the hole.

Figs. 15-17 exemplify C2C12 in the flow at 0.3 $\times 10^{-10}$ m^3/s, at 0.7 $\times 10^{-10}$ m^3/s and at 1.4 $\times 10^{-10}$ m^3/s, respectively. In the figure, the circle shows interested cells, which are trapped in the hole.

The observed behavior of cells is as follows. The diameter of the suspended cell is approximately 0.01 mm. When cells flow at the flow rate of 0.3 $\times 10^{-10}$ m^3/s and 0.7 $\times 10^{-10}$ m^3/s, cells roll on the surface of the disk and are trapped in the holes. When cells flow at the flow rate of 1.4 $\times 10^{-10}$ m^3/s, some of cells are trapped in the holes, while others flow pass over the holes. When cells flow at the flow rate of 2.8 $\times 10^{-10}$ m^3/s, every cell passes over the holes on the surface of the disk. Several cells, which are trapped in a hole, flow out from the hole with the flow (Fig. 14).

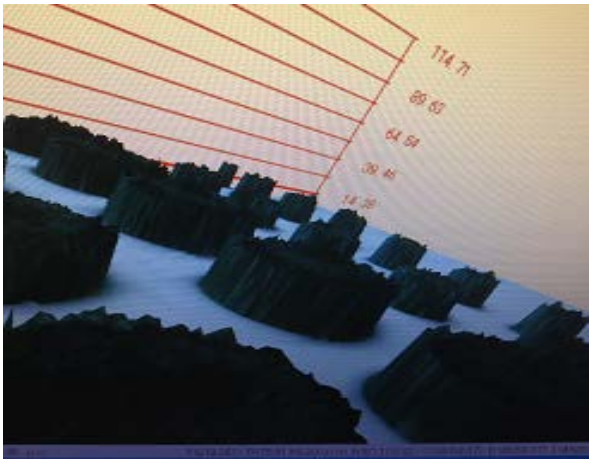

Fig. 10: Laser measurement of morphology of convex column at surface of the mold.

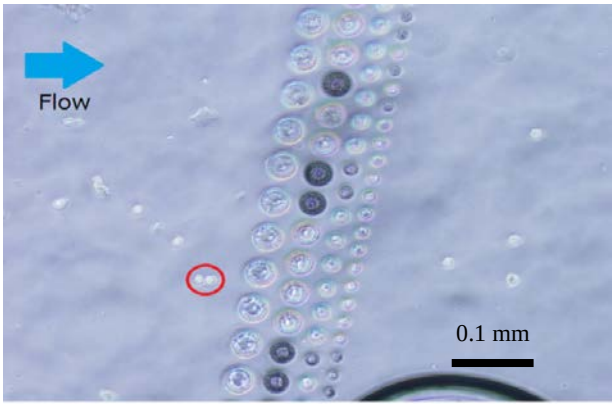

Fig. 11: L929 flows (1.4 $\times 10^{-10}$ m^3/s).

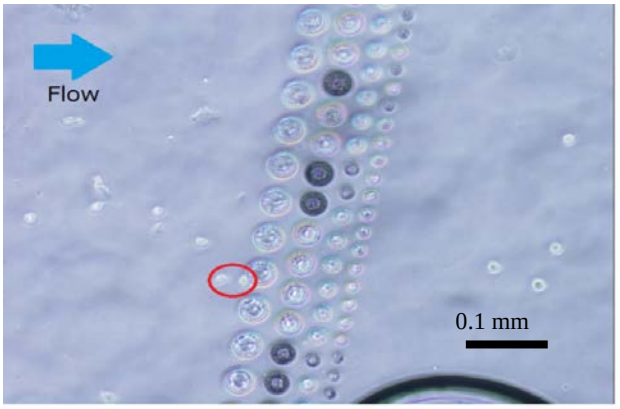

Fig. 12: L929 is going to be trapped (1.4 $\times 10^{-10}$ m^3/s).

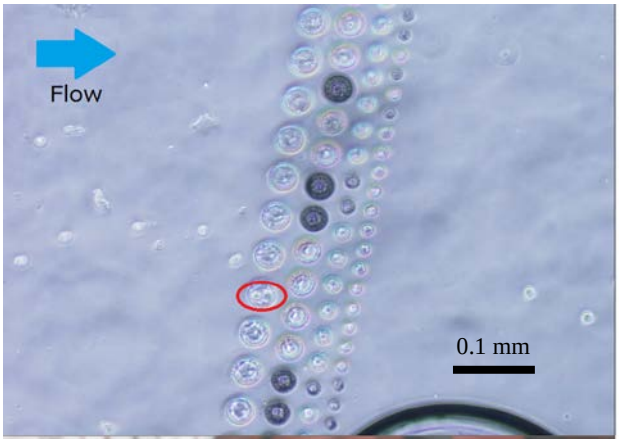

Fig. 13: L929 cells are trapped in the hole (1.4 $\times 10^{-10}$ m^3/s).

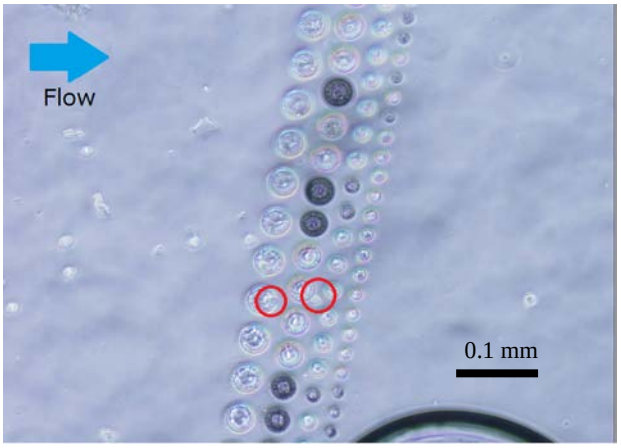

Fig. 14: L929 gets out from the hole (1.4 $\times 10^{-10}$ m^3/s).

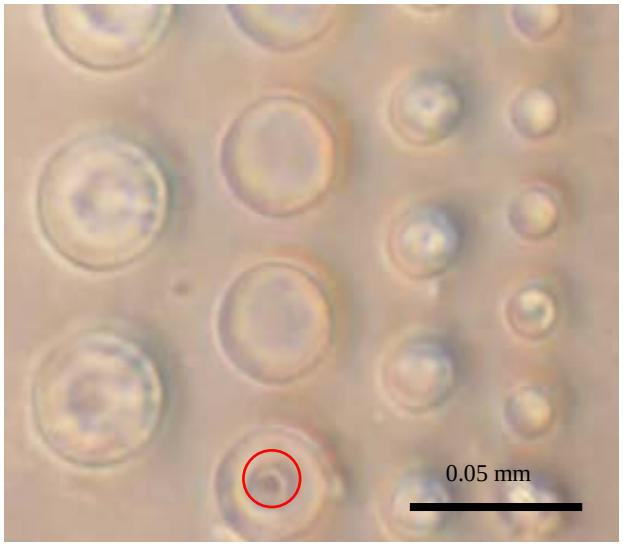

Fig. 15: C2C12 is trapped in the hole (0.3 $\times 10^{-10}$ m^3/s). Flow from right to left.

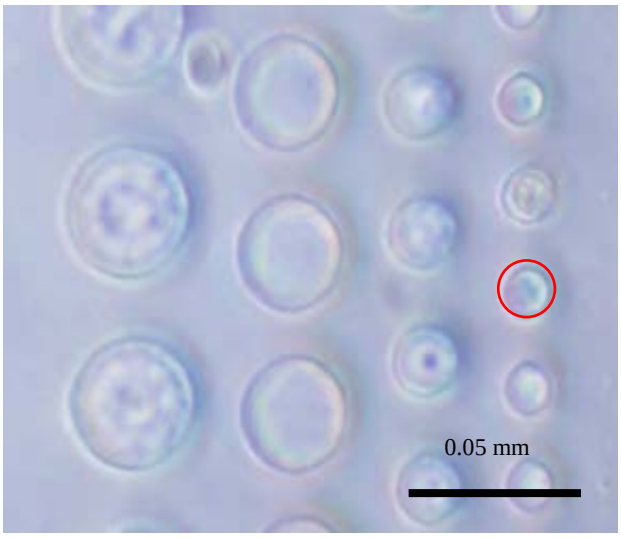

Fig. 16: C2C12 is trapped in the hole (0.7 $\times 10^{-10}$ m^3/s). Flow from right to left.

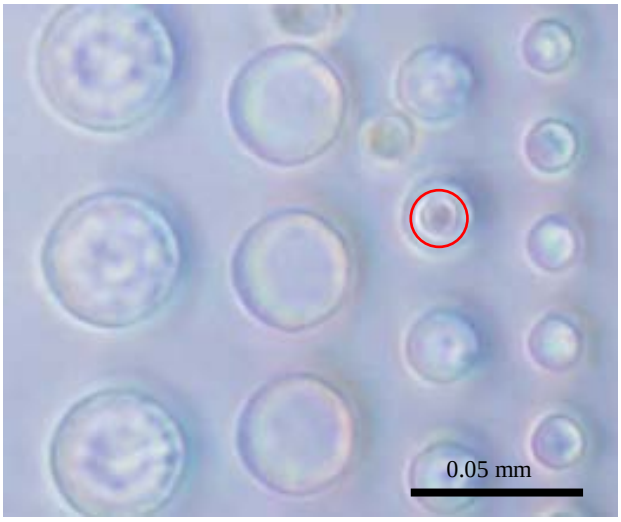

Fig. 17: C2C12 is trapped in the hole (1.4 $\times 10^{-10}$ m^3/s). Flow from right to left.

The experimental results show that a cell can be trapped with a micro hole under the wall shear rate less than 80 s^{-1}. A cell is trapped in a hole with diameter of every size between 0.01 mm and 0.04 mm, regardless of the order of holes in the flow.

4. DISCUSSION

The photolithography technique has been applied to fabricate the micro channel [1, 2]. The microfluidic system has been applied to sort biological cells [11], and to trap biological cells [12]. The system also used to study local environment around the cultured cell [3, 6]. The micro pattern of the surface has been applied to study the surface effect of adhesion of cells [4, 5].

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

5. CONCLUSION

A micro channel has been designed to trap flowing biological cells *in vitro*. Several micro traps of a cylindrical hole of 0.01 mm depth (diameter between 0.01 mm and 0.04 mm) have been fabricated on the polydimethylsiloxane disk with the photolithography technique. The experimental results show that a cell is trapped in the micro hole with the diameter of every size between 0.01 mm and 0.04 mm under the wall shear rate less than 80 per second.

6. ACKNOWLEDGMENT

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