Micro Hole for Trapping Flowing Cell

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ABSTRACT

Micro holes have been designed to trap a biological cell, which flows through a micro channel *in vitro*. Several micro traps of a half cylindrical hole of 0.002 mm depth (0.02 mm diameter) have been fabricated on the surface of the polydimethylsiloxane (PDMS) disk with the photolithography technique. A rectangular flow channel (0.1 mm depth × 1 mm width × 20 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 MC3T3-E1 (mouse osteogenic cell line). A constant flow (2.8×10^{-10} m^3/s or 8.3×10^{-10} m^3/s) 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 results show that the cell is trapped with the micro hole under the wall shear rate less than 100 s^{-1} for several seconds.

Keywords: Biomedical Engineering, C2C12, MC3T3-E1, 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 tumor in another place. 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. The effect of the surface of the scaffold on cell culture has been studied in the previous studies [1-4]. Several micro-fabrication processes have been designed to control adhesion of biological cells *in vitro* [2-8], and to simulate morphology of microcirculation [9]. The micro-fabrication technique has also been applied to design microfluidic systems *in vitro* [10-16]. Cells are rolling on the surface of the wall in the shear flow, and make adhesion to the wall [6]. The surface was modified to capture flowing cells [8, 17, 18]. The technique will also be applied to handle cells in diagnostics *in vitro* [19]. In the present study, micro holes have been designed to trap a biological cell, which flows through a micro channel *in vitro*.

2. METHODS

Micro Traps

Several micro traps of a half cylindrical hole of 0.002 mm depth (Fig. 1) have been fabricated on the surface of the polydimethylsiloxane (PDMS) disk with the photolithography technique (Fig. 2). The diameter of the half cylinder is 0.02 mm. Sixteen holes are arranged in two lines. The interval between holes is 0.02 mm. Variation is made on the direction of the half cylindrical holes (Fig. 3).

A rectangular flow channel has been constructed with a silicone film, which has been sandwiched by two transparent PDMS disks (Fig. 4).

A silicon wafer (Type P, 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 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 was baked on the heated plate with two processes: at 338 K for 1 minute, before at 368 K for 3 minutes.

The pattern of holes (Fig. 3) to make half cylindrical 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 0.01 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 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.

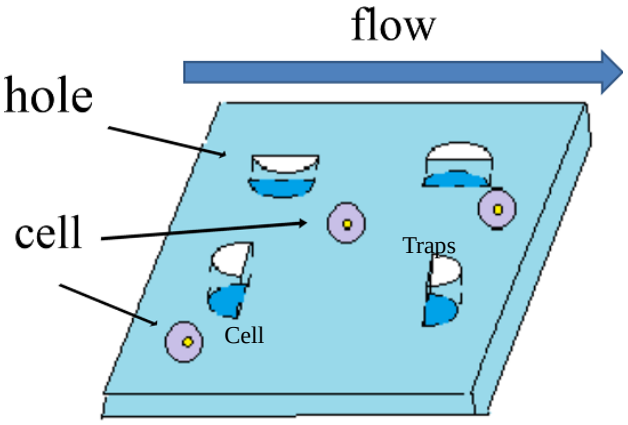

Fig. 1: Several micro traps in flow channel.

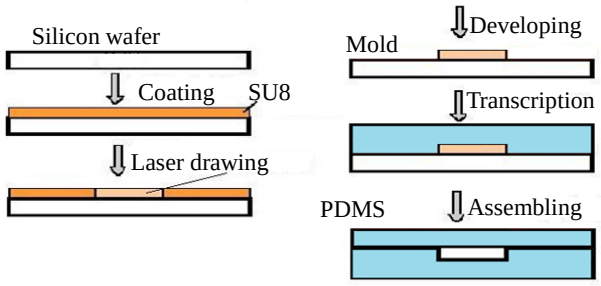

Fig. 2: Photolithography technique.

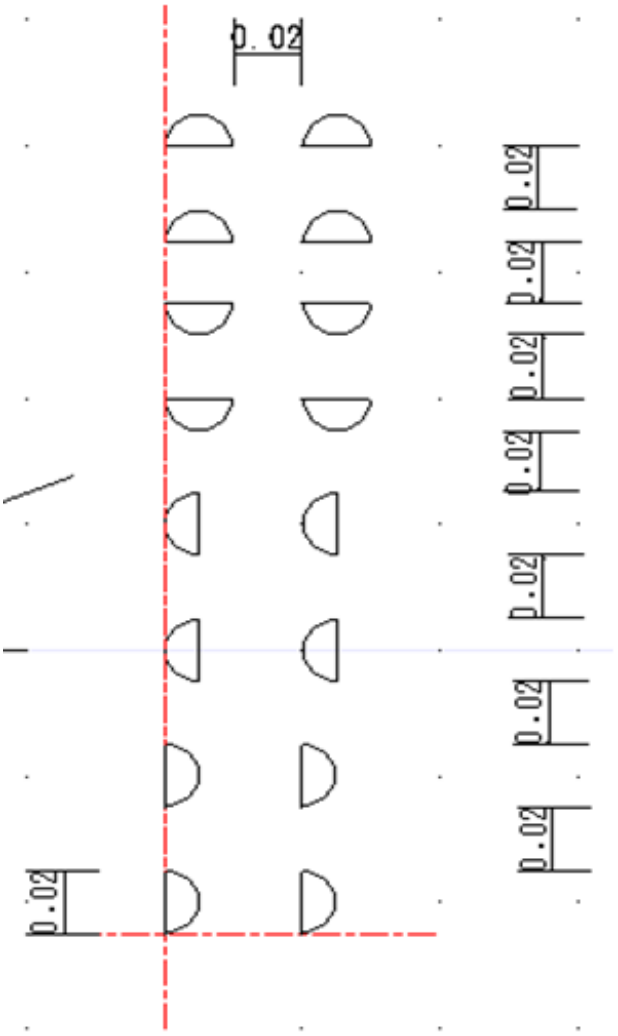

Fig. 3: Pattern of holes: dimension (mm).

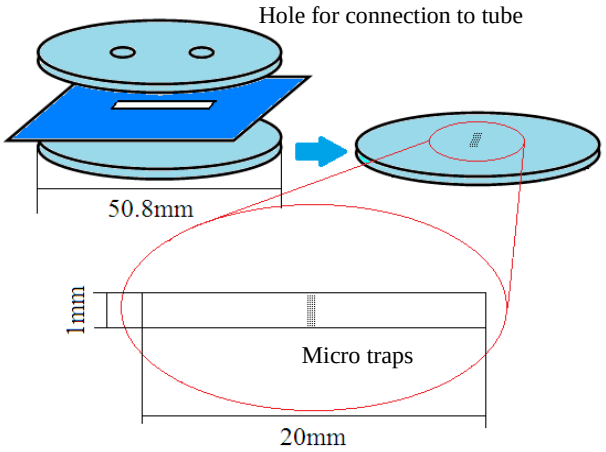

Fig. 4: The flow channel consists of two transparent polydimethylsiloxane (PDMS) disks and a thin silicone rubber sheet.

After development, the dimension of the half cylindrical micro columns on the mold was measured with a laser microscope (VK-X200, Keyence Corporation, Osaka, Japan). The convex mold with micro pattern is used only for the lower disk of PDMS to make the micro holes on the surface.

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.

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. 4) are machined by a punching tool. The silicone tubes are stuck at the holes without an adhesive.

Flow System

A one-way flow system is designed to control the wall shear rate at the disk of PDMS (Fig. 5) [14]. The system consists of a flow channel, a micro syringe pump, tubes and a microscope (Fig. 6). A plastic 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 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. 4). 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 channel 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 syringe pump (Fig. 6). 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: C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse), or MC3T3-E1 (mouse osteogenic cell line, Riken Bio Resource Center, Tsukuba).

C2C12 was cultured 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.

MC3T3-E1 was cultured with the alpha-MEM (alpha modified Eagle's minimal essential medium) containing 10% FBS (fetal bovine serum), in the incubator for one week. The medium also ontains 2.4% of sodium bicarbonate aqueous solution ($NaHCO_3$, 75 g/L) and 1% of Antibiotic-Antimycotic (penicillin, streptomycin and amphotericin B, Life Technologies). Before the flow test, the cells were exfoliated from the plate of the culture dish with trypsin, and suspended in the alpha-MEM.

The suspension was introduced to the chamber at the constant flow with the micro syringe pump. Variation was made on the flow rate: 2.8×10^{-10} m^3/s and 8.3×10^{-10} m^3/s.

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 holes in the channel was observed with the microscope.

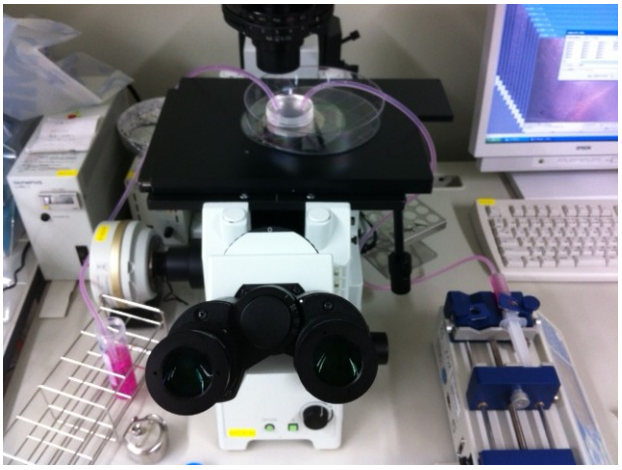

Fig. 5: Flow test system: flow chamber and microscope (middle), syringe pump (right).

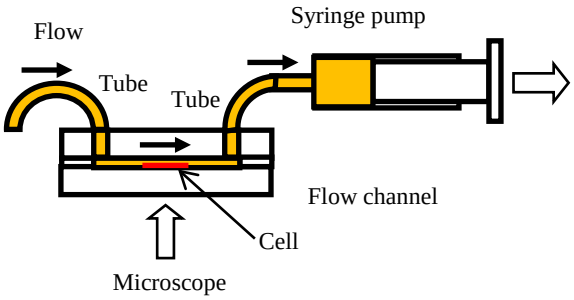

Fig. 6: Flow to syringe pump through flow chamber.

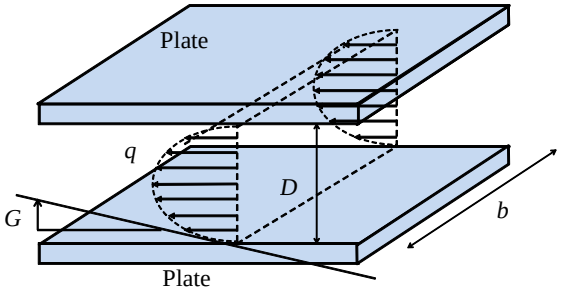

Fig. 7: Parabolic velocity profile between parallel plates.

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. 7).

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

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. 7). The wall shear rate G varies from 35 to 100 per second, when the flow rate q varies from 2.8 $\times 10^{-10}$ m^3/s to 8.3 $\times 10^{-10}$ m^3/s.

3. RESULTS

The laser measurement of surface morphology of the mold shows that the diameter of half cylindrical column is 0.02 mm, as the designed dimension. The laser measurement also shows that the mean height of micro columns is 0.0015 mm (Fig. 8).

The flow tests show the following results.

The moving cells over the holes are able to be observed with the microscope in the flow test system. The diameter of the suspended cell is approximately 0.01 mm. The trapped time of the cell is measured compared with the movement of floating cell.

In the case of C2C12, one of the cells is trapped in the hole for 5 seconds in the flow rate of 2.8 $\times 10^{-10}$ m^3/s (Fig. 9). Another cell is trapped in the hole for 8 seconds in the flow rate of 8.3 $\times 10^{-10}$ m^3/s (Fig. 10).

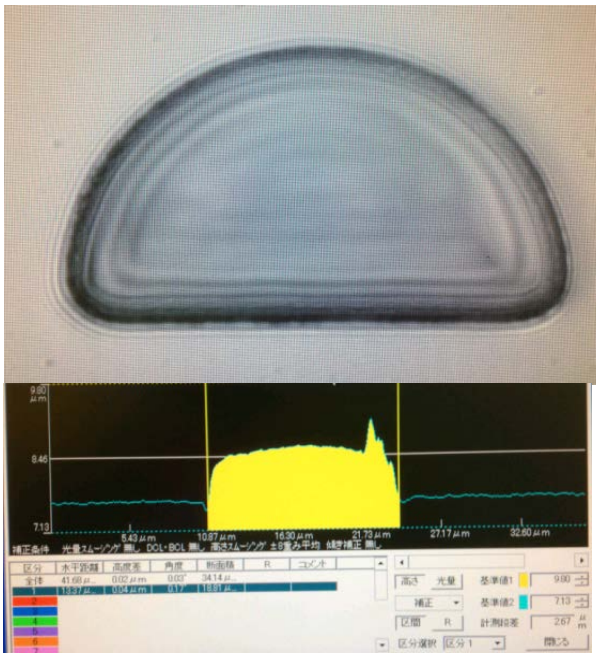

Fig. 8: Laser measurement of morphology of convex column at surface of the mold. Cross section at line A-B (bottom).

In the case of MC3T3-E1, one of the cells is trapped in the hole for one second in the flow rate of 2.8 $\times 10^{-10}$ m^3/s (Fig. 11). Every cell rolls over the hole in the flow rate of 8.3 $\times 10^{-10}$ m^3/s (Fig. 12).

The time of trapped in the hole is longer with C2C12 than with MC3T3-E1.

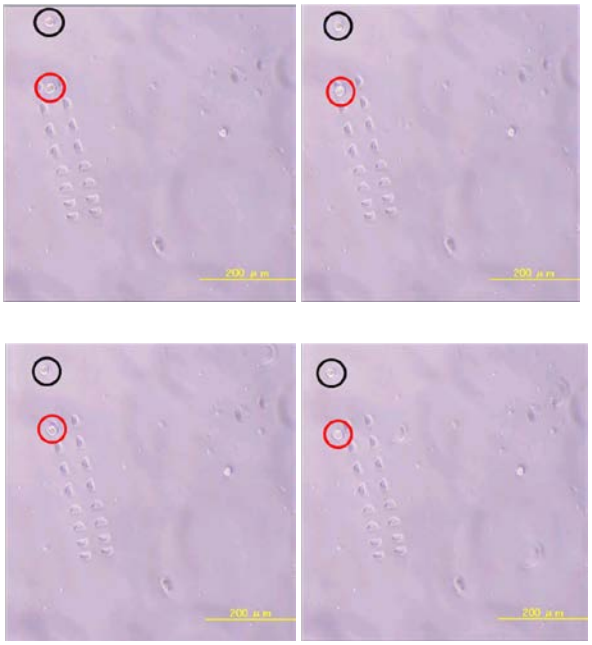

Fig. 9: C2C12 in red lower circle approaches to the hole (upper left), enters (upper right), is trapped for 5 s (lower left), and exits (lower right). Flow (2.8 $\times 10^{-10}$ m^3/s) from right to left.

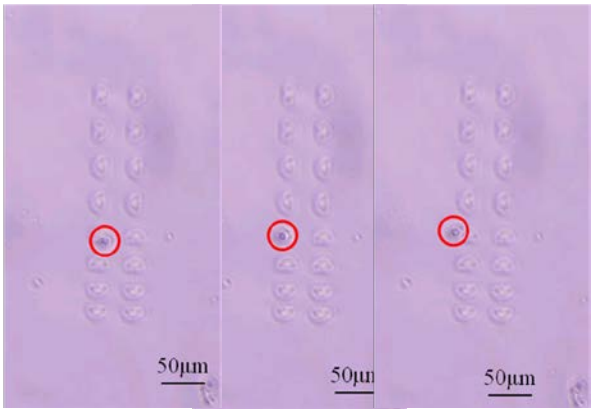

Fig. 10: C2C12 enters (left), is trapped for 8 s (middle), and exits (right). Flow (8.3 $\times 10^{-10}$ m^3/s) from right to left.

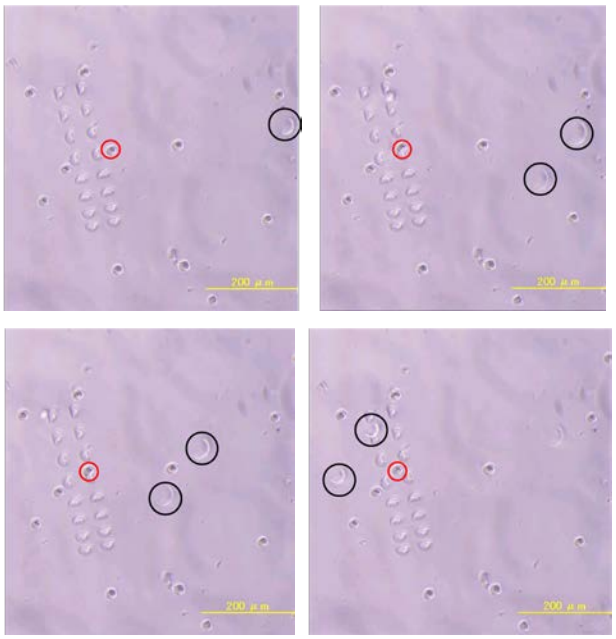

Fig. 11: MC3T3-E1 in red small circle approaches to the hole (upper left), enters (upper right), is trapped for 1 s (lower left), and exits (lower right). Flow (2.8×10^{-10} m^3/s) from right to left.

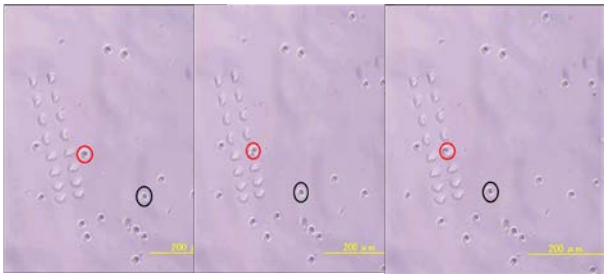

Fig. 12: MC3T3-E1 in red small circle approaches to the hole (left), is on the hole (middle), exits from hole (right). Flow (8.3×10^{-10} m^3/s) from right to left.

4. DISCUSSION

The photolithography technique has been applied to fabricate the micro channel. The microfluidic system has been applied to sort biological cells [13], and to trap biological cells [14, 17, 18]. The system also used to study local environment around the cultured cell [1, 4]. The micro pattern of the surface has been applied to study the surface effect of adhesion of cells [2, 3].

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 holes were used for trap of cells. The half cylindrical holes are designed in the present study. The asymmetrical hole might be suitable for trap than symmetrical hole.

The depth of the micro holes was 0.01 mm in the previous study [8]. In the present study, the depth of the micro holes 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.

Behavior of biological cells might depend on several factors: magnetic field [20], electric field [21], or mechanical field [22-24].

5. CONCLUSION

Micro holes have been designed to trap a biological cell, which flows through a micro channel *in vitro*. Several micro traps of a half cylindrical hole of 0.002 mm depth (0.02 mm diameter) have been fabricated on the surface of the polydimethylsiloxane (PDMS) disk with the photolithography technique. Two types of biological cells were used in the experiment alternatively: C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse), or MC3T3-E1 (mouse osteogenic cell line). The experiments show that the cell is trapped with the micro hole under the wall shear rate less than 100 s^{-1} for several seconds.

6. ACKNOWLEDGMENT

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REFERENCES

[1] G.D. Smith, S. Takayama and J.E. Swain, “Rethinking In Vitro Embryo Culture: New Developments in Culture Platforms and Potential to Improve Assisted Reproductive Technologies”, **Biology of Reproduction**, Vol.86, No. 3: 62, 2012, pp. 1-10.

[2] C.S. Chen, M. Mrksich, S. Huang, G.M. Whitesides and D.E. Ingber, “Geometric Control of Cell Life and Death”, **Science**, Vol. 276, No. 5317, 1997, pp. 1425-1428.

[3] S. Raghavan, R. A. Desai, Y. Kwon, M. Mrksich and C. S. Chen, “Micropatterned Dynamically Adhesive Substrates for Cell Migration”, **Langmuir**, Vol. 26, 2010, pp. 17733-17738.

[4] Y. Rondelez, G. Tresset, K.V. Tabata, H. Arata, H. Fujita, S. Takeuchi and H. Noji, “Microfabricated Array of Femtoliter Chambers Allow Single Molecule Enzymology”, **Nature Biotechnology**, Vol. 23, No. 3, 2005, pp. 361-365.

[5] H. Liu and Y. Ito, “Cell Attachment and Detachment on Micropattern-Immobilized Poly (N-isopropylacrylamide) with Gelatin”, **Lab on a Chip**, Vol. 2, 2002, pp. 175-178.

[6] G. Simone, G. Perozziello, E. Battista, F. De Angelis, P. Candeloro, F. Gentile, N. Malara, A. Manz, E. Carbone, P. Netti, E. Di Fabrizio, “Cell Rolling and Adhesion on Surfaces in Shear Flow. A Model for an Antibody-Based Microfluidic Screening System”, **Microelectronic**

Engineering, Vol. 98, 2012, pp. 668-671.

[7] A. Efremov, J. Cao, "Bistability of Cell Adhesion in Shear Flow", **Biophysical Journal**, Vol. 101, No. 5, 2011, pp. 1032–1040. cell & wall

[8] S. Hashimoto, R. Nomoto, S. Shimegi, F. Sato, T. Yasuda and H. Fujie, "Micro Trap for Flowing Cell", **Proc. 17th World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 1, 2013, pp. 1-6.

[9] M. Abdelgawad, C. Wu, W.Y. Chien, W.R. Geddie, M.A.S. Jewett and Y. Sun, "A Fast and Simple Method to Fabricate Circular Microchannels in Polydimethylsiloxane (PDMS)", **Lab on a Chip**, Vol. 11, 2011, pp. 545–551.

[10] A. Kamiya, R. Bukhari and T. Togawa, "Adaptive Regulation of Wall Shear Stress Optimizing Vascular Tree Function", **Bulletin of Mathematical Biology**, Vol. 46, No.1, 1984, pp. 127-137.

[11] J.C. McDonald, D.C. Duffy, J.R. Anderson, D.T. Chiu, H. Wu, O.J.A. Schuller and G.M. Whitesides, "Fabrication of Microfluidic Systems in Poly (dimethylsiloxane)", **Electrophoresis**, Vol. 21, No. 1, 2000, pp. 27-40.

[12] E. Delamarche, A. Bernard, H. Schmid, B. Michel and H. Biebuyck, "Patterned Delivery of Immunoglobulins to Surfaces Using Microfluidic Networks", **Science**, Vol. 276, No. 5313, 1997, pp. 779-781.

[13] A.Y. Fu, C. Spence, A. Scherer, F.H. Arnold and S.R. Quake, "A Microfabricated Fluorescence-Activated Cell Sorter", **Nature Biotechnology**, Vol. 17, 1999, pp. 1109-1111.

[14] D. Di Carlo, L.Y. Wu and L.P. Lee, "Dynamic Single Cell Culture Array", **Lab on a Chip**, Vol. 6, 2006, pp. 1445-1449.

[15] L. Liu, K. Loutherback, D. Liao, D. Yeater, G. Lambert, A. Estvez-Torres, J.C. Sturm, R.H. Getzenberg and R.H. Austin, "A Microfluidic Device for Continuous Cancer Cell Culture and Passage with Hydrodynamic Forces", **Lab on a Chip**, Vol. 10, 2010, pp. 1807-1813.

[16] K. Funamoto, I.K. Zervantonakis, Y. Liu, C.J. Ochs, C. Kimfg and R.D. Kamm, "A Novel Microfluidic Platform for High-resolution Imaging of a Three-dimensional Cell Culture under a Controlled Hypoxic Environment", **Lab on a Chip**, Vol. 12, 2012, pp. 4855-4863.

[17] H. Miyoshi, J. Ju, S.M. Lee, D.J. Cho, J.S. Ko, Y. Yamagata, T. Adachi, "Control of Highly Migratory Cells by Microstructured Surface Based on Transient Change in Cell Behavior", **Biomaterials**, Vol. 31, No. 33, 2010, pp. 8539-8545.

[18] A. Khademhosseini, J. Yeh, S. Jon, G. Eng, K.Y. Suh, J.A. Burdick and R. Langer, "Molded Polyethylene Glycol Microstructures for Capturing Cells within Microfluidic Channels", **Lab on a Chip**, Vol. 4, No. 5, 2004, pp. 425-430.

[19] M.R. King, L.T. Western, K. Rana and J.L. Liesveld, "Biomolecular Surfaces for the Capture and Reprogramming of Circulating Tumor Cells", **Journal of Bionic Engineering**, 2009, Vol. 6, No. 4, pp. 311-317.

[20] S. Hashimoto and K. Tachibana, "Effect of Magnetic Field on Adhesion of Muscle Cells to Culture Plate", **Journal of Systemics, Cybernetics and Informatics**, Vol. 11, No. 4, 2013, pp. 7-12.

[21] S. Hashimoto, F. Sato, R. Uemura and A. Nakajima, "Effect of Pulsatile Electric Field on Cultured Muscle Cells in Vitro", **Journal of Systemics Cybernetics and Informatics**, Vol. 10, No. 1, 2012, pp. 1-6.

[22] S. Hashimoto, F. Sato, H. Hino, H. Fujie, H. Iwata and Y. Sakatani, "Responses of Cells to Flow in Vitro", **Journal of Systemics Cybernetics and Informatics**, Vol. 11, No. 5, 2013, pp. 20-27.

[23] M. J. Levesque and R. M. Nerem, "The Elongation and Orientation of Cultured Endothelial Cells in Response to Shear Stress", **Journal of Biomechanical Engineering**, Vol. 107, No. 4, 1985, pp. 341-347.

[24] K.A. Barbee, P.F. Davies and R. Lal, "Shear Stress-Induced Reorganization of the Surface Topography of Living Endothelial Cells Imaged by Atomic Force Microscopy", **Circulation Research**, Vol. 74, No. 1, 1994, pp. 163-71.