Deformation of Cell Passing through Micro Slit

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ABSTRACT

Deformation of a biological cell passing through a micro slit has been observed *in vitro*. The photolithography technique enables manufacturing the micro slit. A silicon wafer is used for a mold, and a dry etching process is applied for the micro-fabrication of the surface. The slit, of which width is 0.05 mm and height is 0.0006 mm or 0.003 mm, has been designed between two parts of transparent polydimethylsiloxane disks, which have micro ridges. The slit is placed at the middle part of a flow channel: 0.05 mm height, 3 mm width, and 30 mm length. The suspension of swine red blood cells, C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse), or 3T3-L1 (mouse fat precursor cells) was alternatively introduced to the slits by drawing with a syringe pump at a constant flow rate of 2.8×10^{-10} m^3/s. The deformation of cells passing through the micro slit was observed with an inverted phase-contrast microscope. The experimental results show that several red blood cells deform and pass through the micro slit of 0.0006 mm height, and that some cells of C2C12 and of 3T3-L1 deform and pass through the micro slit of 0.003 mm height.

Keywords: Biomedical Engineering, Red Blood Cell, C2C12, 3T3-L1, Photolithography and Micro-slit.

1. INTRODUCTION

An erythrocyte has flexibility [1, 2] and deforms in the shear flow [3-5]. It also passes through micro-circulation, of which the dimension is smaller than the diameter of the red blood cell. After circulation through the blood vessels for days, the red blood cell is trapped in the micro-circulation systems.

One of the systems, which trap red blood cells, is a spleen. The spleen has special morphology in the blood flow path to sort injured red blood cells [6-8].

The photolithography technique enables manufacturing a micro-channel [9-16]. Several micro-fabrication processes have been designed to simulate morphology of microcirculation. The technique also will be applied to handle cells in diagnostics *in vitro* [17].

In the present study, micro slits have been designed to observe deformation of biological cells passing through the slit *in vitro*.

2. METHODS

Micro Slits

The slits, of which width (*W*) is 0.05 mm and height (*H*) is 0.0006 mm or 0.003 mm, have been designed between two parts of transparent polydimethylsiloxane (PDMS) disks. Both of the disks have micro ridges (Fig. 1). The lower part has ten ridges of 0.2 mm width (1 mm length) with the interval (*W*) of 0.05 mm. Variation is made of the height (*H*) of the lower ridges: 0.0006 mm or 0.003 mm. The upper part has a ridge of 0.05 mm height: 0.05 mm width and 2 mm length. These ridges make contact each other in the perpendicular position, and make slits between the ridges.

Mold Base

A silicon wafer (Type N, Matsuzaki Seisakusyo, Co., Ltd., Tokyo, Japan) is 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 surface of the wafer is cleaned by a spin dryer (Japan Create Co., Ltd., Tokorozawa, Japan).

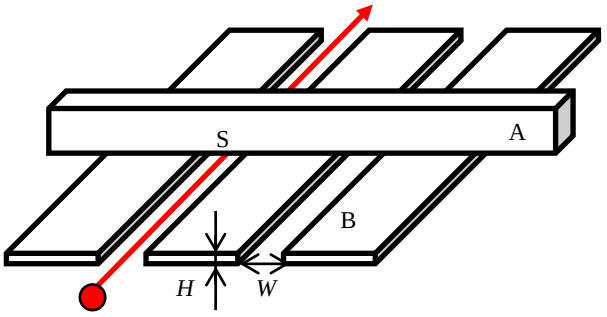

Fig. 1A: Upper (A) and lower (B) micro ridges to make micro slits (S) for cell passing.

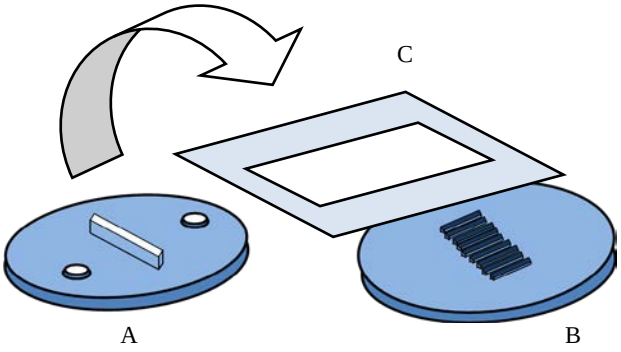

Fig. 1B: Upper (A) and lower (B) micro ridges to make micro slits. Thin sheet of silicone rubber (C) with a rectangular hole is sandwiched between A and B.

Photolithography

To increase affinity between the wafer and the photo-resist material, hexamethyldisilazane (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) was coated on the wafer at 3000 rpm for 30 s with a spin coater (Mikasa Co., Ltd., Tokyo, Japan).

The photo-resist material of OFPR-800 (Tokyo Ohka Kogyo Co., Ltd, Tokyo, Japan) was coated on the wafer at 3500 rpm for 5 s with the spin coater. The photo-resist was baked on the heated plate at 373 K for 90 s.

The pattern of ridges was drawn on the wafer by a laser (wave length of 405 nm) drawing system (DDB-201K-KH, Neoark Corporation, Hachioji, Japan). The width of the trace of laser is proportional to the voltage, although the width is inversely proportional to the velocity. To control the dimension of the ridges of the mold with the laser drawing system, the parameters were selected as follows: the voltage of 3 V, the velocity of 0.02 mm/s, the acceleration of 0.3 mm/s^2. To increase the adhesiveness of the coating, the wafer was baked at 393 K for 300 s.

The photo-resist was developed with tetra-methyl-ammonium hydroxide (NMD-3, Tokyo Ohka Kogyo Co., Ltd., Kawasaki, Japan) for one minute. The wafer was rinsed with the ultrapure water and with the distilled water, and dried with the spin dryer.

For the upper disk, the wafer was etched with the plasma gas using Si Deep RIE System (MUC-21 ASE-SRE, Sumitomo Precision Products Co., Ltd., Amagasaki, Japan) to make the deeper micro grooves. On the upper disk, the switching mode between C_4F_8 gas and SF_6 gas was applied.

For the lower disk, RIE-10NR (Samco International, Kyoto, Japan) was used. For etching of 0.0006 mm depth, the gas of SF_6 (20 cm^3/min at 1013 hPa) with O_2 (10 cm^3/min at 1013 hPa) and with Ar (10 cm^3/min at 1013 hPa) was applied at 50 W at 20 Pa for three minutes. For etching of 0.003 mm depth, the gas of SF_6 (20 cm^3/min at 1013 hPa) with O_2 (10 cm^3/min at 1013 hPa) and with Ar (10 cm^3/min at 1013 hPa) was applied at 200 W at 20 Pa for three minutes.

The residual photo-resist was exfoliated in the compact etcher FA-1 (Samco International, Kyoto, Japan). The gas of O_2 (30 cm^3/min) was applied at 100 W for five minutes.

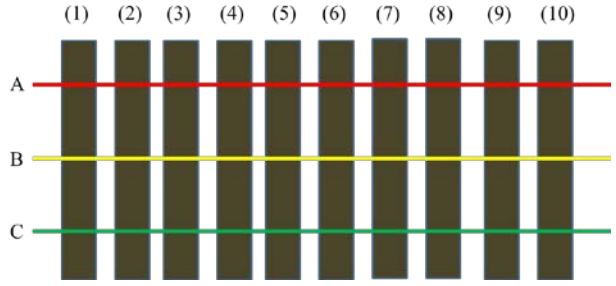

Fig. 2: Lines for measurement of surface micro morphology of lower disk.

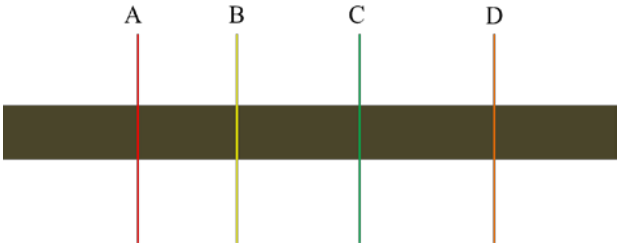

Fig. 3: Lines for measurement of surface micro morphology of upper disk.

The surface of the wafer with micro pattern was coated with 0.001 mm thickness of parylene in the parylene coater (PDS-2010, Speciality Coating Systems, Indianapolis).

The wafer with the grooves was used as the concave mold to make micro convex ridges in the following process. The dimension of the grooves on manufactured mold without parylene coating was measured with the laser microscope (VK-X200, Keyence Corporation, Osaka, Japan) (Figs. 2 & 3).

PDMS Disk

After the wafer was enclosed with a peripheral wall of polyimide, PDMS (Sylgard 184 Silicone Elastomer Base, Dow Corning Corporation) was poured together with the curing agent (Dow Corning Corporation) on the wafer. The volume ratio of curing agent is ten percent of PDMS. The volumes of PDMS are 8.8 cm^3 for the upper disk and 4.4 cm^3 for the lower disk, respectively.

After degassing, PDMS was baked at 433 K for two hours in an oven (DX401, Yamato Scientific Co., Ltd, Tokyo, Japan). The baked disk of PDMS is exfoliated from the mold. The dimension of the ridges on manufactured PDMS was measured with the laser microscope.

Two holes of 5 mm diameter were machined with a punching tool at the upper disk to make the inlet and the outlet for the flow channel.

Flow Test System

A one-way flow system was designed to observe the behavior of cells through the micro slits *in vitro* (Fig. 4). The system consists of a flow channel, a syringe pump, tubes and a microscope.

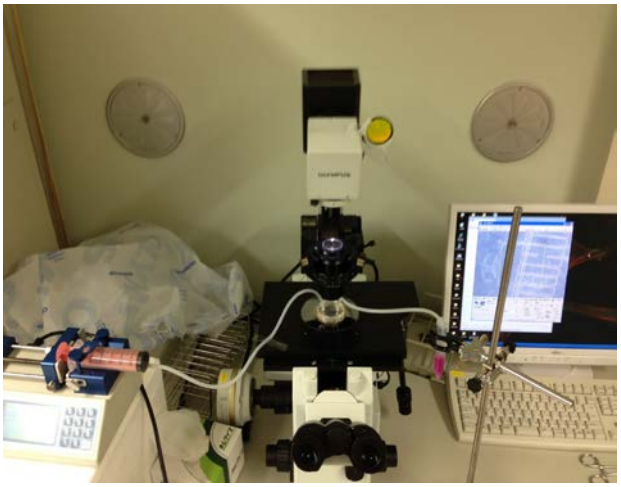

Fig. 4: Flow test system: pump (left), channel (middle), and microscope.

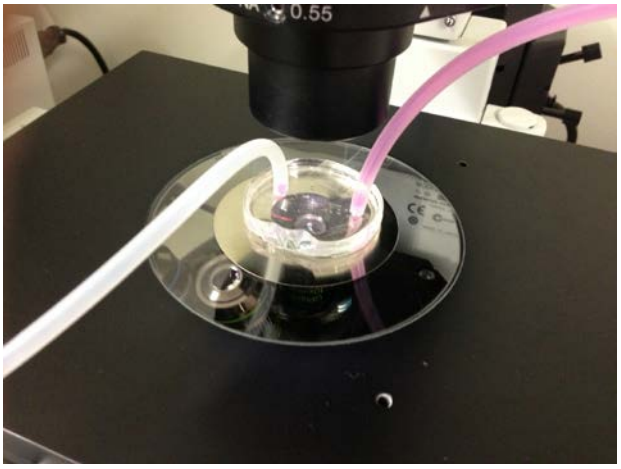

Fig. 5: Flow channel on microscope.

The micro-syringe-pump (Fusion200, CXF1020, ISIS Co., Ltd., Osaka) was used for the syringe pump. The silicone tube of 3 mm internal diameter and of 5 mm external diameter was used for the connector to the flow channel.

The flow channel consists of two transparent PDMS disks and a thin sheet of silicone rubber (thickness of 0.05 mm, ARAM Corporation, Osaka) (Fig. 1B, Fig. 5).

A rectangular open space of 3 mm × 30 mm is cut off in the sheet, and sandwiched between the PDMS disks. The open space forms a channel of 30 mm length × 3 mm width × 0.05 mm depth.

The three parts stick together with their surface affinity. The diameter of two PDMS disks is 50 mm. The thicknesses of the upper and the lower disks are 4 mm and 2 mm, respectively.

The lower PDMS disk has ten micro ridges on the upper surface. The upper PDMS disk has one micro ridge on the lower surface. The upper disk has two holes. The silicone tubes are stuck at the holes for the inlet and the outlet. To seal the circumferential micro gap between disks, extra PDMS was painted to fill the gap from the outside, and baked at 373 K for one hour in the oven.

Flow Test

Three kinds of cells were used in the flow test: the passage fourth of C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse), the passage seventh of 3T3-L1 (mouse fat precursor cells), and swine red blood cells.

In the case of C2C12 and of 3T3-L1, experimental protocol is as follows. The cells were exfoliated from the bottom of the culture dish with trypsin. Cells were suspended in the medium of D-MEM (Dulbecco's Modified Eagle's Medium) with the density of 5000 cells/cm^3. To adjust the density of cells, the number of cells is counted with Burker-Turk hemocytometer under a phase contrast microscope (IX71, Olympus, Tokyo).

The swine blood (Tokyoshibaurazouki Co., Ltd., Tokyo, Japan) was diluted by the phosphate buffer solution without calcium to make a suspension of red blood cells at the volume ratio of 0.02 percent. The red blood cells were used immediately, or used after preservation in a refrigerator at 277 K in four weeks.

The behavior of cells near the slits was observed with an inverted phase-contrast microscope (IX71, Olympus Co., Ltd., Tokyo), while the suspension of cells was pumped at the flow rate of 1 cm^3 /hour at 298 K (Fig. 5). In the flow path of depth of 0.05 mm (width of 3 mm), the flow rate makes mean velocity of 2 mm/s.

3. RESULTS

The dimension of the 0.0006 mm grooves on the manufactured mold of the lower part measured by the laser microscope is as follows. Although the data of depth at tracings along three lines (Fig. 2) across ten grooves show slightly scattered values between 0.00051 mm and 0.00085 mm, the mean depth is 0.00063 mm.

The dimension of the 0.003 mm grooves on the manufactured mold of the lower part measured by the laser microscope is as follows. Although the data of depth at tracings along three lines (Fig. 2) show slightly scattered values between 0.0026 mm and 0.0030 mm, the mean depth is 0.0028 mm.

The data of the depth at the trace along four lines (Fig. 3) across the groove on the manufactured mold of the upper parts scatter between 0.044 mm and 0.055 mm, and the mean depth is 0.0049 mm.

Fig. 6 exemplifies the laser microscopic image of the 0.0006 mm ridges on the manufactured PDMS disks of the lower part. Although the heights at the tracings along three lines (Fig. 2) across ten ridges show scattered values between 0.00053 mm and 0.00077 mm, the mean height is 0.00066 mm.

Fig. 7 exemplifies the tracing of the 0.003 mm ridges on the manufactured PDMS disks of the lower part measured by the laser microscope. Although the heights at the tracings along three lines (Fig. 2) across ten ridges show slightly scattered values between 0.0028 mm and 0.0031 mm, the mean height is 0.0029 mm.

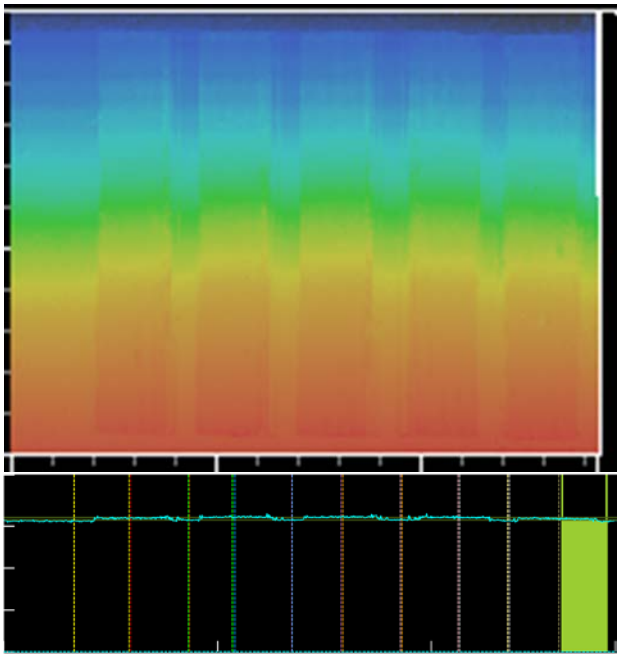

Fig. 6: Laser microscope image of micro ridges on lower disk to make micro slit for red blood cells (upper), and tracing along transverse line (Fig. 2) of ridges (lower). Dimension from left to right is 1.5 mm.

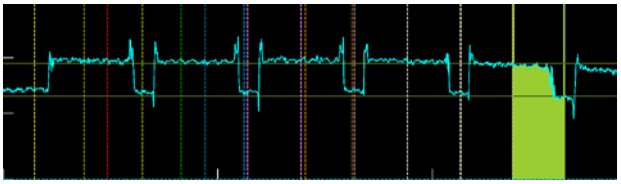

Fig. 7: Tracing along transverse line of micro ridges on lower disk (Fig. 3) to make micro slit for cells (Fig. 1). Dimension from left to right is 1.5 mm.

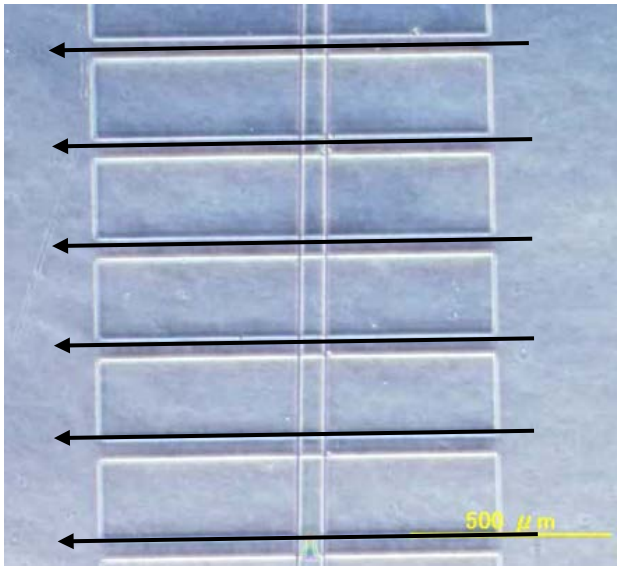

Fig. 8: Micro slits. Each arrow shows the path for cell. Dimension from left to right is 1.5 mm.

The data of the height at the trace along four lines (Fig. 3) across the ridge of the upper parts slightly scatter between 0.054 mm and 0.055 mm, and the mean height is 0.055 mm.

Fig. 8 shows the microscopic view of the slits assembled between the ridges of upper and lower disks.

Figs. 9-11 exemplify the red blood cell passing through the slit. The cell flows from right to left. The preserved red blood cells pass through slits of 0.0006 mm more easily than the fresh red blood cell. The cell marked with the circle in Fig. 10 cannot pass through the slit.

Fig. 12 exemplifies C2C12 passes through slit of 0.003 mm. One cell (marked with the circle) deforms to a flat disk (B-D), is moving through the slit (E, F), and has passed (G) through the slit.

Fig. 13 exemplifies 3T3-L1 passes through slit of 0.003 mm. One cell (marked with the circle) deforms to a flat disk (A), is moving through the slit (B, C), and has passed (D) through the slit.

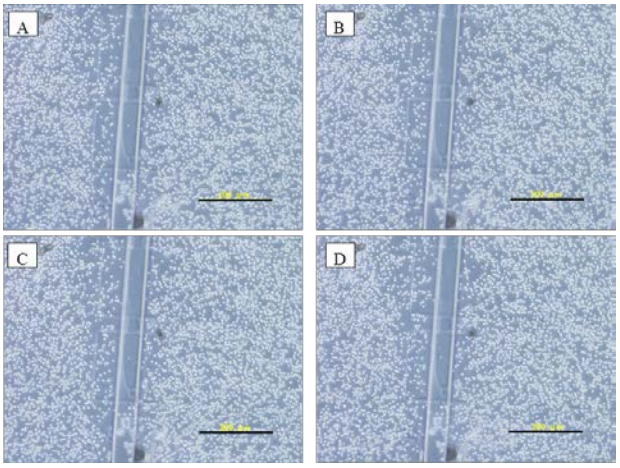

Fig. 9: Fresh red blood cells (photos of every two seconds: A, B, C, D). Flow from right to left. The bar shows 0.2 mm.

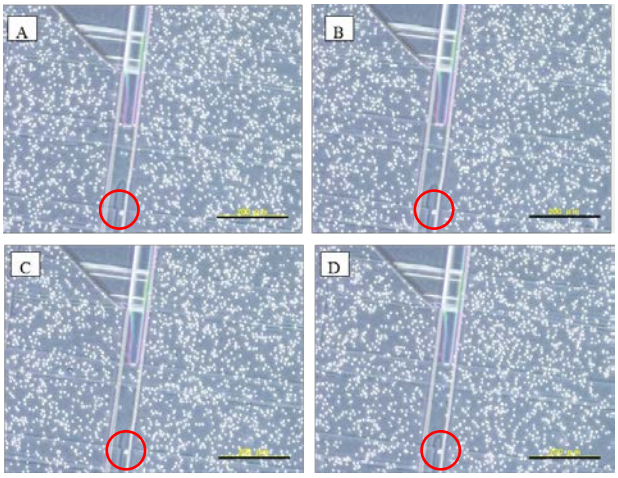

Fig. 10: Red blood cells (preserved for two weeks) passing through slit (photos of every two seconds: A, B, C, D). Flow from right to left. The bar shows 0.2 mm.

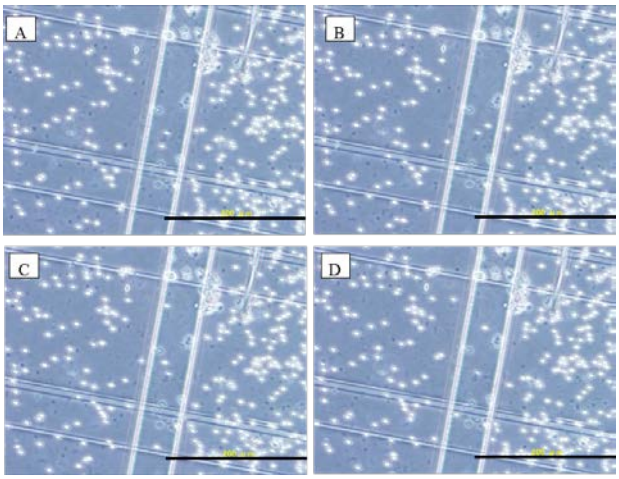

Fig. 11: Red blood cell (preserved for three weeks) passing through slit (photos of every two seconds: A, B, C, D). Flow from right to left. The bar shows 0.2 mm.

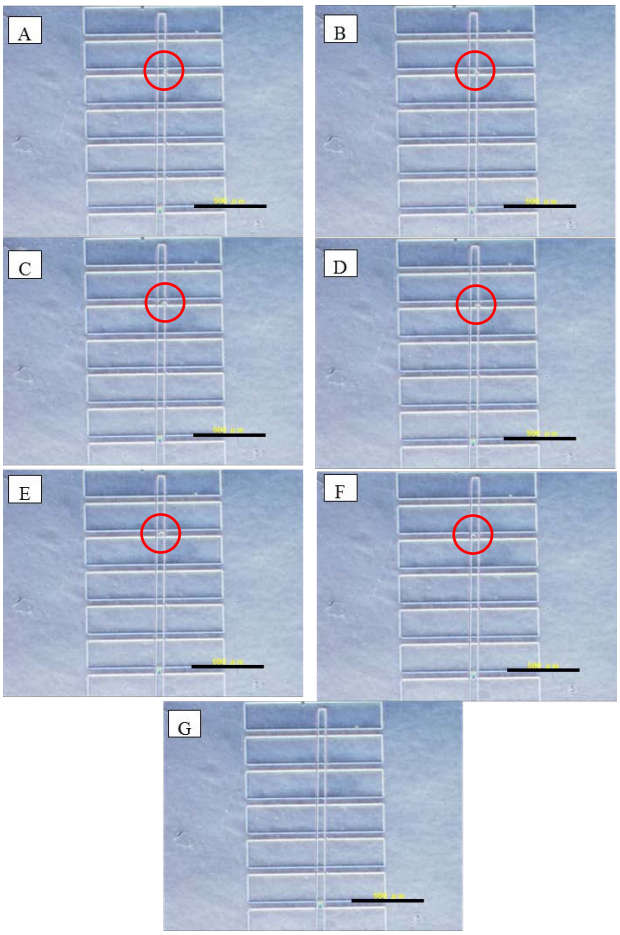

Fig. 12: C2C12 passes through slit. Flow from right to left. The bar shows 0.5 mm. Successive photos: A 0 s, B 6.3 s, C 6.7 s, D 6.9 s, E 9.97 s, F 10.3 s, G 10.3 s.

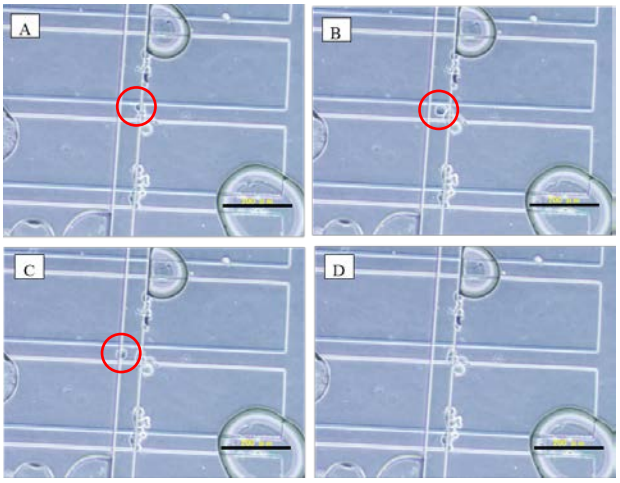

Fig. 13: 3T3-L1 passes through slit. Flow from right to left. The bar shows 0.2 mm. Successive photos: A 0 s, B 3 s, C 6 s, D 6.2 s.

4. DISCUSSION

3T3-L1 is a cell line derived from cells of 3T3 mouse. 3T3-L1 cells have a fibroblast-like morphology. The cells differentiate into an adipocyte-like phenotype under appropriate conditions.

Because the dimension of the slit is mainly governed by the height of the lower ridge, the slight over dimension of the height of the upper ridge might not vary the dimension of the slit. The ridge of the PDMS has elasticity, so that the micro ridge may deform with the small ratio.

In the present study, the micro slit has been designed with narrower dimension than the previous study [18] to observe the deformation of cells or to trap some red blood cells.

The dimension of the slit might be extended during the assembly process of two disks so that most of red blood cells pass through the slit.

The fine architecture of the red pulp of the spleen has been investigated in the previous studies [6-8]. The continuity between capillaries and splenic sinuses has been examined with the microscope. The special morphology might relate to the function for sorting erythrocytes.

In the previous studies, the typical diameter of the micro channel, which simulates the capillary blood vessel, is around 0.005 mm [10]. The red blood cell, on the other hand, passes through micro slit narrower than 0.001 mm in the spleen. The small dimension of passage has been applied to biological cells in the present study.

The effect of flow on cells has been investigated in the previous studies [19, 20]. A micro channel could simulate the microcirculation system. Fatigue of erythrocyte was evaluated through the narrow path [21, 22]. To simulate the microcirculation system with a fabricated channel, the three dimensional curvature of the wall of the flow channel might be important. Cells are responsive to the micro morphology of the scaffold. The micro groove governs the behavior of cells.

The micro-slit is, on the other hand, useful for treatment of cell in diagnostics [17]. The micro-channel devices may contribute to the development of biotechnology.

5. CONCLUSION

Deformation of a biological cell passing through a micro slit has been observed *in vitro*. The slit, of which width is 0.05 mm and height is 0.0006 mm or 0.003 mm, has been designed between two parts of transparent polydimethylsiloxane disks. The experimental results show that several red blood cells deform and pass through the micro slit of 0.0006 mm height, and that some cells of C2C12 and of 3T3-L1 deform and pass through the micro slit of 0.003 mm height.

6. ACKNOWLEDGMENT

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REFERENCES

[1] N. Mohandas, M.R. Clark, M.S. Jacobs and S.B. Shohet, "Analysis of Factors Regulating Erythrocyte Deformability", **The Journal of Clinical Investigation**, Vol. 66, No. 3, 1980, pp. 563-573.

[2] K. Ariyoshi, T. Maruyama, K. Odashiro, K. Akashi, T. Fujino and N. Uyesaka, "Impaired Erythrocyte Filterability of Spontaneously Hypertensive Rats: Investigation by Nickel Mesh Filtration Technique", **Circulation Journal**, Vol. 74, 2010, pp.129-136.

[3] S. Hashimoto, "Detect of Sublethal Damage with Cyclic Deformation of Erythrocyte in Shear Flow", **Journal of Systemics Cybernetics and Informatics**, Vol. 12, No. 3, 2014, pp. 41-46.

[4] O.K. Baskurt, D. Gelmont and H.J. Meiselman, "Red Blood Cell Deformability in Sepsis", **American Journal of Respiratory and Critical Care Medicine**, Vol. 157, 1998, pp. 421-427.

[5] S. Hashimoto, H. Otani, H. Imamura, et al., "Effect of Aging on Deformability of Erythrocytes in Shear Flow", **Journal of Systemics Cybernetics and Informatics**, Vol. 3, No. 1, 2005, pp. 90-93.

[6] B. Steiniger, M. Bette and H. Schwarzbach, "The Open Microcirculation in Human Spleens: A Three-Dimensional Approach", **Journal of Histochemistry Cytochemistry**, Vol. 59, No. 6, 2011, pp. 639–648.

[7] M. Abe, K. Takehana, K. Iwasa and T. Hiraga, "Scanning Electron Microscopic Studies on the Red Pulp of the Mink Spleen", **Japanese Journal of Veterinary Science**, Vol. 51, No. 4, 1989, pp. 775-781.

[8] S. Irino, T. Murakami and T. Fujita, "Open Circulation in the Human Spleen, Dissection Scanning Electron Microscopy of Conductive-Stained Tissue and Observation of Resin Vascular Casts", **Archivum Histologicum Japonicum**, Vol. 40, No. 4, 1977, pp. 297-304.

[9] S.M. Kim, S.H. Lee and K.Y. Suh, "Cell Research with Physically Modified Microfluidic Channels: A Review", **Lab on a Chip**, Vol. 8, 2008, pp. 1015-1023.

[10] J.P. Brody, Y. Han, R.H. Austin and M. Bitensky, "Deformation and Flow of Red Blood Cells in a Synthetic Lattice: Evidence for an Active Cytoskeleton", **Biophysical Journal**, Vol. 68, 1995, pp. 2224-2232.

[11] 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.

[12] S. Hashimoto, Y. Takahashi, H. Hino, R. Nomoto and T. Yasuda, "Micro Hole for Trapping Flowing Cell", **Proc. 18th World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 2, 2014, pp. 114-119.

[13] S. Hashimoto, A. Mizoi, H. Hino, K. Noda, K. Kitagawa and T. Yasuda, "Behavior of Cell Passing through Micro Slit", **Proc. 18th World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 2, 2014, pp. 126-131.

[14] Y.C. Chen, G.Y. Chen Y.C. Lin and G.J. Wang, "A Lab-on-a-chip Capillary Network for Red Blood Cell Hydrodynamics", **Microfluidics and Nanofluidics**, Vol. 9, No. 2, 2010, pp. 585-591.

[15] G. Lim H.W., M. Wortis and R. Mukhopadhyay, "Stomatocyte-discocyte-echinocyte Sequence of the Human Red Blood Cell: Evidence for the Bilayer-Couple Hypothesis from Membrane Mechanics", **Proceedings of the National Academy of Sciences**, Vol. 99, No. 26, 2002, pp. 16766-16769.

[16] S.S. Shevkoplyas, T. Yoshida, S.C. Gifford and M.W. Bitensky, "Direct Measurement of the Impact of Impaired Erythrocyte Deformability on Microvascular Network Perfusion in a Microfluidic Device", **Lab on a Chip**, Vol. 6, No. 7, 2006, pp. 914-920.

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

[18] S. Hashimoto, T. Horie, F. Sato, T. Yasuda and H. Fujie, "Behavior of Cells through Micro Slit", **Proc. 17th World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 1, 2013, pp. 7-12.

[19] S. Hashimoto, H. Oku, N. Komoto, Y. Murashige, S. Manabe, K. Ikegami and C. Miyamoto, "Effect of Pulsatile Shear Flow on Migration of Endothelial Cells Cultured on Tube", **Proc. 6th World Multiconference on Systemics Cybernetics and Informatics**, Vol. 2, 2002, pp. 296-300.

[20] 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.

[21] S. Sakuma, K. Kuroda, C.H.D. Tsai, W. Fukui, F. Arai and M. Kaneko, "Red Blood Cell Fatigue Evaluation Based on the Close-encountering Point between Extensibility and Recoverability", **Lab on a Chip**, Vol. 14, No. 6, 2014, pp. 1135-1141.

[22] T. Tajikawa, Y. Imamura, T. Ohno, F. Muranishi, M. Kubota and K. Ohba, "Measurement and Analysis of the Shape Recovery Process of Each Erythrocyte for Estimation of Its Deformability Using the Microchannel Technique: the Influence of the Softness of the Cell Membrane and Viscosity of the Hemoglobin Solution Inside the Cell", **Journal of Biorheology**, Vol. 27, No. 1-2, 2013, pp. 1-8.