# Behavior of Cell Passing through Micro Slit

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## ABSTRACT

Behavior of a biological cell passing through a micro slit has been observed *in vitro*. The photolithography technique enables manufacturing the micro slit. A silicone disk is used for a mold, and a dry etching process is applied for the micro-fabrication. The slit, of which width is 0.85 mm and height is 0.001 mm, has been designed between two parts of transparent polydimethylsiloxane disks, which have micro ridges. The suspension of swine red blood cells or C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse) has alternatively been introduced to the slits by drawing with a syringe pump. The behavior of cells passing through the micro slit has been observed with an inverted phase-contrast microscope. The experimental results show that several red blood cells can pass through the micro slit, although C2C12 cannot pass through the micro slit.

**Keywords:** Biomedical Engineering, Red Blood Cell, C2C12, Photolithography and Micro-slit.

## 1. INTRODUCTION

An erythrocyte has flexibility [1] and deforms in the shear flow [2, 3]. 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 [4-6].

The photolithography technique enables manufacturing a micro-channel [7-9]. 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* [10].

In the present study, micro slits have been designed to control behavior of biological cells *in vitro*.

## 2. METHODS

**Micro Slits**
The slit, of which width is 0.85 mm and height is 0.001 mm, has been designed between two parts of transparent polydimethylsiloxane disks (Figs. 1 & 2). They have micro ridges. The lower part has three ridges of 0.001 mm height (0.1 mm width, 1 mm length) with the interval of 0.85 mm. The upper part has a ridge of 0.057 mm height: 0.05 mm width and 2 mm length (Fig. 3). These ridges make contact each other in the perpendicular position, and make slits between the ridges.

**Mold Base**
A silicon wafer (Type P, 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 three times in an ultrasonic cleaner: with 2-propanol for five minutes, with hydrogen peroxide solution for five minutes, and with ultrapure water for five minutes. Then, the wafer was dried with an air gun.

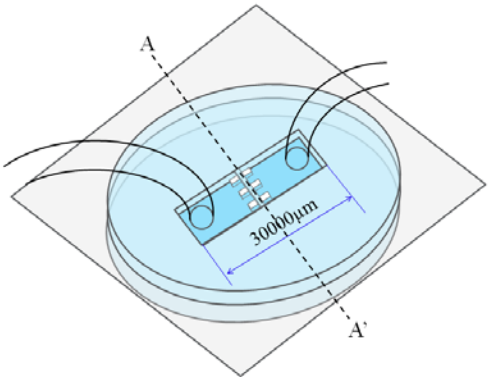



**Fig. 1:** Channel with slit.

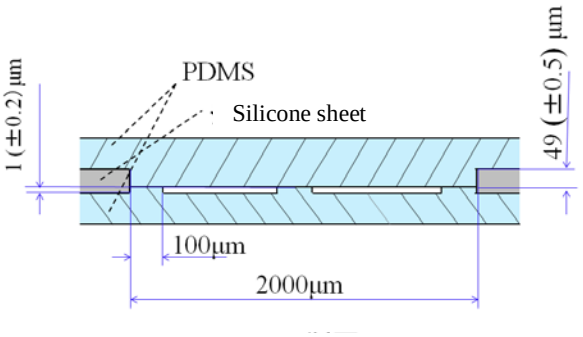



**Fig. 2:** Cross-section view of slit: A-A' in Fig.1.

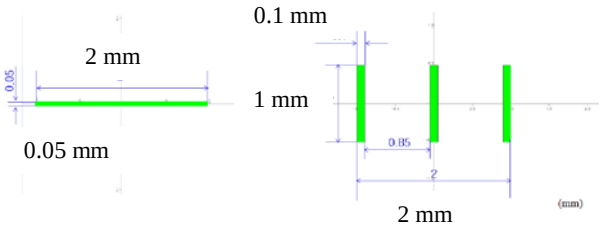



**Fig. 3:** Dimension of micro ridges: upper (left), lower (right).

**Photolithography**

The photo-resist material of OFPR-800 (Tokyo Ohka Kogyo Co., Ltd, Tokyo, Japan) was coated on the wafer with 0.002 mm thick at 5000 rpm for 30 s with a spin coater. The photo-resist was baked on the heated plate at 383 K for 90 s.

The pattern of ridges (Fig. 3) was drawn on the wafer with a laser 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 0.1 mm/s, the acceleration of 0.5 mm/s$^2$.

The photo-resist was developed with tetra-methyl-ammonium hydroxide (NMD-3, Tokyo Ohka Kogyo Co., Ltd., Kawasaki, Japan) for several minutes. The wafer was rinsed with the distilled water, and dried with the air gun. To increase the adhesiveness of the coating, the wafer was baked at 383 K for 5 minutes.

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 micro grooves. On the lower disk, the gas of $SF_6$ (50 cm$^3$/min at 1013 hPa) with $O_2$ (25 cm$^3$/min at 1013 hPa) and with Ar (25 cm$^3$/min at 1013 hPa) was applied at 50 W at 10 Pa for five minutes. On the upper disk, the switching mode between $C_4F_8$ gas and $SF_6$ gas was applied.

The residual photo-resist was exfoliated in the separating solution (Hakuri 105, Tokyo Ohka Kogyo Co., Ltd, Kawasaki, Japan). The wafer was dipped in 2-propanol, before rinsed with the distilled water. Then, the wafer with the grooves was dried with the air gun, and used for the concave mold to make micro convex ridges in the following process. The dimension of the grooves on manufactured mold without coating was measured with the laser microscope (VK-X200, Keyence Corporation, Osaka, Japan).

**PDMS 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). 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 4.4 cm$^3$ for the upper disk and 2.2 cm$^3$ for the lower disk, respectively.

After degassing, PDMS was baked at 383 K for one hour in an oven (DX401, Yamato Scientific Co., Ltd). The baked disk of PDMS is exfoliated from the mold. The dimension of the ridges on manufactured PDMS was measured with the laser microscope.

**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 chamber, a syringe pump, tubes and a microscope. The micro-syringe-pump (Fusion200, CXF1020, ISIS Co., Ltd., Osaka) was used for the syringe pump. A plastic tube of 3 mm internal diameter and of 5 mm external diameter was 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 (thickness of 0.05 mm, ARAM Corporation, Osaka) (Fig. 1).

A rectangular open space of 2 mm × 30 mm is cut off in the sheet, and sandwiched between the PDMS plates. The open space forms a channel of 30 mm length × 2 mm width × 0.05 mm depth.

The three parts stick together with their surface affinity without an adhesive. The diameter of two PDMS plates is 50 mm. The thicknesses of the upper and the lower disks are 4 mm and 2 mm, respectively.

The PDMS disk, which has three micro columns on the upper surface, was placed in the bottom. The upper PDMS disk has one micro column on the lower surface, and has two holes of 5 mm diameter machined with a punching tool. The silicone tubes are stuck at the holes with an adhesive for the inlet and the outlet. The assembled disks are contained on the polystyrene dish of 70 mm diameter and the circumferential crevice between the parts is filled with adhesive from outside (Fig. 4).

**Flow Test**

Two kinds of cells were used in the flow test: C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse), and swine red blood cells.

In the case of C2C12, cells were suspended in the medium of D-MEM (Dulbecco's Modified Eagle's Medium) with the density of the cells 15000 cells/cm$^3$, and pumped at flow rate of 0.005 cm$^3$/hour.

**Fig. 4:** Flow system: channel and microscope.

Swine red blood cells in sodium acid citrate aqueous solution (ACD-A, Terumo Corporation, Japan) were used after preservation in a refrigerator for two days. After centrifugation of the swine blood, the red blood cells were separated from plasma. The cells were suspended in the phosphate buffer solution to make a suspension of red blood cells at the volume ratio of 0.05 percent.

The movement 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 0.003 $cm^3$ /hour at 298 K (Fig. 4). In the flow path of depth of 0.05 mm (width of 2 mm), the flow rate makes mean velocity of 0.08 mm/s.

## 3. RESULTS

Fig. 5 shows the manufactured slit observed with the inverted microscope. Cells flow from left to right over the vertical ridge (0.05 mm height). The horizontal ridge crossing perpendicularly to the vertical ridge makes the slit between vertical ridge and the surface of the lower disk.

Fig. 6 exemplifies the dimension of the groove on the manufactured mold of the lower part measured by the laser microscope. The width and the length are 0.1 mm and 1 mm, respectively. The tracings of the depth along the three lines (Fig. 6(A)) and the cross section (Fig. 6(B)) of the groove show slightly scattered values around the mean value of 0.00088 mm. Fig. 7 exemplifies dimension of the groove of the upper parts. The tracings of the depth along the three lines of the groove show slightly scattered values around the mean value of 0.053 mm.

Figs. 8 & 9 exemplify dimension of the ridges on the manufactured lower and upper parts measured by the laser microscope, respectively. The mean values of the height are 0.0011 mm and 0.055 mm, respectively. The mean value of thickness of the sheet of silicone rubber measured by the laser microscope is 0.057 mm.

Figs. 10-12 exemplify the red blood cell passing through the slit. The cell flows from left to right. The velocity of the cell moving through the slit is not accelerated compared with the decrease of cross section of the flow path. Fig. 13 exemplifies C2C12 approaching to the slit. C2C12 was not able to pass through the slit.

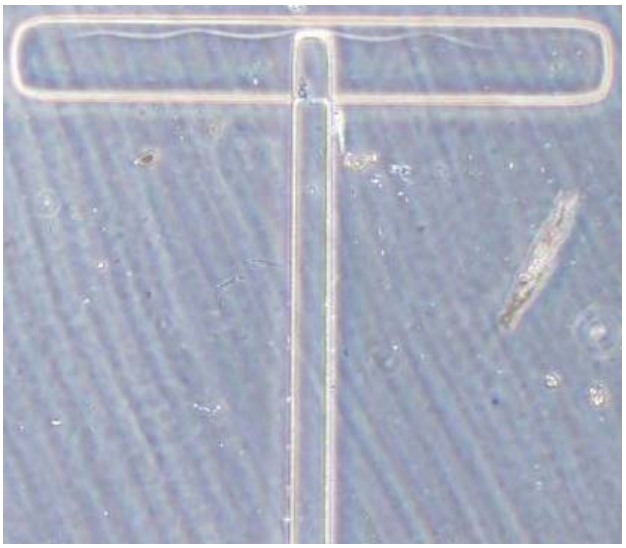
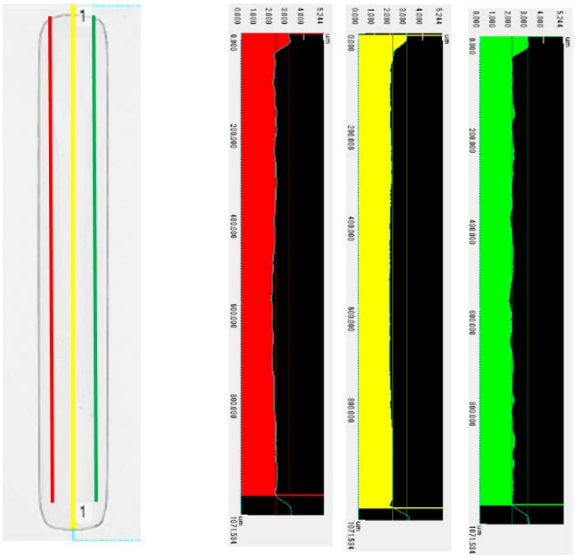


**Fig. 5:** Micro slit. Cells flow from left to right under vertical ridge (0.05 mm height). Horizontal ridge has 0.001 mm height (1 mm length, 0.1 mm width).



Top view Cross-section of three lines

**Fig. 6(A):** Dimension of groove on manufactured mold of lower part measured by laser microscope: tracings of cross section shows mean depth of 0.00088 mm.

## 4. DISCUSSION

In the present study, the micro slit has been designed with narrower dimension than the previous study [11] to observe the deformation of cells or to trap some red blood cells in the present study (Fig. 14). Deformation has not been observed while the red blood cell is passing through the slit. The low velocity of the red blood cell might show the friction between the wall of the slit and the cell during movement.

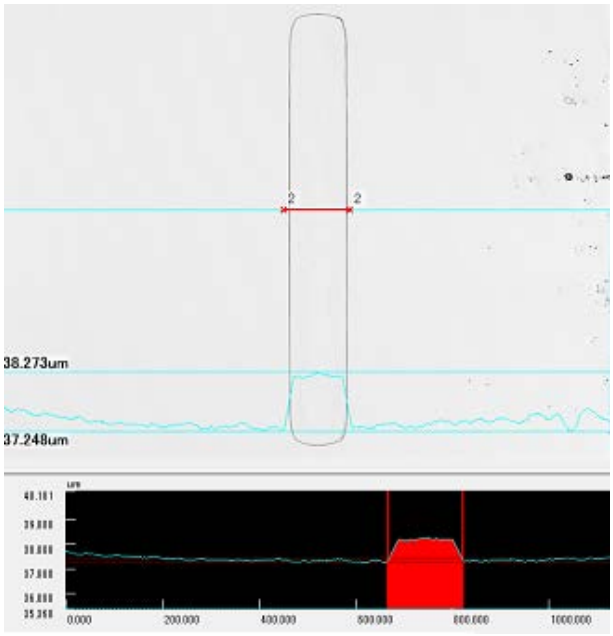
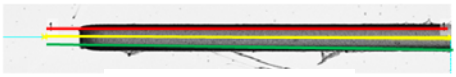
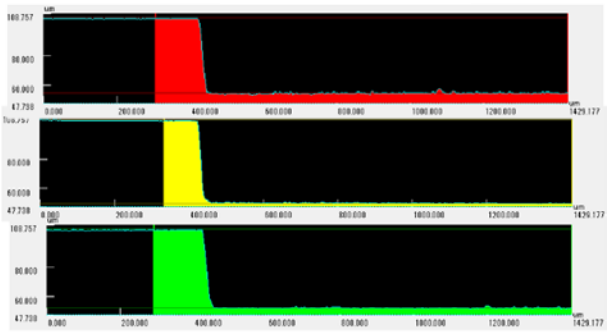



**Fig. 6(B):** Dimension of groove on manufactured mold of lower part measured by laser microscope: top view (upper), and tracings (lower) of cross section between 2 to 2' (upper), which shows mean depth of 0.00088 mm.



Top view



Cross-section of three lines

**Fig. 7(A):** Dimension of groove on manufactured mold of upper part measured by laser microscope: tracings of cross section shows mean depth of 0.053 mm.

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 [4-6]. The continuity between capillaries and splenic sinuses has been examined with the microscope. The special morphology might relate to the function for sorting erythrocytes.

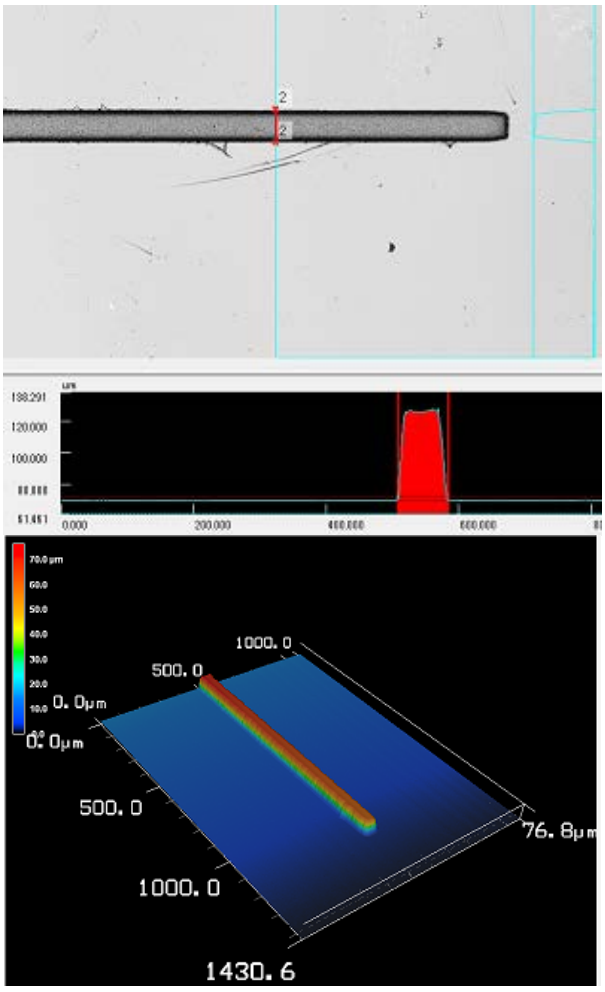
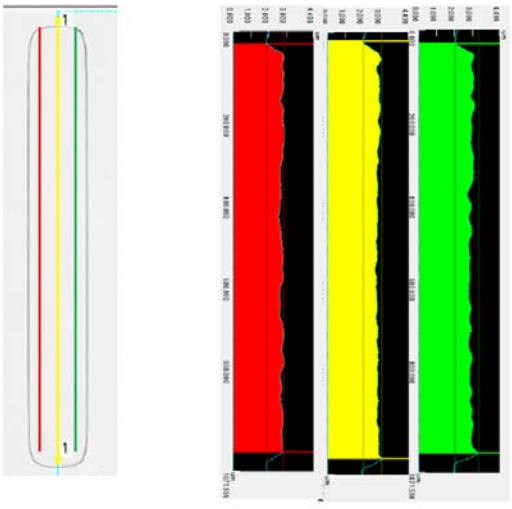
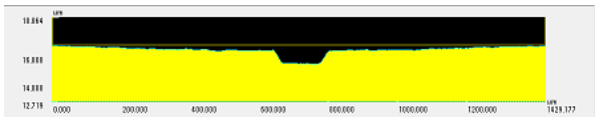



**Fig. 7(B):** Dimension of groove on manufactured mold of upper part measured by laser microscope: top view (upper), cross section (middle) shows mean depth of 0.053 mm, three-dimensional view (lower).

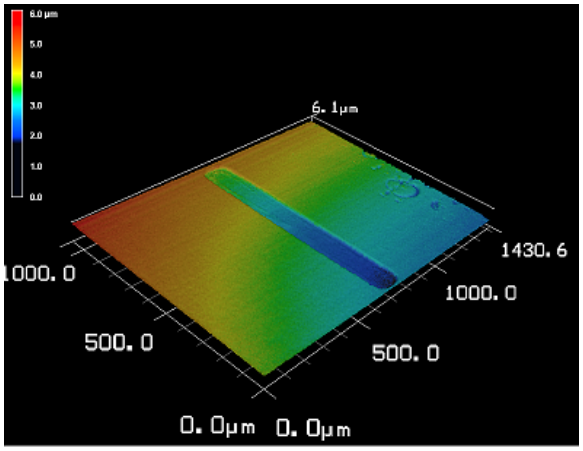


Top view Cross-section of three lines

**Fig. 8(A):** Dimension of ridge on manufactured lower part measured by laser microscope: mean height is 0.0011 mm.



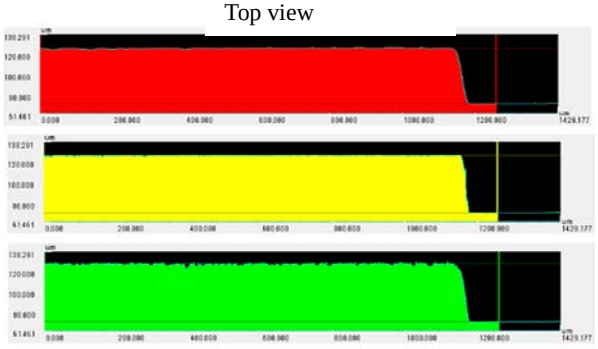
(Tracings of cross-section)



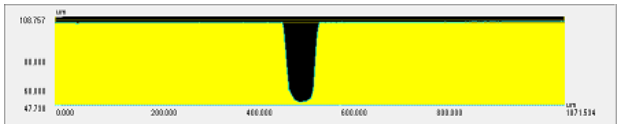

(Three-dimensional view)

**Fig. 8(B):** Dimension of ridge on manufactured lower part measured by laser microscope: mean height is 0.0011 mm.

Top view



Cross-section of three lines



(Cross-section perpendicular to longitudinal direction)

**Fig. 9:** Dimension of ridge on manufactured upper part measured by laser microscope: mean height is 0.055 mm.

In the previous studies, the typical diameter of the micro channel, which simulates the capillary blood vessel, is around 0.005 mm [8]. 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.

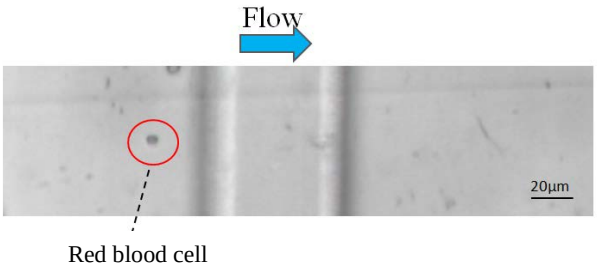



**Fig. 10:** Red blood cell approaches to slit. Flow from left to right. The bar shows 0.02 mm.

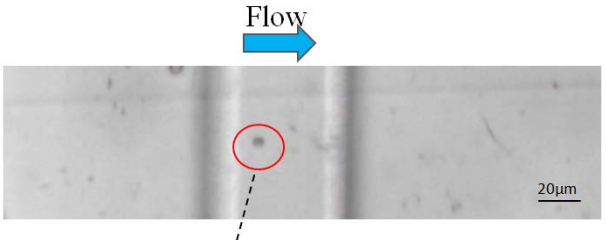



**Fig. 11:** Red blood cell passing through slit. Flow from left to right. The bar shows 0.02 mm.

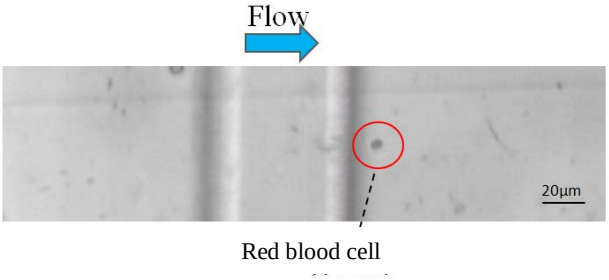



**Fig. 12:** Red blood cell passed through micro slit. Flow from left to right. The bar shows 0.02 mm.

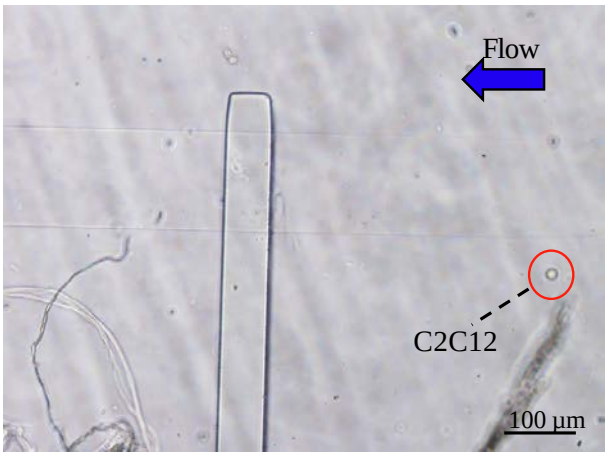



**Fig. 13:** C2C12 approaches to slit. Flow from right to left. The bar shows 0.1 mm.

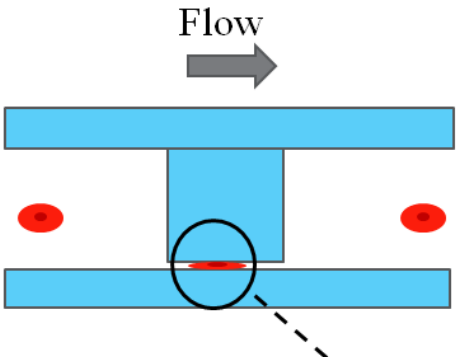



**Fig. 14:** Deformed cell might pass through slit.

The effect of flow on cells has been investigated in the previous studies [12, 13]. A micro channel could simulate the microcirculation system. Fatigue of erythrocyte was evaluated through the narrow path [14, 15]. 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 [10]. The micro-channel devices may contribute to the development of biotechnology.

## 5. CONCLUSION

The behavior of a biological cell through a micro slit has been observed *in vitro*. The slit, of which width is 0.85 mm and height is 0.001 mm, has been designed between two parts of transparent polydimethylsiloxane (PDMS) disks. The suspension of swine red blood cell, or C2C12 has been introduced to the slits, and the movement of cells has been observed with a microscope. The experimental results show that several cells can pass through the micro slit.

## 6. ACKNOWLEDGMENT

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