

# Behavior of Cells through Micro Slit

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## ABSTRACT

The behavior of a biological cell through a micro slit has been observed *in vitro*. An erythrocyte deforms and passes through the micro-circulation, of which the dimension is smaller than the diameter of the erythrocyte. The spleen, for example, has special morphology in the blood flow path to sort erythrocytes. The photolithography technique enables manufacturing the micro slit. A silicone disk was used for a mold, and a dry etching process was applied for the micro-fabrication. The slit, of which width is 0.002 mm and height is 0.08 mm, has been made between micro cylindrical columns of polydimethylsiloxane. The suspension of swine red blood cells or L929 (fibroblast-like mouse cells) has alternatively been introduced to the slits, and the behavior of cells has been observed with a microscope. The experimental results show that several cells can pass through the micro slit of 0.002 mm.

**Keywords:** Biomedical Engineering, Red Blood Cell, L929, 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 erythrocyte. After circulation through the blood vessels for days, the erythrocyte is trapped in the micro-circulation systems.

One of the systems, which trap erythrocytes, is a spleen. The spleen has special morphology in the blood flow path to sort injured erythrocytes [4-6].

The photolithography technique enables manufacturing a micro-channel [7, 8]. 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* [9].

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

## 2. METHODS

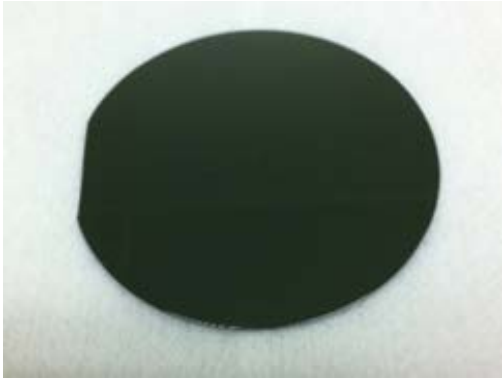
### Micro Slits

Two patterns of micro slits between micro cylindrical columns of 0.05 mm diameter have been designed on a disk of transparent polydimethylsiloxane (PDMS): one with 0.056 mm height columns (type 1) and the other with 0.08 mm height columns (type 2). Variation is made on the interval of the columns, which makes variation of the width of the slits: 0.002 mm, 0.003 mm and 0.004 mm.

### Mold

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 (Fig. 1).

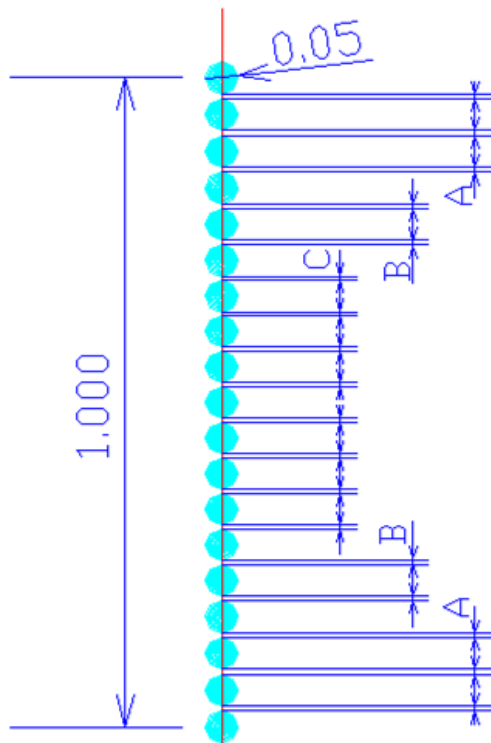
The surface of the wafer is cleaned two times: with 2-propanol for five minutes in an ultrasonic cleaner, and with ultrapure water for ten minutes. Then, the wafer was dried on the hot plate (AHP-300, Asahi-rika, Chiba, Japan) at 383 K for 10 minutes.



**Fig. 1:** Silicon wafer.



**Fig. 2:** Photo-resist material on wafer: before (left) and after spinning.



**Fig. 3:** Pattern of holes (type 1): dimension (mm), 0.005 (A), 0.004 (B), 0.003 (C); depth, 0.056 mm.

### 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 (Fig. 2). The photo-resist was baked on the heated plate at 383 K for 90 s.

The pattern of holes (Fig. 3) was drawn on the wafer with a laser drawing system (DDB-201K-KH, Neoark Corporation, Hachioji, Japan). The 19 cylindrical holes with a diameter of 0.05 mm were arranged on the line with a small interval between 0.003 mm and 0.005 mm. 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 holes 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<sup>2</sup>.

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 on the heated plate. 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 cylindrical holes. The switching mode between C<sub>4</sub>F<sub>8</sub> gas and SF<sub>6</sub> gas was applied on the disk.

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 holes was dried on the hot plate, and used for the concave mold to make micro cylindrical columns in the following process.

### 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 (Fig. 4), PDMS (Sylgard 184 Silicone Elastomer Base, Dow Corning Corporation) was poured with the curing agent (Dow Corning Corporation) on the wafer (Fig. 5). The volume ratio between PDMS and the curing agent is ten to one. The volumes of PDMS are 2 cm<sup>3</sup> for the upper disk and 10 cm<sup>3</sup> 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 (Fig. 6).

### Measurement of Morphology

The dimension of the manufactured slit was measured with EDX WETSEM (SEM, Scanning Electron Microscope, JSM-6360LA), JEOL Ltd., Tokyo, Japan) without coating.

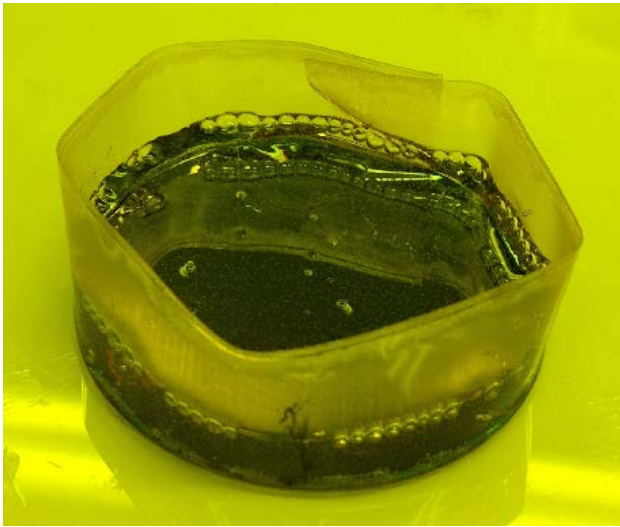
### Flow Test System

A one-way flow system was designed to observe the behavior of cells through the micro slits *in vitro*. 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.

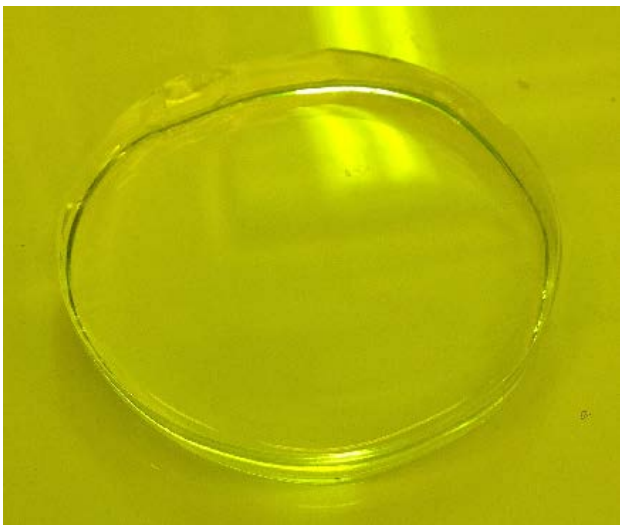
The flow chamber consists of two transparent polydimethylsiloxane (PDMS) disks and a thin sheet of silicone rubber (Fig. 7). The thickness of the sheet is 0.1 mm.



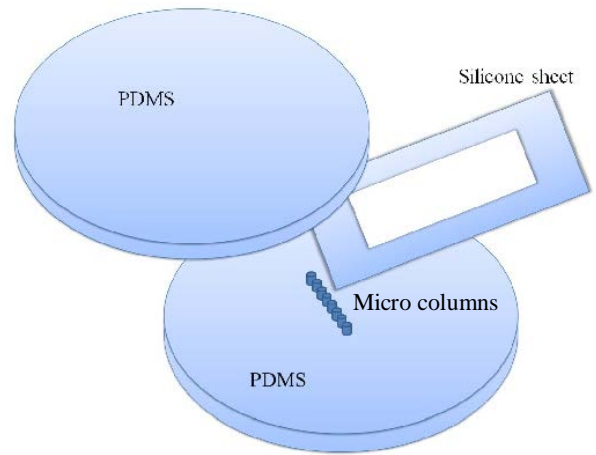
**Fig. 4:** Mold enclosed with peripheral wall of polyimide.



**Fig. 5:** PDMS poured with the curing agent on the wafer.



**Fig. 6:** Disk of PDMS.



**Fig. 7:** Flow chamber consists of two PDMS disks and a thin sheet of silicone rubber.



**Fig. 8:** Upper disk of PDMS with two holes for connection with tube.

The flow chamber consists of two transparent polydimethylsiloxane (PDMS) disks and a thin sheet of silicone rubber (Fig. 7). The thickness of the sheet is 0.1 mm.

A rectangular open space of 1 mm × 20 mm is cut off in the sheet, and sandwiched between the PDMS plates. The open space forms a channel of 20 mm length × 1 mm width × 0.1 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 10 mm and 2 mm, respectively.

The PDMS disk, which has micro columns on the upper surface, was placed in the bottom. The upper PDMS disk has no micro pattern, and has two holes of 5 mm diameter machined with a punching tool (Fig. 8). The silicone tubes are stuck at the holes without an adhesive for the inlet and the outlet (Fig. 9).



**Fig. 9:** Flow chamber with inlet and outlet tubes.



**Fig. 10:** Swine blood.

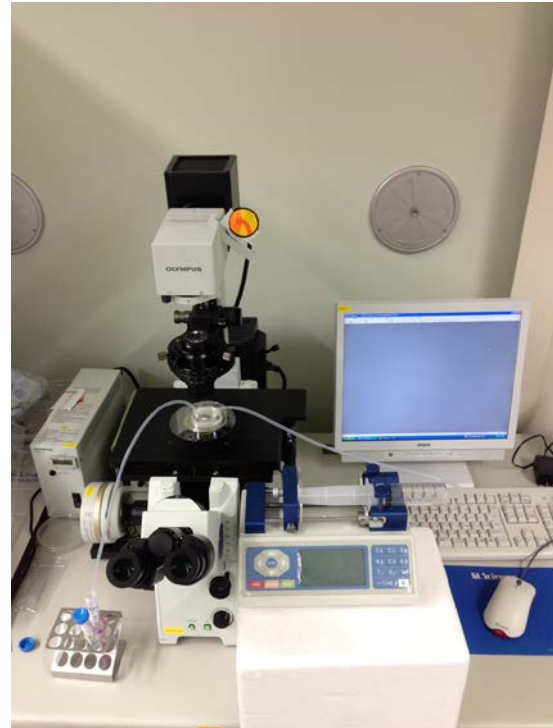
#### Flow Test

Two kinds of cells were used in the flow test: L929 (fibroblast-like, mouse connective tissue, RCB1422, Riken Bio Resource Center, Tsukuba), and swine red blood cells.

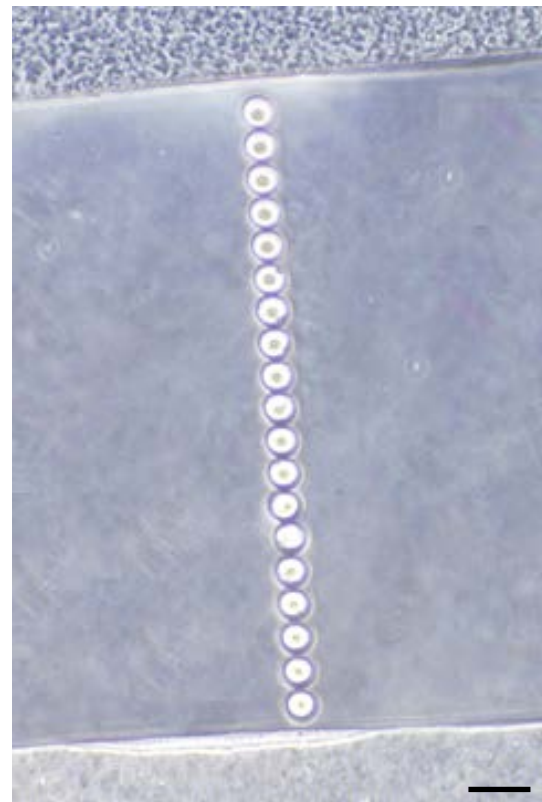
In the case of L929, cells were suspended in the phosphate buffer solution.

Swine red blood cells were used after preservation in the refrigerator for two days. After centrifugation of the swine blood, the red blood cells were separated from plasma (Fig. 10). The cells were suspended in the phosphate buffer solution to make a suspension of red blood cells at the volume ratio of 0.1 percent.

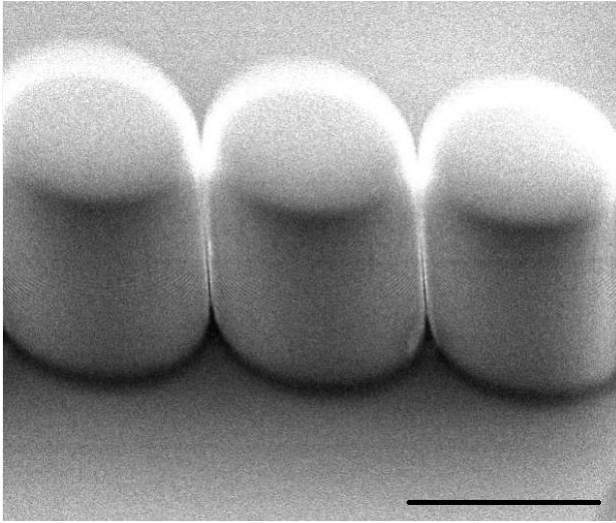
The movement of cells near the slits was observed with an inverted phase-contrast microscope (IX71, Olympus Co., Ltd., Tokyo), while the suspension of cells was pumped at the flow rate of  $1 \text{ cm}^3/\text{hour}$  at  $298 \text{ K}$  (Fig. 11). In the flow path of depth of  $0.1 \text{ mm}$  (width of  $1 \text{ mm}$ ), the flow rate makes mean velocity of  $2.8 \text{ mm/s}$ .



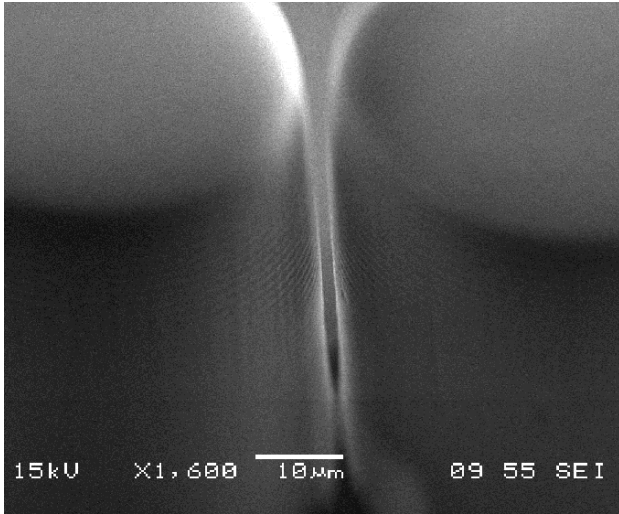
**Fig. 11:** Flow system.



**Fig. 12:** Micro cylindrical columns in the flow path of which width is  $1 \text{ mm}$ . The bar shows  $0.1 \text{ mm}$ .



**Fig. 13:** Micro slits between micro cylindrical columns. The bar shows 0.05 mm.



**Fig. 14:** Micro slits between micro cylindrical columns.

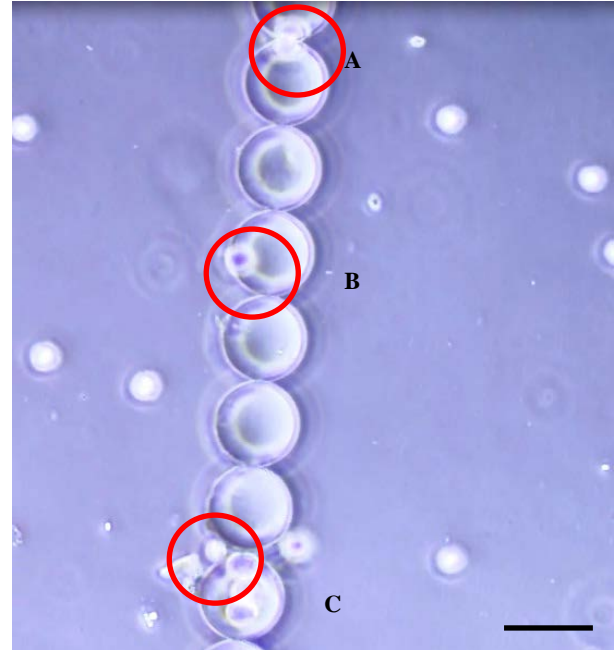
### 3. RESULTS

Fig. 12 shows the top view of the twelve micro cylindrical columns in the flow path.

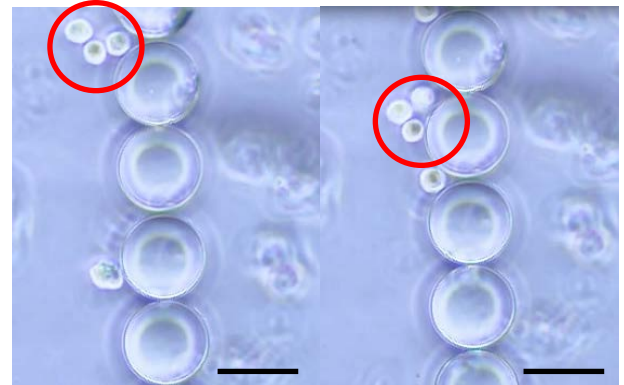
The microscopic measurement shows that the minimum width of the slits between columns is 0.002 mm (Figs. 13 & 14).

In Figs. 15-18, the medium flows from left to right. Figs. 15-17 show L929 flows near the micro columns. Some cells are passing through the micro slit (Fig. 15(A)). Some cells are flowing over the micro column in type 1 (Fig. (B)). Few cells are flowing over the column in type 2. Some cells get stuck between the micro columns (Fig. 15(C)). Some cells move to wider slit (Fig. 16).

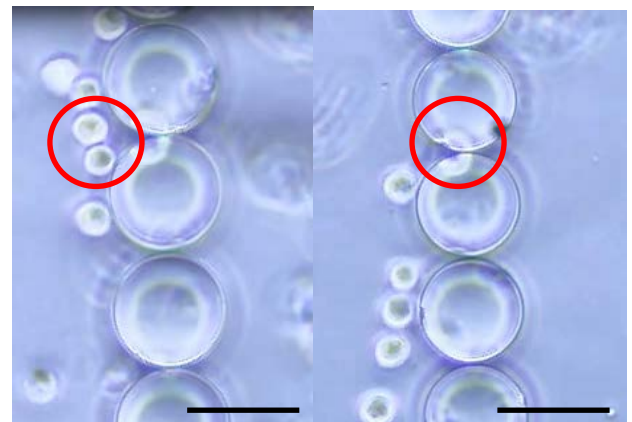
Fig. 18 shows red blood cells flowing near the micro columns. Every cell is passing through the slit.



**Fig. 15:** L929 (A) flows through micro slits of type 1. Cell flows above column (B). Cell stuck in slit (C). The bar shows 0.05 mm.



**Fig. 16:** L929 moves to wider slit of type 2 (from top to bottom). The bar shows 0.05 mm.



**Fig. 17:** L929 flows through micro slits (from left to right) of type 2. The bar shows 0.05 mm.



**Fig. 18:** Red blood cells flow through micro slits of type 2. The diameter of columns is 0.05 mm.

#### 4. DISCUSSION

The micro-slit is useful for treatment of cell in diagnostics [9].

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.

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.

The effect of flow on cells has been investigated in the previous studies [10-11]. A micro channel could simulate the microcirculation system. To simulate the microcirculation system with a fabricated channel, the three dimensional curvature of the wall of the flow channel might be important [12]. Cells are responsive to the micro morphology of the scaffold. The microgroove governs the behavior of cells [13].

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 0.002 mm and height is 0.08 mm, has been made between micro cylindrical columns. The suspension of swine red blood cells, or L929 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 of 0.002 mm.

#### 6. ACKNOWLEDGMENT

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