# Design of Slit between Micro Cylindrical Pillars for Cell Sorting

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## ABSTRACT

Micro slits have been designed between micro cylindrical pillars to sort biological cells. Micro cylindrical pillars of 0.02 mm diameter and 0.055 mm height were fabricated on the glass plate using the photolithography technique. Variation was made on the gap between pillars: 0.01 mm, 0.02 mm, 0.03 mm, and 0.04 mm. The micro pillars are set in the flow path between parallel plates, of which dimension of the cross section has 5 mm width and 0.055 mm height. Three types of biological cells were used in the test alternatively: C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse), Hepa1-6 (mouse hepatoma cell line of C57L mouse), or swine red blood cell. The suspension of cells was introduced into the slits by the syringe pump at the flow rate between 0.4 and 500 $cm^3$/hour. The deformation of the cell at the slit can be observed by the microscope. The experimental results show that the designed slit has capability for sorting cells according to the size and deformability of the cell.

**Keywords:** Biomedical Engineering, Cell Sorting, C2C12, Hepa1-6, Red Blood Cell, Photolithography, and Micro Flow Channel.

## 1. INTRODUCTION

Biological cells can pass through narrow gaps: micro capillaries, or micro slits. The biological system sorts cells according to the size, deformability, and adsorptivity of the cell. Cells are sorted according to deformability through the gap or adsorptivity on the membrane *in vitro*. The technique might be applied to handle cells in diagnostics *in vitro*.

A red blood cell has flexibility and deforms in the shear flow [1]. 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. Some cells can pass through the slit narrower than the capillary.

The photolithography technique enables manufacturing a micro-channel. Several micro-fabrication processes have been designed to simulate the morphology of the microcirculation. In the previous study, the micro slits have designed between micro cylindrical pillars [2], or between micro ridges [3, 4]. The micro-fabrication technique has also been applied to design microfluidic systems *in vitro* [5].

In the present study, micro slits have been designed between micro cylindrical pillars to sort biological cells *in vitro*.

## 2. METHODS

**Micro Slits**

For trapping cells, several micro slits have been designed between micro cylindrical pillars. The dimension of each micro cylindrical pillar is as follows: 0.02 mm diameter, and 0.055 mm height. Variation is made on the dimension of the gap between pillars (the width of slits): 0.01 mm, 0.02 mm, 0.03 mm, and 0.04 mm.

Several cylindrical pillars are made by photolithography technique along the line. The line is perpendicular to the flow direction of the channel. Variation has been made on several parameters for micromachining to control the three dimensional shape of the micro slit.

The arrangement of the micro cylindrical pillars is controlled by a photomask, which is made by the following process (Fig. 1).

The borosilicate glass (Tempax) disk (50 mm diameter, 1mm thickness) was used for the base of the mask. The disk was dipped in the pure water and cleaned by the ultrasonic washer for five minutes. After hydrophilization by the oxygen plasma ashing by RIE (FA-1, Samco International, Kyoto, Japan), titanium was coated on the surface with 100 nm thickness in the sputtering equipment (L-210S-FH, Canon Anelva Corporation). To improve affinity between titanium and photoresist material, HMDS (hexamethyldisilazane: Tokyo Chemical Industry Co., Ltd., Tokyo) was coated on the disk at 3000 rpm for 30 s with a spin coater.

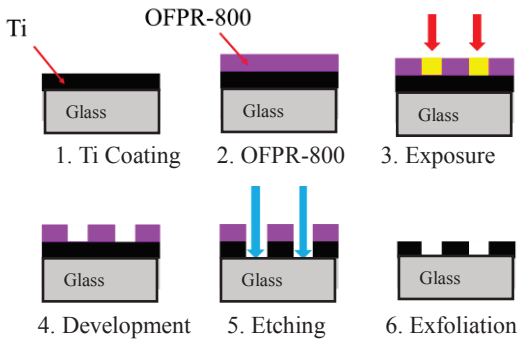



**Fig. 1:** Process for fabrication of photomask.

The positive photoresist material of OFPR-800LB (Tokyo Ohka Kogyo Co., Ltd, Tokyo, Japan) was coated on the disk at 5000 rpm for 30 s with the spin coater. The photoresist was baked in the oven at 368 K for three minutes.

The pattern for pillars was drawn on the mask 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 2.3 V, the velocity of 0.08 mm/s, the acceleration of 0.5 mm/s$^2$. The pattern was baked on the heated plate at 368 K for five minutes. The photoresist was developed with tetra-methyl-ammonium hydroxide (NMD-3, Tokyo Ohka Kogyo Co., Ltd., Kawasaki, Japan) for 8 min.

After confirmation of free of residual resist by the microscope, the titanium coating disk was etched with the plasma gas using RIE-10NR (Samco International, Kyoto, Japan). For etching, the gas of $SF_6$ (50 cm$^3$/min at 1013 hPa) with Ar (50 cm$^3$/min at 1013 hPa) was applied at 100 W at 4 Pa for five minutes. OFPR-800LB was removed by acetone, after confirmation of the pattern of etching by the microscope.

The glass slide (26 mm × 38 mm × 1 mm) was used for the base of micro pillars, after cleaning and hydrophilization. The negative photoresist material of high viscosity (SU8-10: Micro Chem Corp., MA, USA) was coated on the glass slide at 1000 rpm with a spin coater (Fig. 2). The photoresist was baked in the oven at 368 K for ten minutes. The same process of coating with baking (40 min) was repeated one more time. The surface of mask coated with titanium was mounted on the surface of SU8-10, and the photoresist was exposed to the UV light through the mask in the mask aligner (M-1S, Mikasa Co. Ltd., Japan) at 10 V for 20 s. The photoresist was baked in the oven at 368 K for ten minutes. The photoresist was developed with Remover PG (Nippon Kayaku Co., Ltd, Tokyo, Japan) to make micro pillars. The glass slide with the micro pattern was rinsed with acetone, ethanol, and the ultrapure water.

At the end of the process, the dimension of the micro pillars was measured with a laser microscope (VK-X200, Keyence Corporation, Osaka, Japan). The height along the cross sectional line of micro pillars was traced. The micro morphology of the surface with the micro pillars was also observed by a scanning electron microscope (JSM6360, JEOL Ltd., Japan).

**Flow Test System**

A one-way flow system has been designed to introduce the suspension of cells. The system consists of a flow channel, a micro syringe pump, tubes and a microscope. A silicone tube of 3 mm internal diameter and of 5 mm external diameter is used for the connector to the flow channel (Fig. 3).

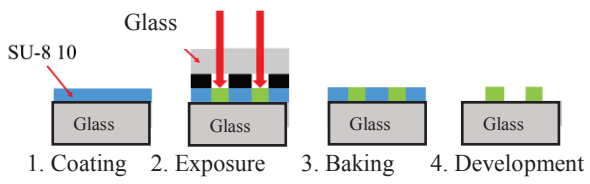



**Fig. 2:** Process for fabrication of pillars.

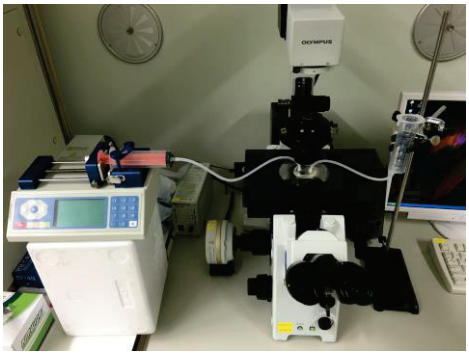


**Fig. 3:** Flow test system.

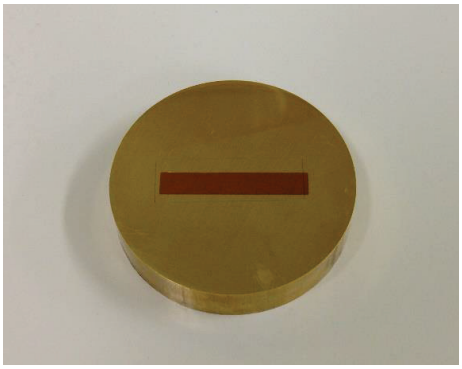


**Fig. 4:** Mold for upper plate of flow channel.

The flow channel consists of the upper plate of transparent polydimethylsiloxane (PDMS) and the lower plate of glass disk with micro pillars.

The upper plate was made of PDMS. A brass disk was used for the mold of the upper plate (Fig. 4). After the polyimide tape of 0.055 mm thickness was adhered on the surface of the blass disk, the tape was cut by laser (Ultrashort Pulse Laser, High power industrial femtosecond laser, IFRIT, Cyber Laser Inc., Tokyo, Japan) to make the rectangle of 30 mm × 5 mm. The rectangular convex part on the mold makes the concave part for the flow channel.

After the polyimide tape was attached around the mold to make the peripheral wall, PDMS (Sylgard 184 Silicone Elastomer Base, Dow Corning Corp., MI, USA) was poured with the curing agent (Sylgard 184 Silicone Elastomer Curing Agent, Dow Corning Corp., MI, USA). The volume ratio of PDMS to curing agent is ten to one. After degassing, PDMS was baked at 373 K for one hour in the oven.

A rectangular parallelepiped channel of 30 mm length × 5 mm width × 0.055 mm height is formed between upper and lower plates. The two plates stick together with their surface affinity.

At the upper plate of PDMS, two holes of 5 mm diameter (Fig. 3) are machined by a punching tool. The silicone tube is inserted to each hole of the upper PDMS disk. To seal the leak at the gap between elements, the liquid of PDMS was pasted on the junction of elements, and baked at 373 K for 20 minutes in an oven.

One of the tubes is connected to the syringe pump, and the other tube is connected to the reservoir of the suspension. The channel is placed on the stage of the inverted phase-contrast microscope (IX71, Olympus Co., Ltd., Tokyo).

**Flow Test**

Three kinds of biological cells were used in the test alternatively: C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse), Hepa1-6 (mouse hepatoma cell line of C57L mouse), or swine red blood cells.

Cells (C2C12 and Hepa1-6) were cultured with the D-MEM (Dulbecco's Modified Eagle's Medium) containing 10% FBS and 1% of Antibiotic-Antimycotic (penicillin, streptomycin and amphotericin B, Life Technologies Japan Ltd., Tokyo, Japan) in the incubator for four days. Before the flow test, the cells were exfoliated from the plate of the culture dish with trypsin, and suspended in the D-MEM.

In the case of swine red blood cells, the blood collected through the carotid artery was diluted by thousand times with the saline solution.

After the channel was prefilled with the saline solution, the suspension of cells was introduced to the channel at the constant flow rate with the micro syringe pump.

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 through the slit in the channel was observed with the microscope for five minute. From the microscopic video image for the selected five minutes, the passing ratio ($R$) was calculated by Eq. 1.

$$R = N_2 / N_1 \quad (1)$$

In Eq. 1, $N_1$ is the number of cells attacking to the slit, and $N_2$ is the number of cells passing through the slit.

## 3. RESULTS

Fig. 5 shows the photomask, which was made after adjustment of the micromachining condition. The designed pattern of circles is successfully etched on the titanium film on the glass. Fig. 6 shows the plane view of micro pillars. After adjustment of the micromachining condition, the micro pillars are arranged on the glass slide.

Fig. 7 shows the tracings of the height along the cross section of the micro-machined pillars on the glass. After adjusting the micromachining conditions of the photolithography, the side surface of cylindrical pillar has successively become perpendicular to the base surface of the glass. The measurement by the laser microscope shows that dimension of each micro cylindrical pillar is as follows: 0.02 mm diameter, and 0.055 mm height. The width of each slit between pillars is 0.01 mm, 0.02 mm, 0.03 mm, or 0.04 mm, respectively.

Fig. 8 shows the scanning electron microscope image of the micro pillars. Although several neighboring top surfaces of the micro cylindrical pillars make connections over the slit of 0.01 mm, the morphology of the gap between pillars keeps rectangular slit. The micro pillars stand perpendicular to the surface of the glass slide.

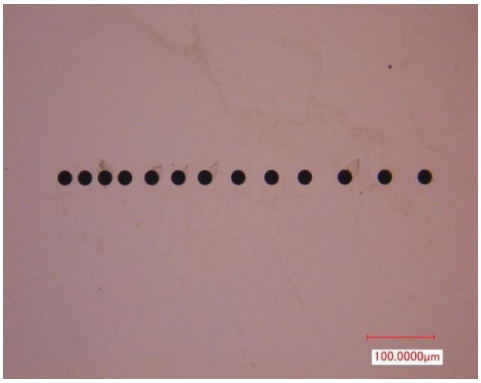



**Fig. 5:** Photomask.

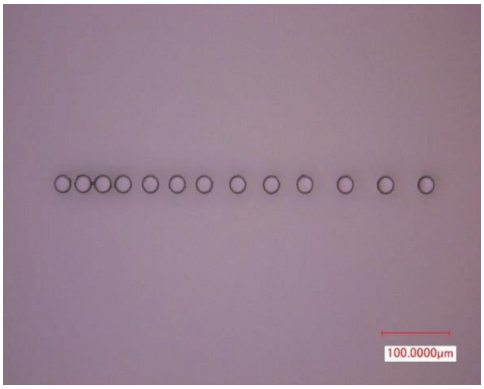



**Fig. 6:** Micro pillars.

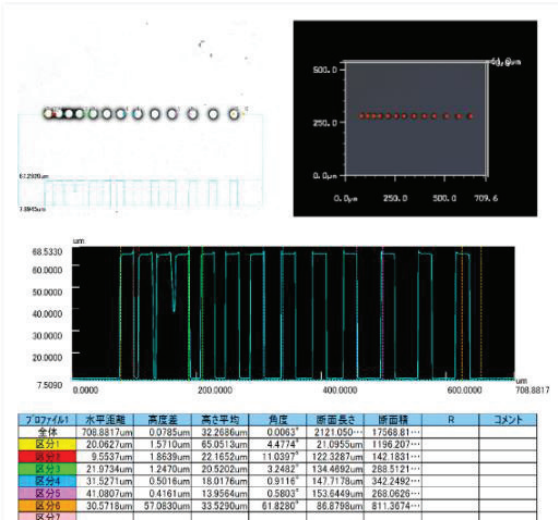


**Fig. 7:** Tracings of the height along the cross section of the micro-machined pillars.

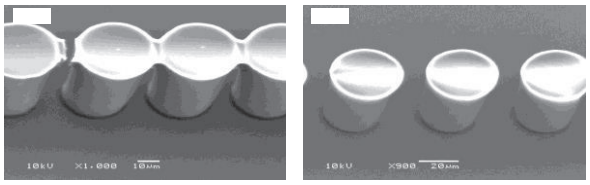


**Fig. 8:** Scanning electron microscope image of slit between micro pillars.

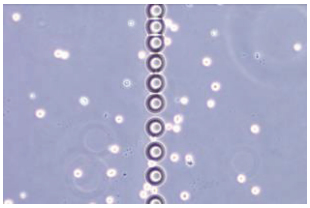
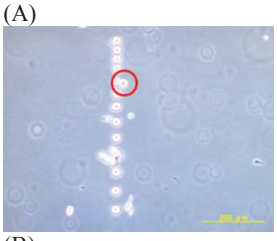
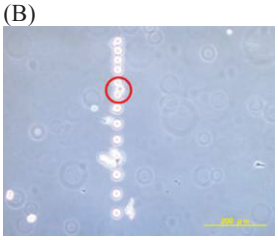


**Fig. 9:** C2C12 deformed in slit.

(A)



(B)



(C)

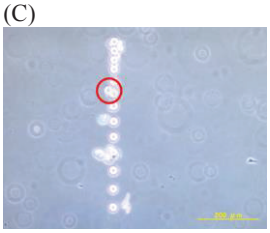


**Fig. 10:** C2C12 attacking (A), trapped at 3 $\times 10^{-10}$ $m^3/s$ (B), slipping out at 1 $\times 10^{-7}$ $m^3/s$ (C). Dimension from left to right is 1 mm.

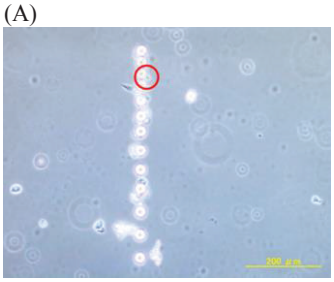
Figs. 9-12 show cells passing through the slit between micro pillars. Some cells deform, and pass through the slit. At the deformation, the projected area decreases 50 percent (Fig. 9). The projected area increases, after passing through the slit. C2C12 trapped at the 0.02 mm slit slips out, after the flow rate increases from 3 $\times 10^{-10}$ $m^3/s$ to 1 $\times 10^{-7}$ $m^3/s$ (Fig. 10C).

The cell trapped at the slit is able to deform and pass through the slit by the flow at higher flow rates. Hepa1-6 is able to pass the narrower slit than C2C12 (Fig. 11).

Fig. 13 shows trapping ratio of cells at each trial, when each width of slits is arranged on each line: 0.01 mm, 0.02 mm, 0.03 mm and 0.04 mm, respectively. The width of the slits is same on each line. Cell flows from wider to narrower slits of line, step by step.

In 6 trials of C2C12, 156 cells of 544 cells were captured at the slit of 0.03 mm, 313 cells of 386 cells were captured at the slit of 0.02 mm, and every cell of 73 cells was captured at the slit of 0.01mm.

(A)



(B)

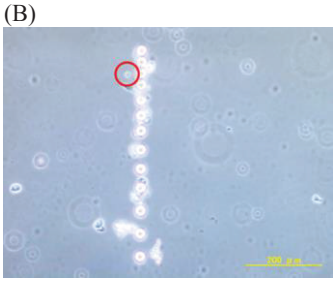
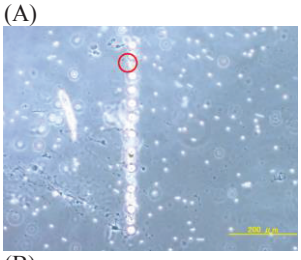


**Fig. 11:** Hepa1-6 attacking (A), slipping out of slit at 3 $\times 10^{-10}$ $m^3/s$ (B). Dimension from left to right is 1 mm.

(A)



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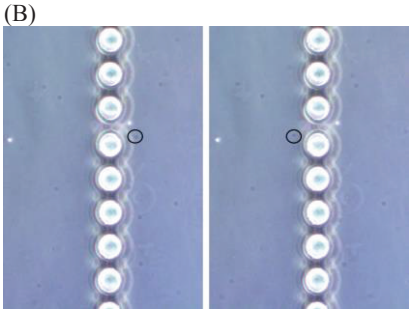

**Fig. 12:** Red blood cells passing through slit at 3 $\times 10^{-10}$ $m^3/s$.

In 7 trials of Hepa1-6, 13 cells of 194 cells were captured at the slit of 0.03 mm, 91 cells of 184 cells were captured at the slit of 0.02 mm, and 89 cells of 90 cells were captured at the slit of 0.03 mm.

In 6 trials of red blood cell, two cells of 127 cells were captured at the slit of 0.01 mm.

Any cells (C2C12, Hepa1-6, and red blood cell) were not captured at the slits of 0.04 mm. The slit of width between 0.01 mm and 0.03 mm can sort Hepa1-6.

Micro pillars fell down on glass plate, when the flow rate is higher than 1 $\times 10^{-7}$ $m^3/s$ (Fig. 14). At the flow rate lower than 1 $\times 10^{-7}$ $m^3/s$, the affinity between micro pillar and the glass plate is enough strong to keep standing.

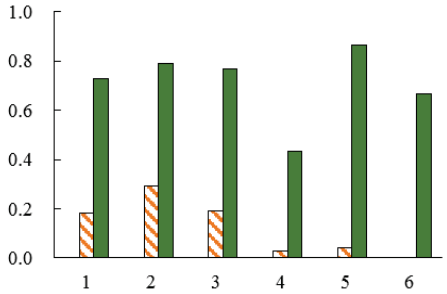


**Fig. 13A:** Passing ratio of C2C12 in 7 trials: at slit of 0.02 mm (middle), 0.003 mm (right).

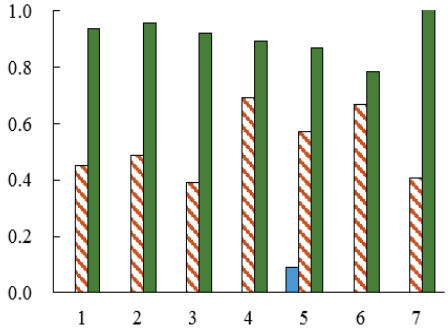


**Fig. 13B:** Passing ratio of Hepa1-6 in six trials: at slit of 0.01 mm (left), 0.02 mm (middle), 0.003 mm (right).

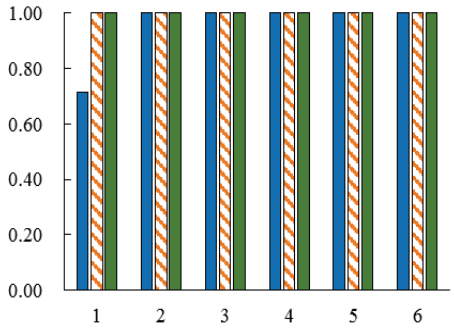


**Fig. 13C:** Passing ratio of red blood cell in six trials: at slit of 0.01 mm (left), 0.02 mm (middle), 0.003 mm (right).

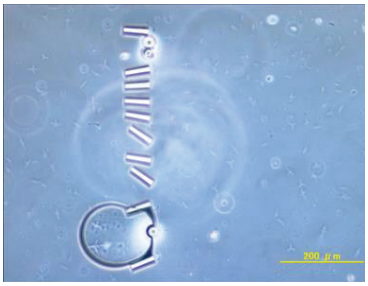

**Fig. 14:** Micro pillars falling down on glass plate by higher flow rate in the channel. Dimension from left to right is 1 mm.

## 4. DISCUSSION

The wall shear stress ($\tau_w$) is estimated by Eq. 2 with the parabolic velocity distribution between parallel piped micro-channel.

$$\tau_w = 6\,\eta\,Q / b\,d^2 \qquad (2)$$

In Eq, 2, $\eta$ is viscosity of the fluid, $Q$ is flow rate, $b$ is width of the flow path, and $d$ is the distance of the parallele wall. When $\eta$ = 0.002 Pa s, $Q = 1 \times 10^{-7}$ m$^3$/s, $b$ = 0.005 m, and $d$ = 0.000055 mm, $\tau_w$ = 80 Pa. When $Q = 1 \times 10^{-10}$ m$^3$/s, $\tau_w$ = 0.08 Pa. The micro pillar keeps standing against the wall shear stress of 80 Pa. The cylindrical shape of the pillar was, on the other hand, confirmed by the fallen pillars in Fig. 14.

The capture of the cell at the slit depends on several factors: the dimension of the cell and the slit, deformation of the cell, the wall sear stress (shear rate) at the slit, and the affinity (charge) of the cell to the wall of the slit.

The diameter calculated from the projected area of the circle of the floating C2C12, Hepa1-6 and red blood cells are 0.018 mm ± 0.001 mm, 0.021 mm ± 0.004 mm and 0.006 mm ± 0.0005 mm (mean ± standard deviation), respectively.

Preparation of the slit of sub micrometer is not easy. In the previous study, the slit of micrometer between pillars was manufactured by photolithography technique [2]. In another study, the micro slit was manufactured between ridges [3, 4].

Only one projection can be traced in the present experimental system. The cell can deform in the perpendicular direction. To observe the deformation in the perpendicular plane, the slit between upper and lower ridges is effective [4].

It is not easy to fabricate micro slit, keeping the rectangular shape smaller than 0.01 mm. The resistance of the flow channel is very high in the present experimental system. The resistance increases, when the cells clogged the slits in the flow channel. Internal pressure of the flow channel should be carefully controlled under the constant flow rate, otherwise the leakage or cavitation would occur.

In the present study, the flow rate is confirmed by the flow speed of the cell during microscopic observation.

There are several methods to sort cells [6-9]. Non-invasive way is preferable. To sort cells with minimum damage. Flow cytometry is one of the technology, which is used for cell sorting [7]. Cells suspended in the fluid are analyzed by the laser. The fluorescently labelled components in the cell are analyzed by the light emission.

The stream line at the entrance of the slit is not always symmetric, and depends on several factors: the dimension of the slit, the condition of the clogged cells at the slit, and the interaction between cells. When the dimensions of the slit at the one side of the pillar is narrower than that at the other side, the asymmetry of the flow makes curved stream line. The cells flow along the curved stream line adjacent to the pillar. Cell can flow through the extra space of the slit, which is clogged with a cell.

The photolithography technique has been applied to fabricate the micro channel. The microfluidic system has been applied to sort biological cells, and to trap biological cells [10, 11]. The micro pattern of the surface has been applied to study the surface effect of adhesion of cells [5, 12, 13].

In the previous study, several kinds of holes were designed for trap of cells: cylindrical [14], half cylindrical [15] holes, and rectangular grooves [5].

The adhesion of the cell in the flow depends on the morphology of the wall and the wall shear stress [11, 16, 17]. The morphology of the defect on the wall of flow path might govern the capture of cells. The capture might depend on the property of the cell. The mechanism of adhesion might be applied to sort cells [18, 11].

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

A red blood cell has flexibility and deforms in the shear flow [1]. 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. The results of the present test shows that swine red blood cells can pass through the slit of 0.01 mm, even after preservation of one week in the refrigerator.

## 5. CONCLUSION

Micro slits have been designed and fabricated between micro cylindrical pillars using the photolithography technique. The selected machining condition has realized the rectangular micro slits between micro pillars, which is confirmed by microscopic measurement. Three kinds of biological cells were used in the test alternatively: C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse), Hepa1-6 (mouse hepatoma cell line of C57L mouse) or swine red blood cells. The experimental results show that the designed slit has capability for sorting cells according to the size and deformability of the cell.

## 6. ACKNOWLEDGMENT

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