Deformation of Cell Passing through Micro Slit between Micro Ridges

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ABSTRACT

The micro slits have been designed between micro ridges, and deformation of a biological cell passing through a micro slit has been observed *in vitro*. The slit, of which width is 1.3 mm and height is 0.0004 mm or 0.010 mm, has been designed between the transparent polydimethylsiloxane disk and glass disk. Each disk has the micro ridge. The slit is placed at the middle part of a flow channel: 0.05 mm height, 3 mm width. The suspension of swine red blood cells, or Hepa1-6 (mouse hepatoma cell line of C57L mouse) was alternatively introduced to the slits by drawing with a syringe pump at a constant flow rate of 2.8×10^{-11} m^3/s (2.8×10^{-10} m^3/s for Hepa1-6). The deformation of cells passing through the micro slit was observed with an inverted phase-contrast microscope. The experimental results show that cells deform to the flat disk and pass through the micro slit: red blood cell at 0.0004 mm height, and Hepa1-6 at 0.010 mm height. The deformation is evaluated with the ratio of the projected area of the disk during the passing through the slit. The designed slit between micro ridges has capability to evaluate the deformability of cells.

Keywords: Biomedical Engineering, Red Blood Cell, Hepa1-6, Deformation, Photolithography, and Micro-slit.

1. INTRODUCTION

The behavior of biological cells depends on the flow [1]. The deformability of the biological cell plays an important role *in vivo*. An erythrocyte, for example, has high flexibility [2-7] and deforms in the shear flow [8, 9]. 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.

Several systems sorts cells according to the deformability *in vivo*. 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 [10-14].

The photolithography technique enables manufacturing a micro-channel [15-21]. 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*.

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

2. METHODS

Micro Slit

The slit, of which width (W) is 1.30 mm and height (H) is 0.0004 mm (narrow) or 0.010 mm (wide), has been designed between a transparent polydimethylsiloxane (PDMS) disk and a borosilicate glass (Tempax) disk (Figs. 1 & 2). Both of the disks have micro ridges. The upper disk of PDMS has a ridge of 0.06 mm height: 0.10 mm width and 2 mm length. The lower disk of glass has two ridges of 0.3 mm width (2.3 mm length) with the interval (W) of 1.3 mm. At the ridges of the lower part, variation is made on the height (H): 0.0004 mm (narrow, H_1) or 0.010 mm (wide, H_2). These ridges make contact each other in the perpendicular position, and make slits between the ridges.

Upper Disk

A silicon wafer (Type N, Matsuzaki Seisakusyo, Co., Ltd., Tokyo, Japan) is used for a surface mold for the upper disk (Fig. 3). The diameter and the thickness of the wafer are 50 mm and 0.30 mm, respectively.

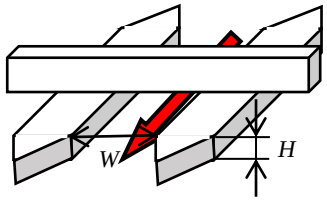

Fig. 1: Slit between ridges.

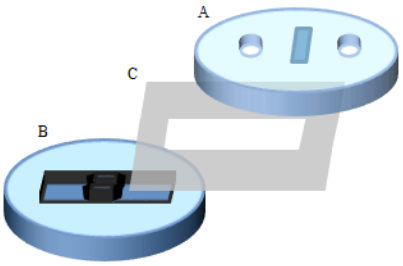

Fig. 2: Upper PDMS (A) and lower glass (B) disks, and silicone sheet (C).

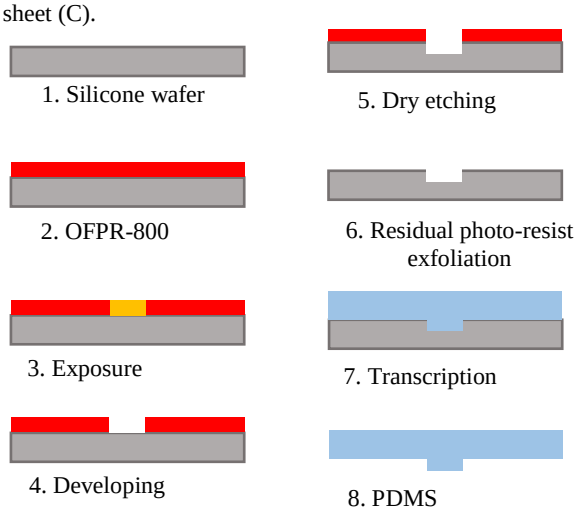

Fig. 3: Photolithography process of upper disk.

To increase affinity between the wafer and the photo-resist material, hexamethyldisilazane (HMDS, 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 positive 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 in an oven.

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). To increase the adhesiveness of the coating, the wafer was baked in the oven. The photo-resist was developed with tetra-methyl-ammonium hydroxide (NMD-3, Tokyo Ohka Kogyo Co., Ltd., Kawasaki, Japan).

For the upper disk, the wafer was etched with the Deep RIE System (MUC-21 ASE-SRE, Sumitomo Precision Products Co., Ltd., Amagasaki, Japan) to make the deeper micro grooves. To exfoliate the residual photo-resist material from the surface, the disk was exposed to the oxygen gas of 30 milliliter per minute at power of 100 W for five minutes using a compact etcher (FA-1, Samco Inc., Kyoto): the oxygen plasma ashing.

The dimension of the grooves on manufactured mold was measured with the laser microscope (VK-X200, Keyence Corporation, Osaka, Japan) (Fig. 8). After the mold of 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 volume of PDMS is 8.8 cm^3 for the upper disk.

After degassing, PDMS was baked at 353 K for one hour 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 (Fig. 9). 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.

Photomask for Lower Disk

Three kinds of the photomask were designed with variation of patterns: a vacancy of 3 mm × 30 mm (A), two vacancies of 0.3 mm × 2.3 mm with the interval of 1.3 mm (B), and two patterns of 0.3 mm × 2.3 mm with the interval of 1.3 mm (C). The borosilicate glass (Tempax) disk (50 mm diameter, 1.1 mm thickness) was used for the base of the mask (Fig. 4). Titanium was coated on the surface with 150 nm thickness in the electron beam vapor deposition apparatus (JBS-Z0501EVC, JEOL Ltd., Japan). The positive photoresist material of OFPR-800LB (Tokyo Ohka Kogyo Co., Ltd, Tokyo, Japan) was coated on the disk at 3500 rpm for 5 s with the spin coater. The photoresist was baked in the oven at 368 K for three minutes.

The pattern was drawn on the mask with a laser drawing system. The pattern was baked on the heated plate at 368 K for three minutes. The photoresist was developed with tetra-methyl-ammonium hydroxide (NMD-3, Tokyo Ohka Kogyo Co., Ltd., Kawasaki, Japan) for 8 minutes. The disk was rinsed by the ultrapure water, and dried by the spin-dryer. The titanium coating disk was etched with the plasma gas using RIE-10NR (Samco International, Kyoto, Japan).

Lower Disk (Narrow H_1)

The positive photo-resist material of OFPR-800 (Tokyo Ohka Kogyo Co., Ltd, Tokyo, Japan) was coated on the Tempax glass (50 mm diameter) at 3500 rpm for 5 s with the spin coater (Fig. 5). The photo-resist was baked in the oven at 368 K for three minutes.

Two kinds of masks (A and C) coated with titanium were combined and adhered on the surface of OFPR-800, and the photoresist was exposed to the UV light through the mask in the single sided mask aligner (M-1S, Mikasa Co. Ltd., Japan) at 10 V for 30 s. The photoresist was baked in the oven at 368 K for three minutes.

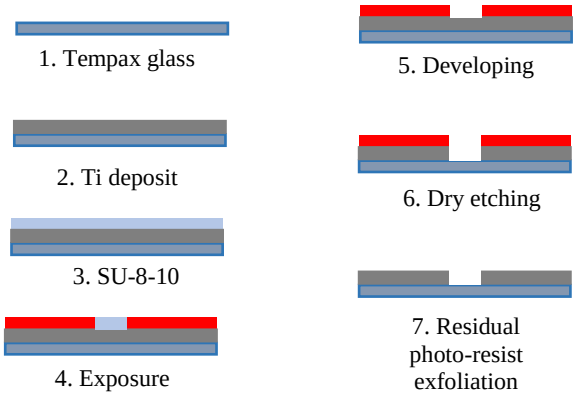

Fig. 4: Photomask for lower disk.

The photoresist was developed with tetra-methyl-ammonium hydroxide (NMD-3, Tokyo Ohka Kogyo Co., Ltd., Kawasaki, Japan) for eight minutes. The glass was immersed in a buffered hydrofluoric acid solution on a mixture of hydrofluoric acid and ammonium fluoride for 20 minutes for etching.

Lower Disk (Wide H_2)

The negative photoresist material of high viscosity (SU8-10: Micro Chem Corp., MA, USA) was coated on the Tempax glass (50 mm diameter) at 2500 rpm for 30 s with a spin coater. The photo-resist was baked in the oven at 333 K for three minutes.

The mask (B) coated with titanium was adhered on the surface of SU8-10, and the photoresist was exposed to the UV light through the mask in the single sided mask aligner. The aluminum sheet of 3 mm × 50 mm was adhered on the surface of the photoresist, and the resist was exposed to the UV light through the mask in the single sided mask aligner to form the flow channel.

After the resist was baked in the oven at 333 K for three minutes, the photoresist was developed with SU8 developer (Nippon Kayaku Co., Ltd, Tokyo, Japan).

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 channel, a syringe pump, tubes and a microscope (Fig. 6).

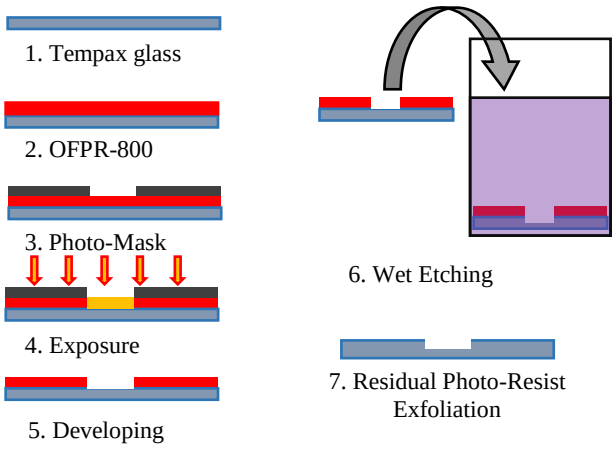

Fig. 5: Photolithography process of lower disk.

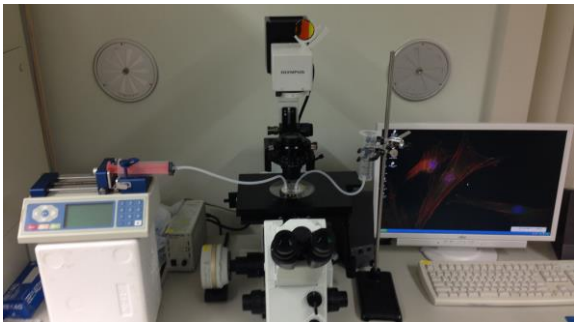

Fig. 6: Flow (from right to left) test system: reservoir (right), flow channel on the microscope, syringe pump (left).

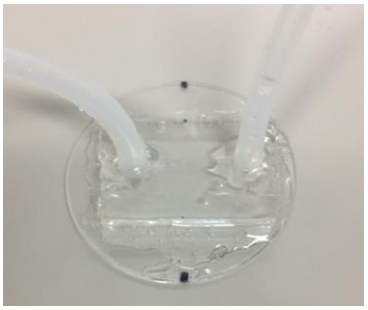

Fig. 7: Flow channel with micro slit.

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 (Fig. 2) consists of two transparent upper and lower disks and a thin sheet of silicone rubber (thickness of 0.05 mm, ARAM Corporation, Osaka).

A rectangular open space of 5 mm × 30 mm is cut off in the sheet, and sandwiched between the disks. The open space forms a channel of 30 mm length × 5 mm width × 0.05 mm depth.

After hydrophilization by the oxygen plasma ashing by RIE (FA-1, Samco International, Kyoto, Japan), the three parts stick together with their surface affinity. The diameter of the glass disks is 50 mm. The thicknesses of the upper and the lower disks are 4.5 mm and 1.1 mm, respectively.

The lower PDMS disk has two 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 (Fig. 7).

Flow Test

Two kinds of cells were used in the flow test: swine red blood cells, and Hepa1-6 (passage < 10, mouse hepatoma cell line of C57L mouse)

The swine blood (Tokyoshibaurazouki Co., Ltd., Tokyo, Japan) was diluted by the saline solution to make a suspension of red blood cells at the volume ratio of 0.03 percent. The red blood cells were used immediately, or used after preservation in a refrigerator at 277 K in four weeks.

In the case of Hepa1-6, experimental protocol is as follows. The cells were exfoliated from the bottom of the culture dish with trypsin, and suspended in the medium of D-MEM (Dulbecco's Modified Eagle's Medium).

After the saline solution was prefilled in the flow channel, the suspension of the cells was introduced into the flow channel.

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 admitted by the

syringe pump at 298 K (at the flow rate of 2.8 $\times 10^{-11}$ m^3/s for red blood cell, 2.8 $\times 10^{-10}$ m^3/s for Hepa1-6). In the flow path of height of 0.05 mm (width of 5 mm), the flow rate makes mean velocity of 1.1 mm/s at the flow rate of 2.8 $\times 10^{-10}$ m^3/s.

At the microscopic image, the outline of each cell was traced with "Image J", and the area (A) was calculated. The deformation ratio (A_2 / A_1) was calculated from the projected area of each cell before slit (A_1) and that in the slit (A_2).

3. RESULTS

Fig. 8 exemplifies the tracing across the groove on the mold for the ridge of the upper disk measured by the laser microscope (VK-X200, Keyence Corporation, Osaka, Japan). The tracing shows that the depth of the groove is 0.061 mm. Fig. 9 exemplifies the tracing across the ridge on the upper disk of PDMS measured by the laser microscope. The tracing shows that the height of the ridge is 0.061 mm. Fig. 10 exemplifies the tracing across the groove (for the narrow slit H_1) etched on the glass measured by the stylus profiler (Dektak XT-E, Bruker Corporation). The tracing shows that the depth is 0.00038 mm. Fig. 11 exemplifies the tracing across the ridge (for the wide slit H_2) on the lower disk measured by the laser microscope. The tracing shows that the height of the ridge is 0.0105 mm.

Fig. 12 exemplifies red blood cell flowing through slit H_1. The red blood cell deforms and passes through the slit of 0.0004 mm. Fig. 13 exemplifies Hepa1-6 flowing through slit H_2. The cell deforms and passes through the slit of 0.010 mm.

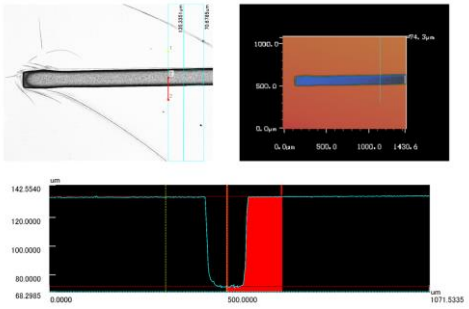

Fig. 8: Tracing across groove on mold for upper disk.

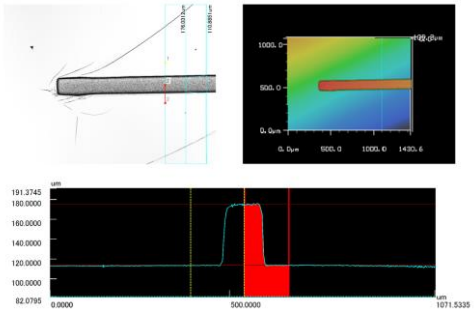

Fig. 9: Tracing across ridge on upper disk.

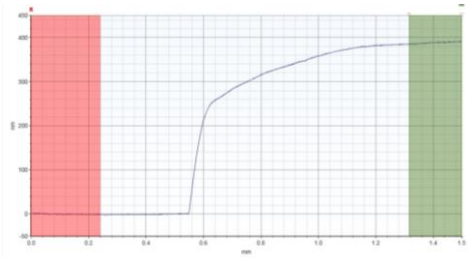

Fig. 10: Tracing across groove (H_1) on lower disk.

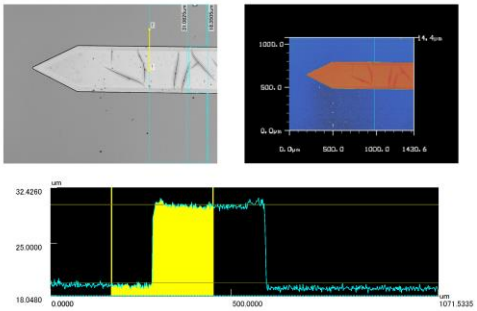

Fig. 11: Tracing across ridge (H_2) on lower disk.

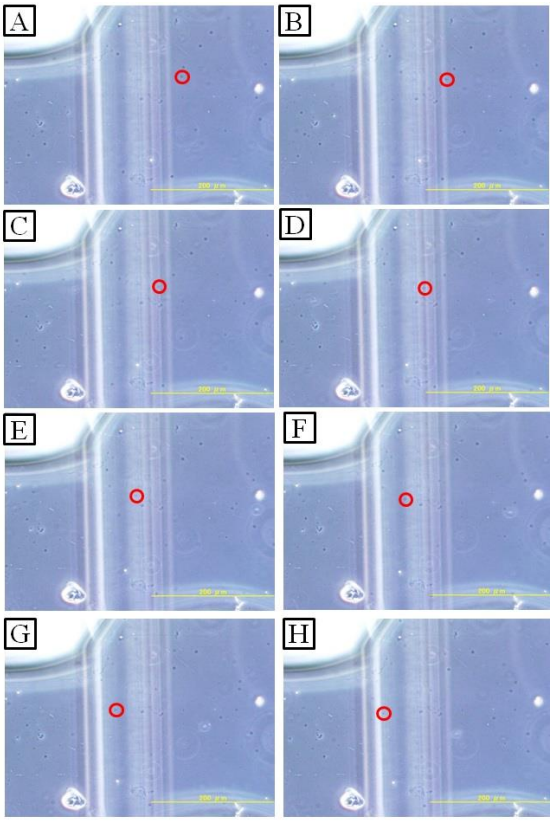

Fig. 12: Red blood cell flows through slit H_1 from right to left (A→H). Dimension from left to right is 0.4 mm.

Fig. 14 shows the projected area of the red blood cell: before the slit (left), and in the slit (right). Every cell deforms to the flat disk, while passing through the slit. The projected area of the cell increases under deformation, and the mean deformation ratio (A_2 / A_1) is 1.31 (Fig.15). Fig. 16 shows the projected

area of Hepa1-6: before the slit (left), and in the slit (right). Every cell deforms to the flat disk, while passing through the slit. The projected area of the cell increases under deformation, and the mean deformation ratio (A_2 / A_1) is 1.10 (Fig. 17).

4. DISCUSSION

The biological cells are sorted according to the shape, and deformability *in vivo*. Several cells pass through the micro slit. Some cells or fragments, which pass through the slit, are decomposed. Some cells, which cannot pass through the narrow channel, are captured, on the other hand. Erythrocyte deforms from the biconcave disk to the parachute like shape, when it is passing through micro capillary. Erythrocyte rotates in the shear field. The most of biological cells, on the other hand, keep the spherical shape, when they are flowing in the medium.

When passing through a micro slit, erythrocyte deforms to the thin disk and struggles to go through. The mechanical property of erythrocyte is maintained with energy consumption. To keep the biconcave shape, energy is consumed between structural proteins in the red blood cell. Because the dimension of the slit is mainly governed by the height of the lower ridge in the present study, the slight over dimension of the height of the upper ridge does 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.

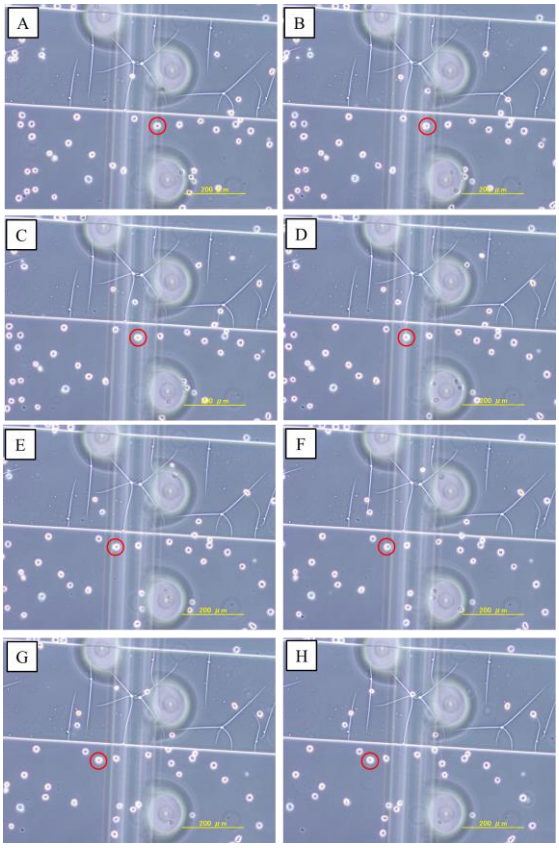

Fig. 13: Hepa1-6 flows through slit H_2 from right to left (A→H). Dimension from left to right is 0.8 mm.

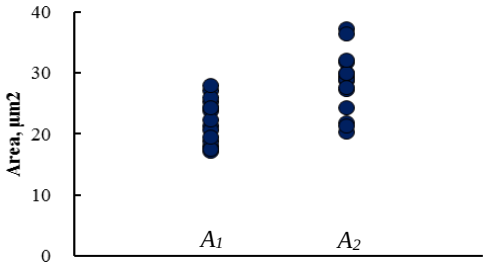

Fig. 14: Projected area of red blood cell [μm^2]: before slit (A_1; left), in slit (A_2; right).

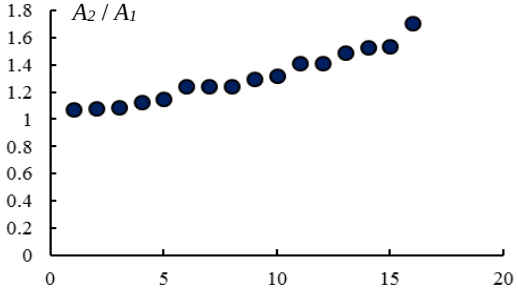

Fig. 15: Deformation ratio (A_2 / A_1) (mean: 1.31) of 16 red blood cells.

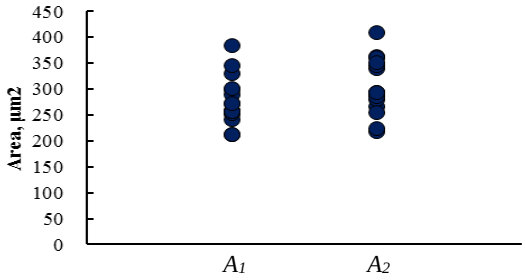

Fig. 16: Projected area of Hepa1-6 [μm^2]: before slit (A_1; left), in slit (A_2; right)

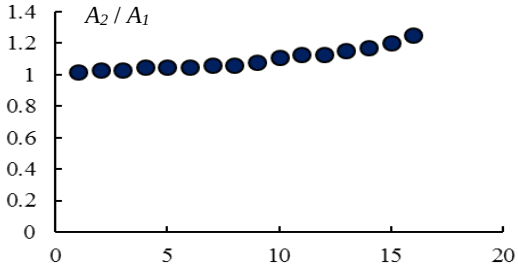

Fig. 17: Deformation ratio (A_2 / A_1) (mean: 1.10) of 16 cells of Hepa1-6.

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. Some red blood cells can pass through the position out of the slit. In the present study, the micro slit has been designed with narrower dimension than the previous study [16] to observe the deformation of cells or to trap some red blood cells. The fine architecture of the red pulp of the spleen has been investigated in the previous studies [13]. 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. 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 deformation is evaluated with the ratio of the projected area of the disk during the passing through the slit in the present study. The mean ratio of red blood cell and Hepa1-6 are 1.1 (1.02 - 1.25) and 1.3 (1.07-1.71), respectively. The deformation in the perpendicular direction can be observed with slit between micro cylindrical pillars [16].

5. CONCLUSION

Deformation of a biological cell passing through a micro slit has been observed *in vitro*. The slit, of which width is 1.3 mm and height is 0.0004 mm or 0.010 mm, has been designed between a ridge at transparent polydimethylsiloxane disk and ridges at glass disk. The experimental results show that red blood cells deform to the flat disk and pass through the micro slit of 0.0004 mm height, and that Hepa1-6 deform to the flat disk and pass through the micro slit of 0.010 mm height. The deformation is evaluated with the ratio of the projected area of the disk during the passing through the slit. The mean ratio of red blood cell and Hepa1-6 are 1.1 and 1.3, respectively. The designed slit between micro ridges has capability to evaluate the deformability of cells.

6. ACKNOWLEDGMENT

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