Design of Micro-channel for Controlling Behavior of Cells in Vitro

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ABSTRACT

A micro-channel has been designed to control behavior of biological cells *in vitro*. An erythrocyte deforms and passes through the micro-circulation, of which the dimension is smaller than its diameter. The spleen, for example, has special morphology in the blood flow path to sort injured erythrocytes. The micro-channel is useful for treatment of cell in diagnostics, too. The photolithography technique enables manufacturing the micro-channel. A borosilicate glass disk was used for a mold, and a wet etching process was applied for micro-fabrication. The study shows that the curvature of the micro groove can be controlled with the isotropic etching process in the photolithography machining and that the photolithography technique is effective to manufacture the micro-channel for the manipulation of a biological cell.

Keywords: Biomedical Engineering, Cell, Micro-channel and Etching.

1. INTRODUCTION

An erythrocyte has flexibility [1] and deforms in the shear flow [2]. It also passes through micro-circulation, of which the dimension is smaller than its diameter. 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 [3-5].

The photolithography technique enables manufacturing a micro-channel [6-13]. 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, a micro-channel has been designed to control behavior of biological cells *in vitro*. The fabrication technique has been investigated with the photolithography process.

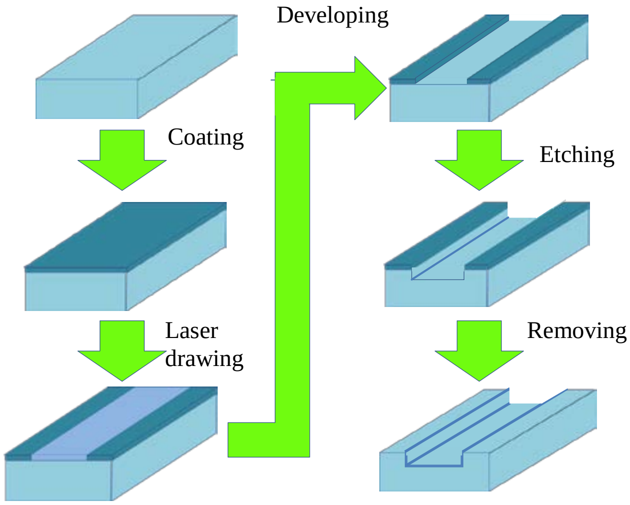

Fig. 1: Photolithography and etching processes.

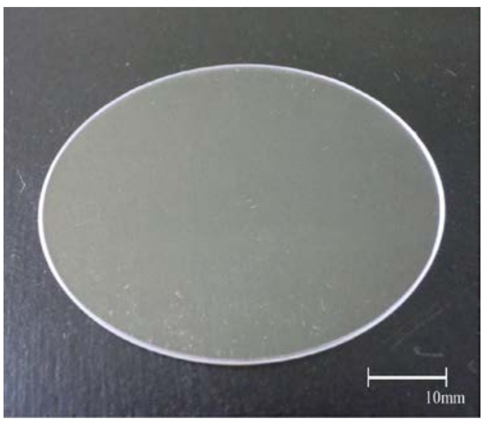

Fig. 2: Borosilicate glass disk.

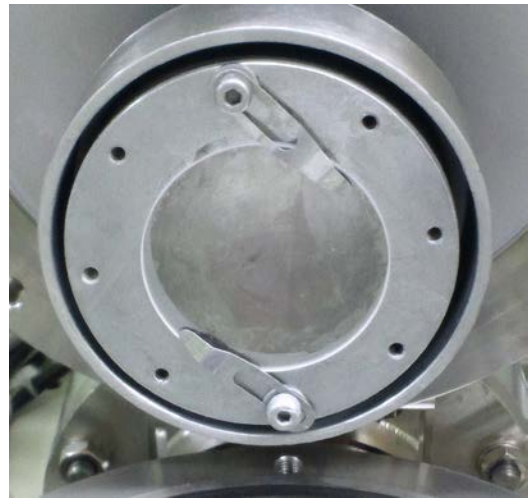

Fig. 3: Disk coated with chromium.

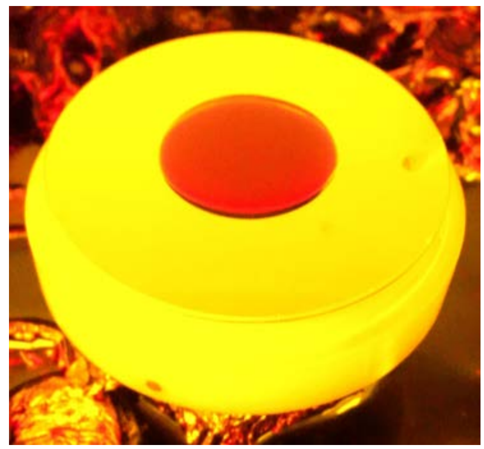

Fig. 4: Disk coated with photo-resist material.

2. METHODS

Photolithography

The micro channel has been fabricated with the photolithography and etching processes (Fig. 1).

A borosilicate glass disk (Tempax, Matsunami Glass Ind., Ltd., Tokyo, Japan) of 50 mm diameter and 1.1 mm thick was used for a mold (Fig. 2).

The disk was cleaned with 2-propanol (IPA, Taisei Kagaku, Tokyo, Japan) in an ultrasonic cleansing apparatus for five minutes. After cleaning, the disk was rinsed with distilled water, and dried on a heated plate (PXW-4, Asahi-rika, Chiba, Japan).

The disk was coated with chromium in the sputtering system (L-210S-FH, Canon Anelva Corporation, Kawasaki, Japan) (Fig. 3). In the system, the thickness of coating increases in proportion to the time. The time for coating was adjusted to 3 min to control the thickness to 0.1 mm.

The photo-resist material of Ethyl-Cellosolve-Acetate (2-ethoxyethyl acetate) (OFPR-800, Tokyo Ohka Kogyo Co., Ltd, Kawasaki, Japan) was coated with a spin coater (Fig. 4). The photo-resist was baked for ninety seconds on the plate heated at 383 K.

The groove pattern (Fig. 5) was drawn on the disk with a laser drawing system (DDB-201K-KH, Neoark Corporation, Hachioji, Japan). The pattern was baked for five minutes on the plate heated at 393 K.

To control the width of the groove of the mold with the laser drawing system, variations were made in the parameters: the voltage, the velocity, the acceleration, and the focus. In the present study, the target minimum value of the width is one micrometer for the channel of cells. Several bars were drawn with the variations in the parameters, and the each width of the grooves was measured (Fig. 6).

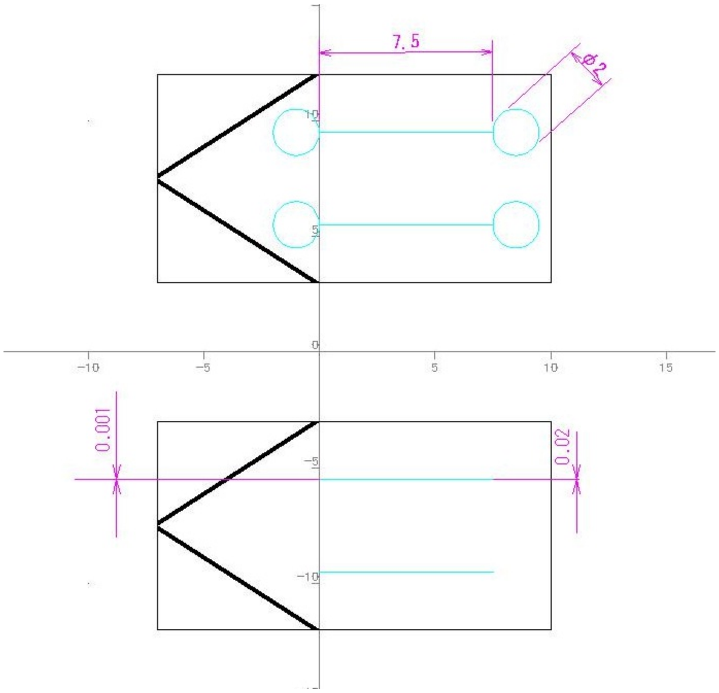

Fig. 5: Groove pattern. Unit of dimension is mm.

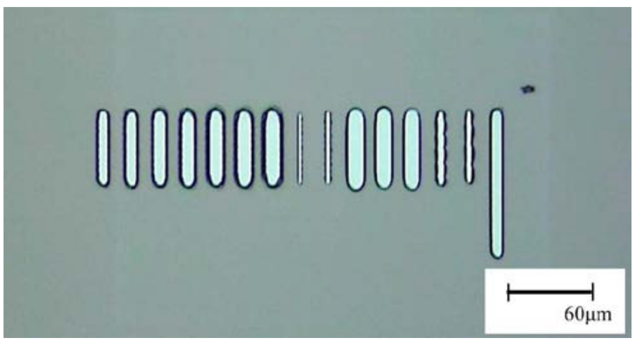

Fig. 6: Control the width of the groove.

The photo-resist was developed with tetra-methyl-ammonium hydroxide (NMD-3, Tokyo Ohka Kogyo Co., Ltd, Kawasaki, Japan) for five minutes (Fig. 7). The disk was rinsed with the distilled water, and dried on the heated plate.

The disk was dipped in TW (Nissin-Kasei Co., Ltd, Tokyo, Japan) to etch chrome (Fig. 8). The disk was rinsed with the distilled water, and dried on the heated plate.

Wet Etching

The disk was etched with the hydrofluoric acid (LAM2680, Wako Pure Chemical Industries, Ltd., Osaka, Japan) (Fig. 9). Variation was made on the time for etching. After etching, the disk was rinsed with the distilled water to stop etching.

The photo-resist was exfoliated in separating solution (Hakuri 105, Tokyo Ohka Kogyo Co., Ltd, Kawasaki, Japan) heated at 353 K., and dipped in IPA, before rinsed with the distilled water (Fig. 10).

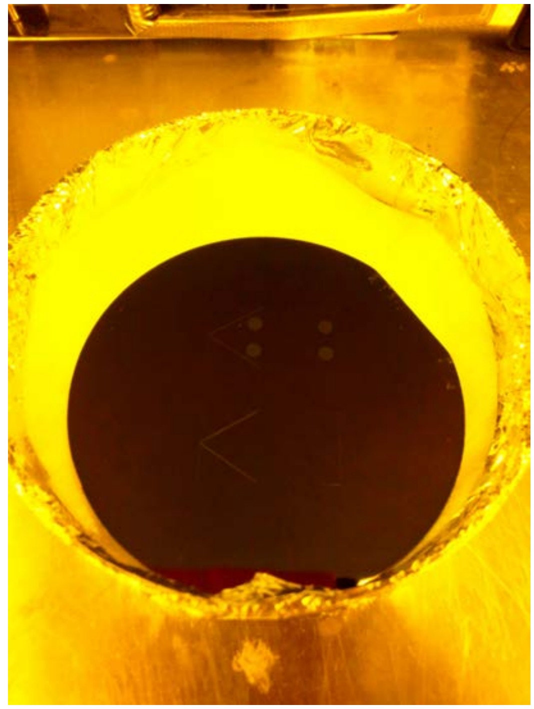

Fig. 7: Developed pattern.

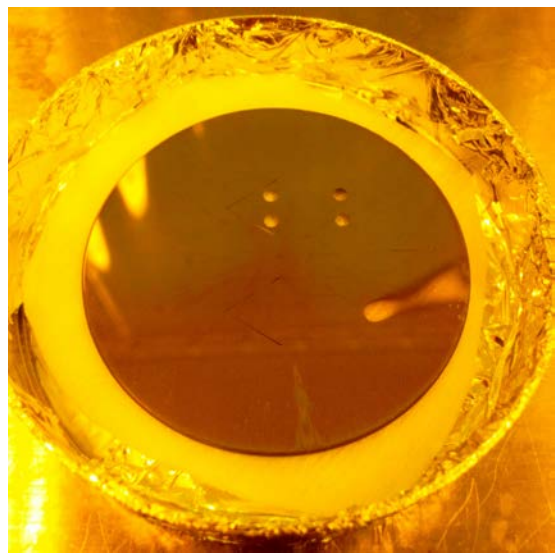

Fig. 8: After chrome etching.

The disk, again, was dipped in TW (Nissin-Kasei Co., Ltd, Tokyo, Japan) to etch chrome. The disk was rinsed with the distilled water, and dried on the heated plate.

Measurement of Morphology

The dimension of the groove was measured with a laser microscope (VK-9510, Keyence Corporation, Osaka, Japan). The width of the groove was measured, when the parameters of the laser drawing system were varied. Both the width and the depth of the groove were measured, when the time of etching was varied (Fig. 11).

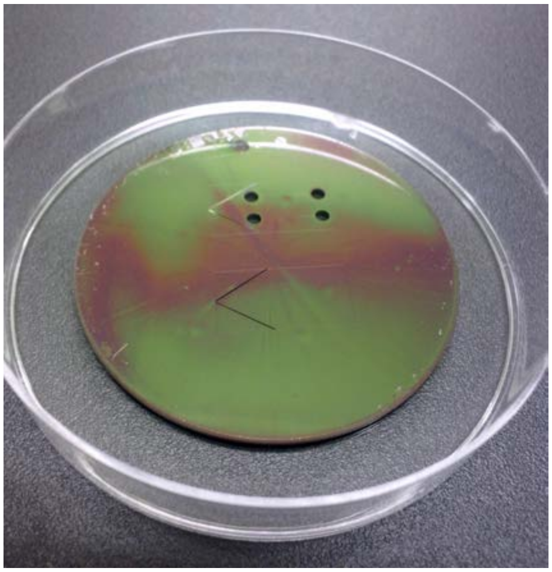

Fig. 9: After hydrofluoric acid etching.

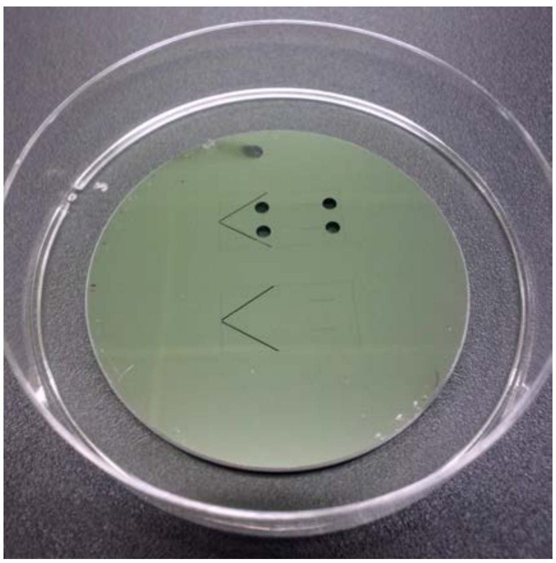

Fig. 10: After photo-resist removal.

3. RESULTS

The experimental results show following relations between the width of the groove and the parameters of the drawing system: the width increases with the voltage, the width decreases with the velocity, and the width decreases with focus. To decrease the voltage of the single drawing, the each pattern was traced three times. The experimental results show that the too high velocity makes some patches along the narrow groove (Fig. 12).

The minimum width of 540 nm has been attained in laser drawing of three times at the voltage of 3.15 V, the velocity of 2 mm s^{-1}, acceleration of 0.5 mm s^{-2}, and the focus offset of 0.4 V (Fig. 13). The width of 0.022 mm has been attained in laser drawing of three times at the voltage of 5.0 V, the velocity of 0.5 mm s^{-1}, acceleration of 0.5 mm s^{-2}, and the focus offset of -0.9 V (Fig. 14).

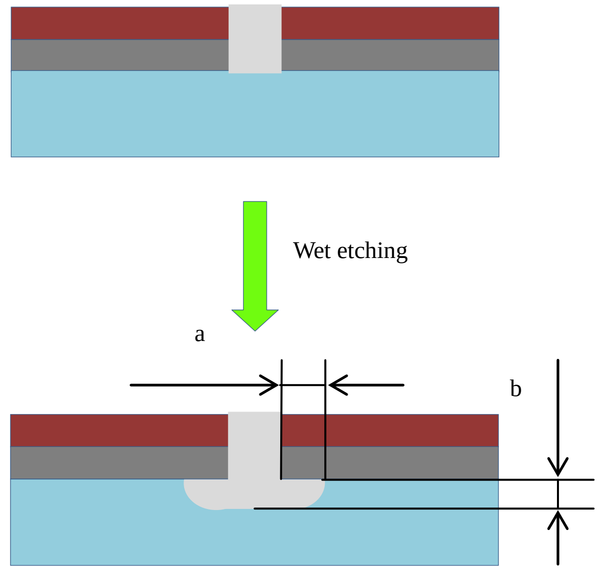

Fig. 11: Extension of width (a) and depth (b) were measured, after wet etching.

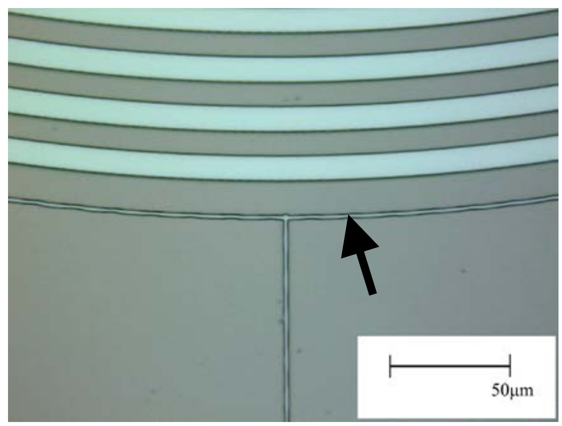

Fig. 12: Some patches along the narrow groove.

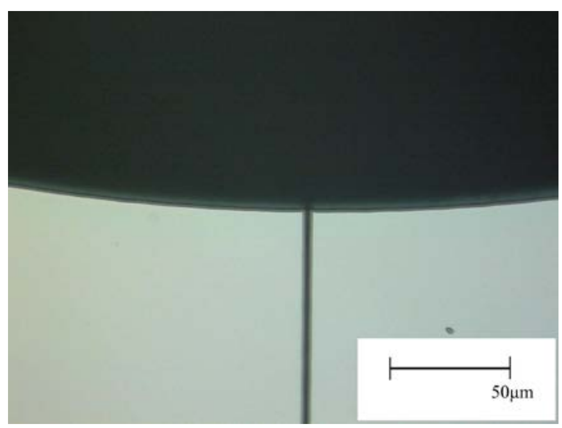

Fig. 13: Channel of minimum width of 540 nm before wet etching.

Fig. 14: Channel of width of 0.022 mm before wet etching. Magnification ratio is the same as Fig. 13.

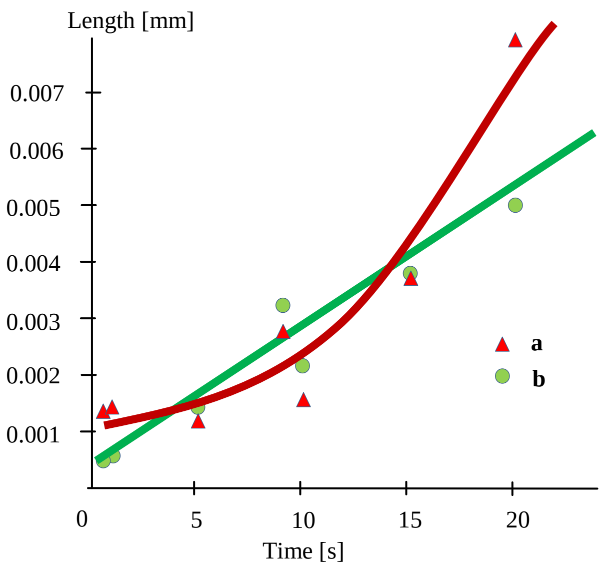

Fig. 15: Extension of width (a) and depth (b) related to the time of etching. See Fig. 11.

Both the width and the depth after application of the wet etching on the above mold (Figs. 13&14) have been measured (Fig. 12) and illustrated for the wider channel (Fig. 14) in Fig. 15.

The depth of the groove increases in proportion to the time of etching. The width of the groove also increases with the time of etching, but extension of width is above one micrometer even at the 0.5 s of etching.

4. DISCUSSION

The fine architecture of the red pulp of the spleen has been investigated in the previous studies [3-5]. The continuity between capillaries and splenic sinuses has been examined with the microscope. The special morphology might relate to the function for sorting erythrocytes.

The effect of flow on cells has been investigated in the previous studies [14-16]. 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. Cells are responsive to the micro morphology of the scaffold [17]. The microgroove governs the behavior of cells [18].

The Tempax has enough thermostability during the photolithography process. Several factors govern the morphology of the groove at etching on glass [19]. The control of the temperature during the photolithography process is important to control the uniform dimension of the groove. The wet etching process has been investigated on glass and applied to a monitoring system for a cell [19].

The uniformity of thickness of coating of the photo-resist material governs the uniformity of the laser drawing through the focusing position. To control the penetration depth in the wet etching process, the timing of using the stop solution is important. The narrow groove decreases the speed of penetration during the etching process.

When the width of the micro groove is narrow enough, the ratio of penetration of the hydrofluoric acid to the laminar direction increases. The isotropic etching produces round sidewalls. This process controls curvature of the micro groove on the mold. It enables making semi-cylindrical grooves.

After etching process, the mold will be applied to polydimethylsiloxane [16] to make a micro-channel for biological cells. The micro-channel devices may contribute to the development of biotechnology [10].

5. CONCLUSION

A micro-channel has been designed to control behavior of cells *in vitro*. The study shows that the curvature of the micro groove can be controlled with the isotropic wet etching process after the photolithography machining and that the photolithography technique is effective to manufacture the micro-channel for the manipulation of a biological cell.

6. ACKNOWLEDGMENT

This work was supported by a Grant-in-Aid for Strategic Research Foundation at Private Universities (2008-2012) from the Japanese Ministry of Education, Culture, Sports and Technology.

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