

# Effect of Micro Ridges on Cell Culture

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## ABSTRACT

The effect of micro ridges on the cell culture has been studied *in vitro*. Several patterns of micro ridges have been fabricated on a transparent polydimethylsiloxane disk with the photo lithography technique: a single line or ten parallel lines of micro ridges (0.001 mm height, 0.003 mm width). C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse) was cultured on the disk for one week and was observed with an inverted phase contrast microscope. The experimental results show that cells align to the direction of micro ridges, and that the differentiation of cells can be accelerated with micro ridges.

**Keywords:** Biomedical Engineering, Cell Culture, C2C12, Lithography, Micro Ridge and Polydimethylsiloxane.

## 1. INTRODUCTION

Biological cells respond to various environmental factors, such as electric [1], magnetic [2] and mechanical [3, 4] fields.

Cell culture technique has been developed and several methodologies have been clinically applied to regenerative medicine. The acceleration technique for orientation and proliferation of cells has been studied to make a biological tissue *in vivo* or *in vitro* [1-3]. Control methodology for behavior of cells would be applied to regenerative tissue technology: orientation, proliferation and differentiation [5].

The morphology of the surface of the scaffold might affect the orientation of cells and might govern organization of cells. Several methods have been applied to make orientation of cell culture in the previous studies: fibers and grooves [6-8].

The photo lithography technique is effective to make micro patterns on the cell culture plate. In the present study, the effect of micro ridges on the cell culture has been studied with the photo lithography technique *in vitro*.

## 2. METHODS

### Micro Ridges

Three patterns of micro ridges have been made on a disk of transparent polydimethylsiloxane (PDMS): a single line or ten parallel lines of micro ridges. The height and the width of the ridge are around 0.001 mm and 0.003 mm, respectively. Variation is made on the interval of the parallel lines of the ridges: 0.002 mm and 0.004 mm.

A silicon wafer (Type P, Matsuzaki Seisakusyo, Co., Ltd., Tokyo, Japan) is used for a surface mold for the disk (Fig. 1). The diameter and the thickness of the wafer are 50 mm and 0.30 mm, respectively.

The surface of the wafer was cleaned three times: with the acetone for five minutes in an ultrasonic cleaner, with the isopropyl alcohol for five minutes in an ultrasonic cleaner, and with the 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, and exposed to the oxygen gas in a reactive ion etching system (FA-1, Samco Inc., Kyoto) to be characterized as hydrophilic (the oxygen plasma ashing).

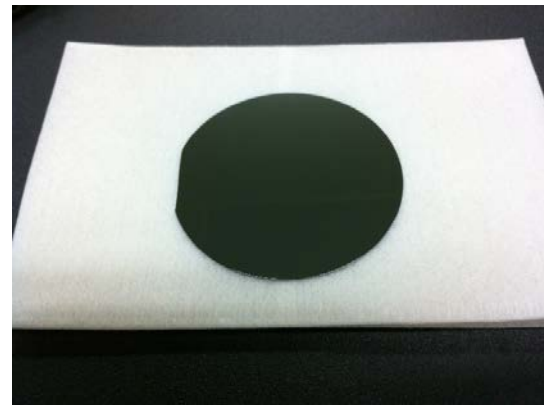


Fig. 1: Silicon wafer (diameter: 50 mm).



**Fig. 2:** Silicon wafer coated with photo-resist material.

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 with a spin coater (Fig. 2). The photo-resist was baked on the heated plate at 338 K for 3 minutes.

The pattern of lines was drawn on the wafer with a laser drawing system (DDB-201K-KH, Neoark Corporation, Hachioji, Japan). To control the dimension of the lines of the mold with the laser drawing system, the parameters were selected as follows: the voltage of 3.5 V, the velocity of 3 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 decrease the remaining stress and to increase the adhesiveness of the coating, the wafer was baked at 393 K for 3 minutes.

The wafer was etched with the plasma gas using the Si Deep RIE System (MUC-21 ASE-SRE, Sumitomo Precision Products Co., Ltd., Amagasaki, Japan) to make lines of the micro groove. 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 the isopropyl alcohol, before rinsed with the distilled water. Then, the wafer with grooves was dried on the hot plate, and used for the concave mold to make micro ridges in the following process.

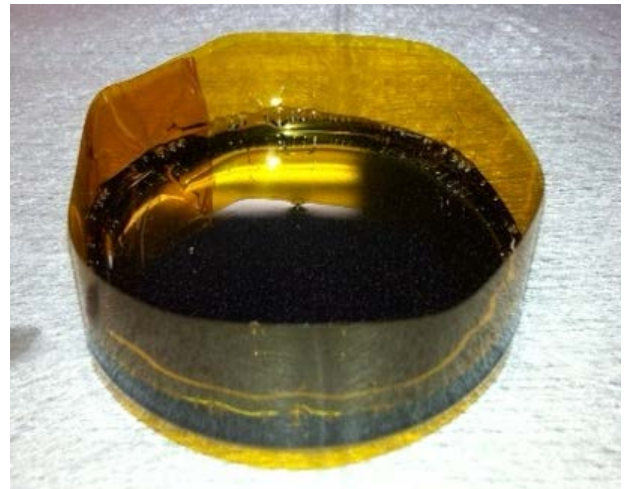
The dimension of the micro groove was measured with a laser microscope (VK-9510, Keyence Corporation, Osaka, Japan).

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 with the curing agent (Dow Corning Corporation) on the wafer. The volume ratio between PDMS and the curing agent is ten to one. After degassing (Fig. 3), PDMS was baked at 383 K for one hour in an oven (DX401, Yamato Scientific Co., Ltd) (Fig. 4).



**Fig. 3:** Degassing of PDMS on mold of silicon wafer with micro pattern.



**Fig. 4:** PDMS baked at 383 K for one hour.

The baked disk of PDMS is exfoliated from the mold, and sterilized in an autoclave. The disk was exposed to the oxygen gas in a reactive ion etching system (FA-1, Samco Inc., Kyoto) to be characterized as hydrophilic (oxygen plasma ashing), just before the cell culture.

#### Cell Culture

The culture dish, which consists of 6 cylindrical wells of 35 mm diameter, was used for cell culture (Fig. 5). The PDMS disk, which has micro ridges on the upper surface, was placed in the bottom of each well. C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse) was used in the experiment. Cells were seeded on the culture plate with D-MEM (Dulbecco's Modified Eagle Medium) at density of  $1.5 \times 10^3$  cells per cm<sup>2</sup>. Fetal bovine serum (FBS) was added to the medium with the volume rate of 10 percent. Cells were cultured in the incubator for one week.

The cells near the micro ridges were observed with an inverted phase-contrast microscope (IX71, Olympus Co., Ltd., Tokyo) day by day.

### 3. RESULTS

Figs. 6-9 show the results of the measurement of the micro grooves of the manufactured mold of A, B, C and D, respectively. The dimension of micro grooves measured with the micro scope is summarized in Table 1.

Table 1. Dimension of micro grooves of mold

Mold	Width (W) [mm]	Interval (I) [mm]	Depth (D) [mm]
A	0.0034		0.0002
B	0.0037	0.0016	0.0003
C	0.0038	0.0017	0.0013
D	0.0041	0.0031	0.0011

Figs. 10-13 show cells near the single micro ridge of "A" after one, two, three and seven days cultivation, respectively. The figure shows that cells adhere over the ridge.

Figs. 14-17 show cells near the micro ridges of "B" after cultivation for one, two, three and seven days, respectively. Figs.18-21 show cells near the micro ridges of "D" after cultivation for one, two, three and seven days, respectively. The figure shows orientation of cells along the ridges. The figure also shows that some cells are differentiated to myotubes near the ridges.

The experimental results show that cells align to the direction of micro ridges, and that the differentiation of cells can be accelerated with micro ridges. The tendency is accelerated on the multiple parallel ridges.



Fig. 5: Culture dish with 6 cylindrical wells.

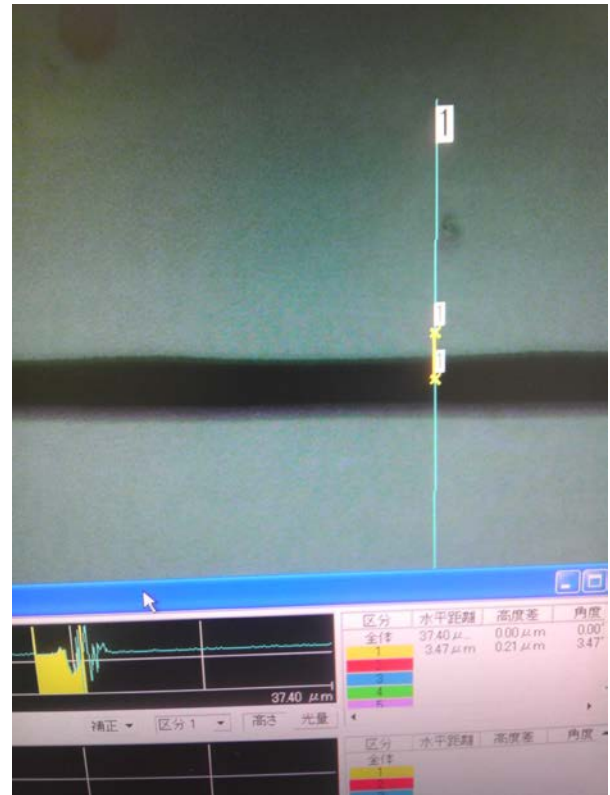


Fig. 6: Dimension of the single groove (A) measured with a laser microscope.

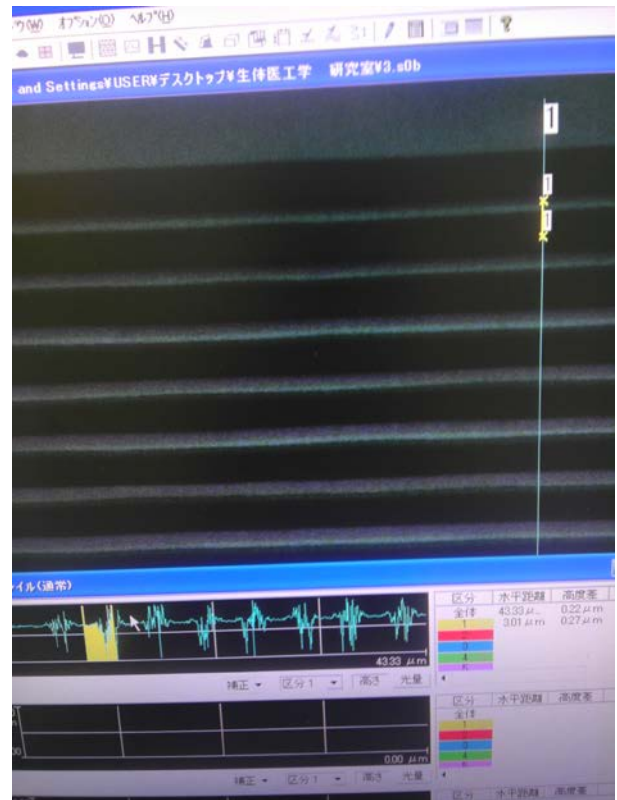


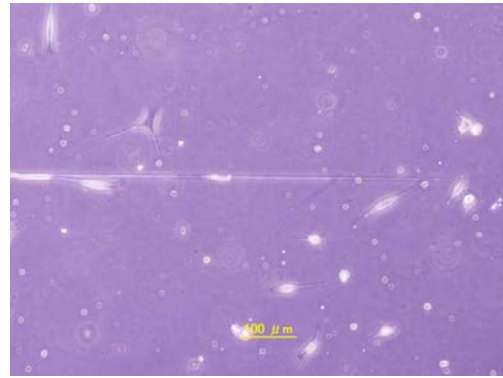
Fig. 7: Dimension of the micro groove (B) measured with a laser microscope.



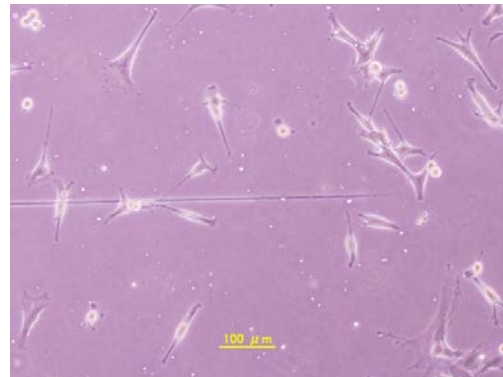
**Fig. 8:** Dimension of the micro groove (C) measured with a laser microscope.



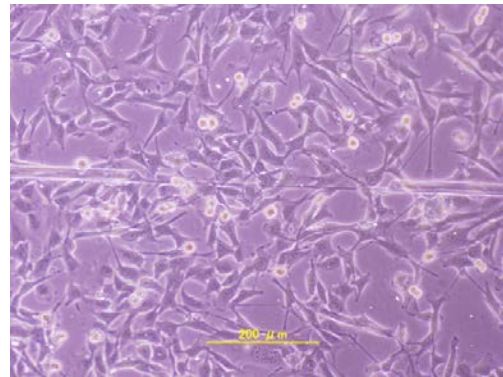
**Fig. 9:** Dimension of the micro groove (D) measured with a laser microscope.



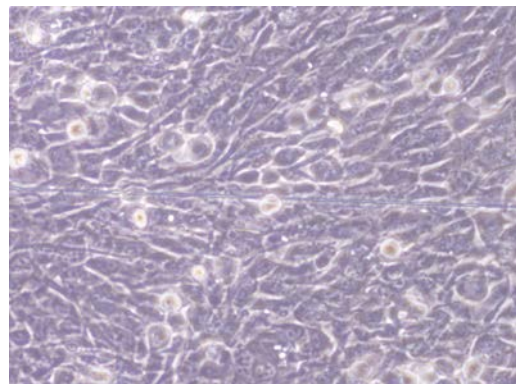
**Fig. 10:** Cell near the single ridge (one day).



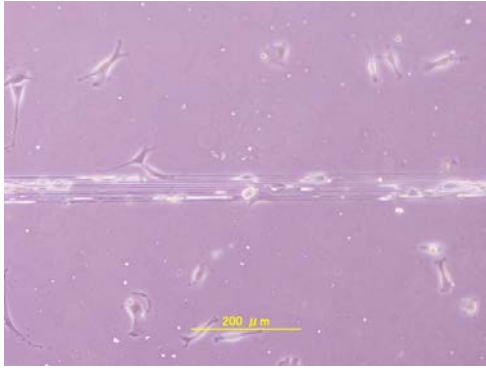
**Fig. 11:** Cell near the single ridge (two days).



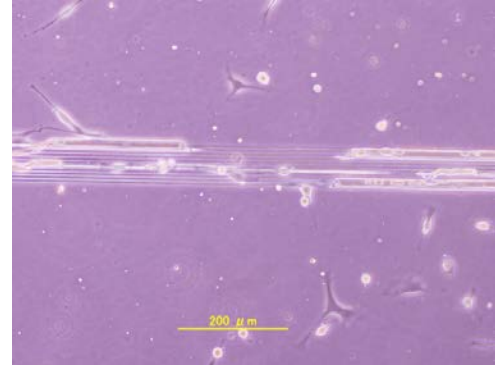
**Fig. 12:** Cell near the single ridge (three days).



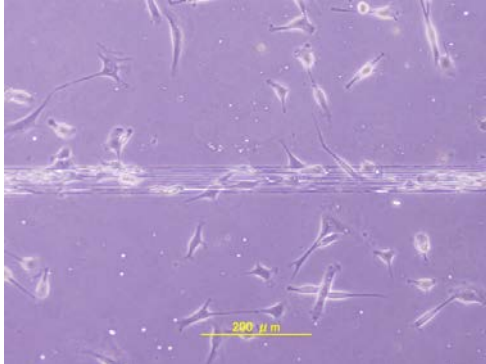
**Fig. 13:** Cell near the single ridge (seven days). Dimension from left to right is 1.0 mm.



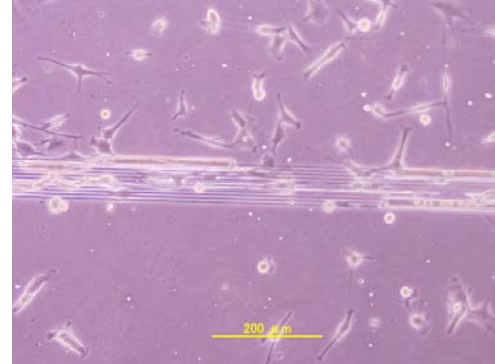
**Fig. 14:** Cell near the multiple ridges (B) (one day).



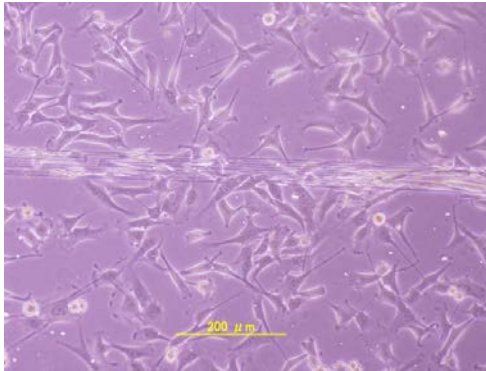
**Fig. 18:** Cell near multiple ridges (D) (one day).



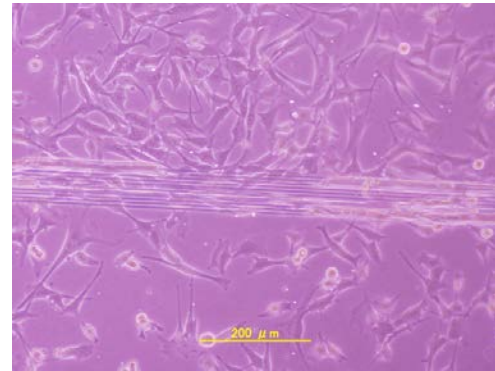
**Fig. 15:** Cell near the multiple ridges (B) (two days).



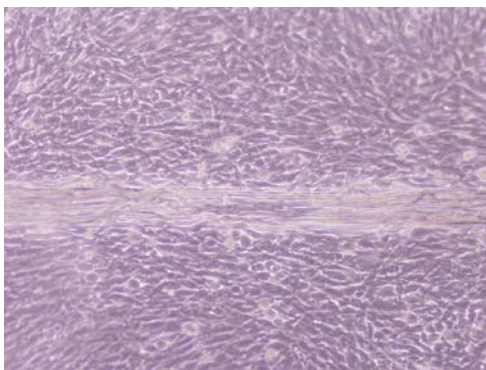
**Fig. 19:** Cell near multiple ridges (D) (two days).



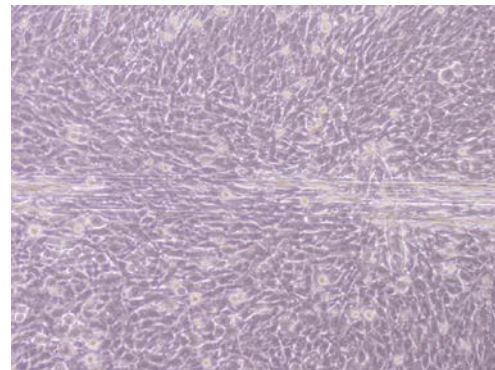
**Fig. 16:** Cell near the multiple ridges (B) (three days).



**Fig. 20:** Cell near the multiple ridges (D) (three days).



**Fig. 17:** Cell near the multiple ridges (B) (seven days).  
Dimension from left to right is 1.0 mm.



**Fig. 21:** Cell near the multiple ridges (D) (seven days).  
Dimension from left to right is 1.0 mm.

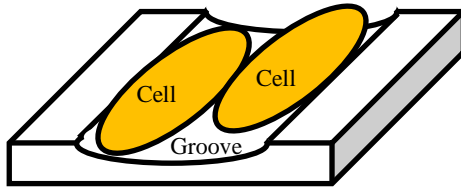


Fig. 22: Orientation of cell in groove.

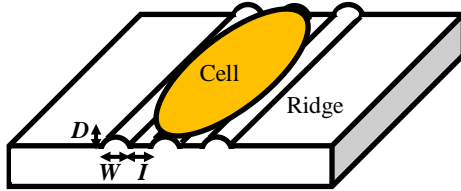


Fig. 23: Orientation of cell on multiple ridges ( $W$ ,  $I$ ,  $D$ : see Table 1).

#### 4. DISCUSSION

Several morphologies were applied to cell culture in the previous studies: a micro capillary of glass and a groove of sub-millimeter. In these experiments, the cells orient along the wall (Fig. 22).

The biological cells, on the other hand, might sense micro morphology of the surface smaller than their own dimension [6-9]. The effect of the curvature of grooves of micro meter order on the behavior of cell was studied in the previous study [10]. Morphology of nanometer order of the surface might affect behavior of cells [11, 12]. In the present study, the effect of smaller dimension of morphology on the orientation of cells has been studied.

Control methodology for orientation and proliferation of cells has a potential to be applied to a bio-actuator [13].

The experimental results show that cells move and elongate according to the ridge. Although the single ridge is not enough to make orientation of cells, multiple ridges affect the cells orientation (Fig. 23). The surface morphology of micrometer affects cells behavior. The differentiation of cells can be accelerated with micro ridges.

#### 5. CONCLUSION

Responses of cells to micro ridges have been studied *in vitro*. Several patterns of micro ridges have been fabricated on a transparent polydimethylsiloxane disk with photo lithography technique. The experimental results show that cells align to the direction of micro ridges, and that the differentiation of cells can be accelerated with micro ridges.

#### 6. ACKNOWLEDGMENT

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

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