

Design of Scaffold with Taper-Striped Pattern to Observe Durotactic Migration of Cell

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ABSTRACT

The scaffold with the taper-striped pattern has been designed to observe durotactic migration of a biological cell. The surface of the scaffold with the micro taper-striped ($0.05 \text{ mm} < \text{width} < 0.2 \text{ mm}$) pattern was made by the photolithography technique. The variation of hardness of the surface of the band has been made of the materials alternately: polydimethylsiloxane (PDMS), and epoxy based negative photoresist material (SU-8). C2C12 (mouse myoblast) was seeded on the micro pattern, and incubated for 48 hours in the medium. The projected area of each cell was traced in each band at the microscopic images, and the total area was compared between bands of two kinds of materials. The experimental result shows that each cell tends to migrate to the area of the band of SU-8. The experimental system is effective to investigate durotaxis of cells *in vitro*.

Keywords: Biomedical Engineering, C2C12, Durotaxis and Photolithography.

1. INTRODUCTION

After adhesion on the scaffold, a biological cell migrates on the scaffold. The cell migration might govern the subsequent orientation of cells. Control methodology for orientation of cells would be applied to the regenerative medicine.

The migration depend on several factors: the micro morphology of the scaffold [1-8], the vibration [8, 9], the shear flow field [10-14], and the gravitational field [15, 16]. The cell migration might also be guided by the rigidity gradients on the scaffold (durotaxis) [17-29].

The photolithography technique is available to make the micro patterns on the scaffold of the cell culture. The surface of the scaffold with the micro stripe pattern of alternative bands (polydimethylsiloxane (PDMS) and epoxy based negative photoresist material (SU-8)) was made by the photolithography technique to observe durotaxis in the previous study [17]. At the parallel stripe pattern, migration to the longitudinal direction of the stripe has no relation to durotaxis. At the band of the gradually decreased width, on the other hand, the longitudinal direction of the stripe has asymmetry.

In the present study, the scaffold with the triangular striped pattern has been designed to observe durotactic migration of a biological cell *in vitro*.

2. METHODS

Micro Hybrid Triangular Striped Pattern

The hybrid taper-striped pattern of scaffold has been designed on a plate of polydimethylsiloxane (PDMS) for a flat scaffold by the photolithography technique. A part of each band has gradual reduction at the width: from 0.2 mm to 0.1 mm , or 0.15 mm to 0.05 mm . The surface of the scaffold consists of bands made of two alternate materials: PDMS, and SU-8 (epoxy based negative photoresist material). The pattern was controlled by a photomask. The surface of the photomask was observed by the laser microscope (VK-X200, Keyence Corporation, Osaka, Japan) (Fig. 1).

Mold

The borosilicate glass (Tempax) disk is used for the base of the mold (Fig. 2). The diameter and the thickness of the disk are 35 mm and 1.1 mm , respectively. The surface of the glass disk was cleaned by the oxygen (0.1 Pa , $30 \text{ cm}^3/\text{min}$) plasma ashing (100 W , for five minutes) in the reactive ion etching system (FA-1, Samco Inc., Kyoto, Japan).

The negative photoresist material of high viscosity (SU-8 10: Micro Chem Corp., MA, USA) was coated on the disk by the spin coater (at 1000 rpm for 1 min). The photoresist was baked in the oven (DX401, Yamato Scientific Co., Ltd) at 368 K for five minutes.

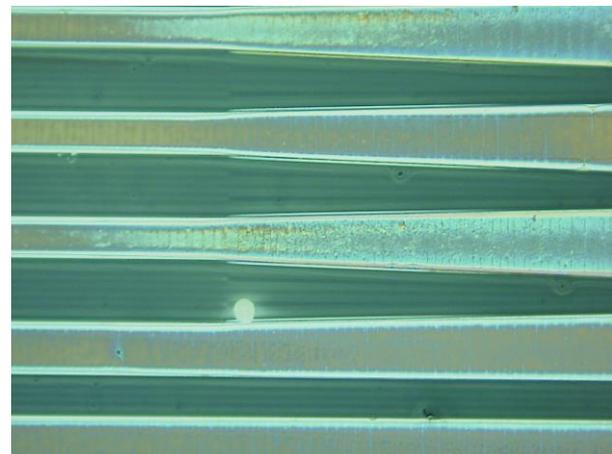


Fig. 1: Laser microscopic image of photomask for taper-stripe pattern. Dimension from left to right is 1.5 mm .

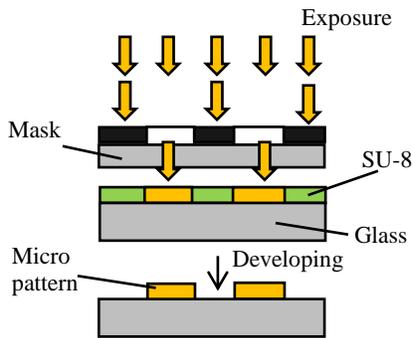


Fig. 2: Photolithography process for mold.

The photomask with the taper-stripe patterns was mounted on the surface of SU-8 10, and the photoresist was exposed to the UV light through the mask in the mask aligner (M-1S, Mikasa Co. Ltd., Japan) at 15 mW/cm² for 20 s. The photoresist was baked in the oven at 368 K for ten minutes. The photoresist was developed with SU-8 developer (Micro Chem Corp., MA, USA) for five minutes. The glass surface with the micro pattern was rinsed with IPA (2-propanol, Wako Pure Chemical Industries, Ltd.) for one minute, and pure water for one minute. The surface was dried by the spin-dryer (SF-250, Japan Create Co., Ltd., Tokorozawa, Japan).

The morphology of the surface of the mold was measured across the bands by the stylus of the contact profilometer (Dektak XT-E, Bruker Corporation) (Fig. 4).

Scaffold with Taper-stripe Micro Pattern

After the mold was enclosed with a peripheral wall of polyimide tape, degassed 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) on the mold. The volume ratio of PDMS to the curing agent is ten to one. PDMS was baked at 373 K for thirty minutes in an oven.

The baked disk of PDMS (3 mm thickness) was exfoliated from the mold, and punched to make a disk (diameter of 25 mm) with the micro pattern. The disk was exposed to the oxygen gas in a reactive ion etching system (FA-1) to be characterized as hydrophilic (oxygen plasma ashing).

On the micro pattern of the disk, another disk without micro pattern, which has two holes, was attached. SU-8 2 was introduced by the capillary effect into the space, which is kept by the micro pattern, between two disks through the hole at the disk with no micro pattern [17]. After the disks with SU-8 2 was baked at 393 K for five hours in the oven, the PDMS disk with no micro pattern was exfoliated, and discarded.

The pattern of the surface of the scaffold was observed by the phase contrast microscope (IX71, Olympus, Tokyo) (Fig. 3).

Before the cell cultivation, the surface of the culture plate was hydrophilized by the oxygen (30 cm³/min, 0.1 Pa) plasma ashing for thirty seconds at 50 W by the reactive ion etching system (FA-1).

Cell Culture

C2C12 of the passage 6 was cultured in D-MEM (Dulbecco's Modified Eagle Medium) containing 10% FBS (fetal bovine serum) and 1% penicillin/ streptomycin.

In each test, cells were seeded on the micro pattern of the disk placed in the culture dish at the density of 2000 cells/cm². The culture dish was kept in the incubator to maintain both the temperature of 310 K and the carbon dioxide partial pressure of 5 percent for 48 hours.

The migration tendency of each cell was checked by the time laps image of the phase contrast microscope during the cell culture. Figs. 7a, 8a, and 9a show microscopic images of cells. On the microscopic image, the contour of each cell was traced, and the projected area of each cell (A_k) was calculated. At the two dimensional image, the total area of cells was evaluated at each band of the micro pattern by the occupation ratio of the area (R).

$$R = 100 (\sum A_k) / S \quad (1)$$

In Eq. 1, S is the total scaffold area of each kind of band in each segment of the microscopic image. R becomes 100 % at the confluent state in each kind of band. When the cell across the border between the bands, the area is divided into two parts at the line of the border. Each partial area is added to each band, respectively.

3. RESULTS

Fig. 3 shows the phase contrast microscopic image of the scaffold. The taper-stripe pattern of SU-8 (wider on the left hand) and PDMS (wider on the right hand) have been successfully made on the surface of the scaffold for the cell culture.

Fig. 4 and Fig. 5 show the tracings of the surface of the mold measured by the stylus of the contact profilometer. Fig. 4b shows the tracing measured by the stylus at the line (Fig. 4a) on the surface of the mold. The tracing shows the height of 0.015 mm, and the pitch of 0.25 mm with the narrower top width.



Fig. 3: Phase contrast microscopic image of hybrid taper-stripe pattern of SU-8 (0.05 mm – 0.15 mm) and PDMS (0.1 mm – 0.2 mm) for scaffold of cell culture. Dimension from left to right is 2.2 mm.

Fig. 5b shows the tracing measured by the stylus at the line (Fig. 5a) on surface of the mold. The tracing shows the height of 0.015 mm, and the pitch of 0.25 mm with the wider top width. The height of 0.15 mm corresponds to the thickness of SU-8 layer on the surface of the scaffold.

Fig. 6 shows the tracings measured by the stylus across the stripe on the scaffold surface with indentation load of 3×10^{-5} N. The tracings show the step of 0.001 mm, and the pitch of 0.25 mm.

Fig. 7a shows C2C12 cultivated on the micro pattern for 24 hours. The contour of each cell is traced to calculate the total area of cells on each band: SU-8 (yellow), and PDMS (red). Fig. 7b shows the occupation ratio of the area (R) of C2C12 cultured on micro pattern for 24 hours. The ratio is higher at SU-8 than at PDMS.

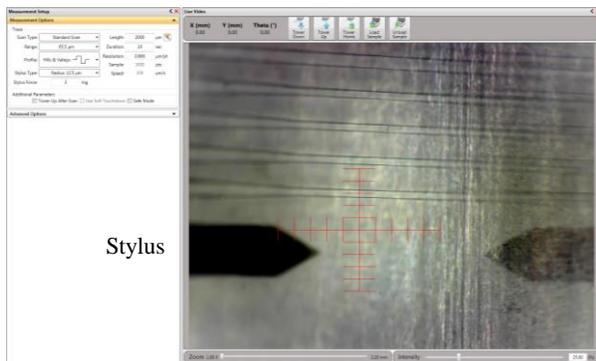


Fig. 4a: Measurement of morphology of surface of mold across bands by stylus (left).

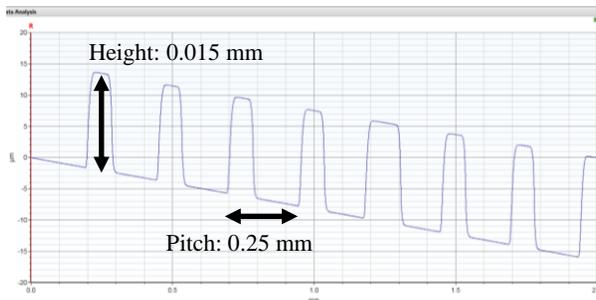


Fig. 4b: Tracing measured by stylus at the line (Fig. 4a) on surface of mold.

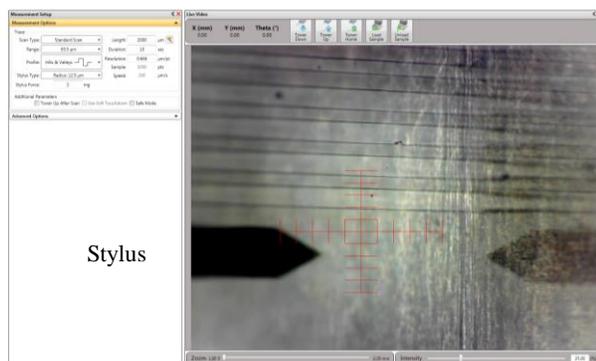


Fig. 5a: Measurement of morphology of surface of mold across bands by stylus (left).

Fig. 8a shows C2C12 cultivated on the micro pattern for 48 hours. The contour of each cell is traced to calculate the total area of cells on each band. Fig. 8b shows the occupation ratio of the area (R) of C2C12 cultivated on micro pattern for 48 hours. The ratio is higher at SU-8 than at PDMS.

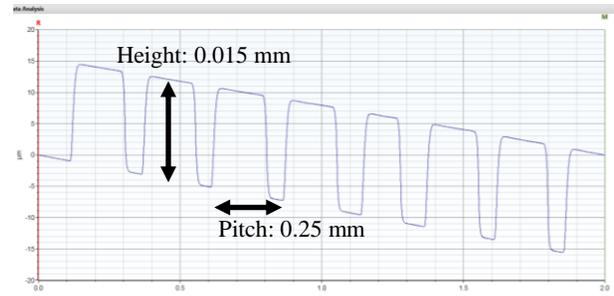


Fig. 5b: Tracing (height, μm ; distance, mm) measured by stylus at the line (Fig. 5a) on surface of the mold.

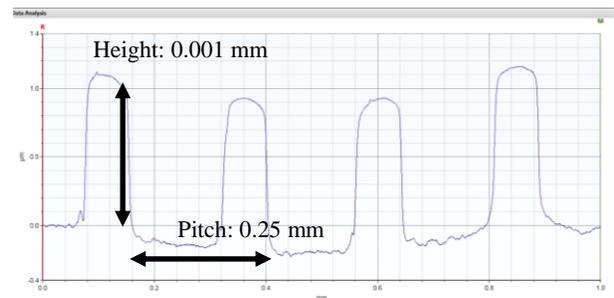


Fig. 6: Tracing (height [μm] vs. distance [mm]) measured by stylus across stripe on scaffold surface with indentation load of 3×10^{-5} N.

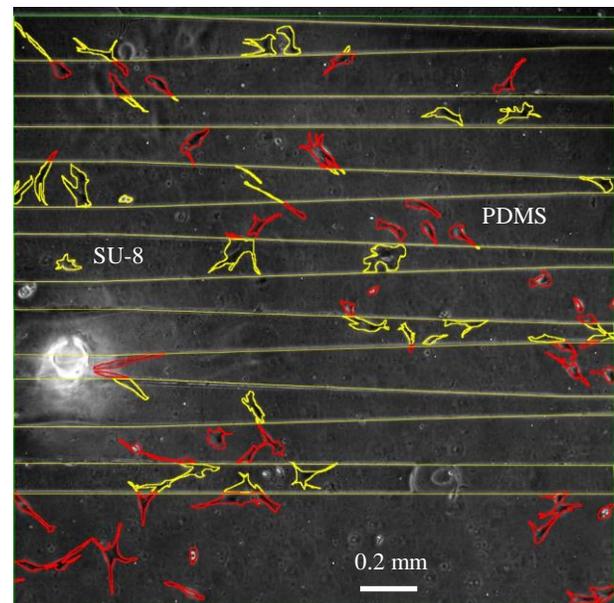


Fig. 7a: C2C12 cultivated on micro pattern for 24 hours. Dimension from left to right is 2.2 mm. Trace of contour of each cell to calculate total area of cells on each band.

Fig. 9a shows C2C12 cultivated on the micro pattern for 24 hours, and the contour of each cell is traced to calculate the total area of cells on left and right sides. Fig. 9b shows the total area of C2C12 cultivated on micro pattern for 24 hours. The total area is wider at the left side than at the right side. The ratios of SU-8 area at left and right sides are 48 % and 32 %, respectively.

Fig. 10 shows the migration of C2C12 at every three hours for 24 hours after seeding. At the initial position, five cells are selected on each band. Migration tends to be active in the wide band of SU-8.

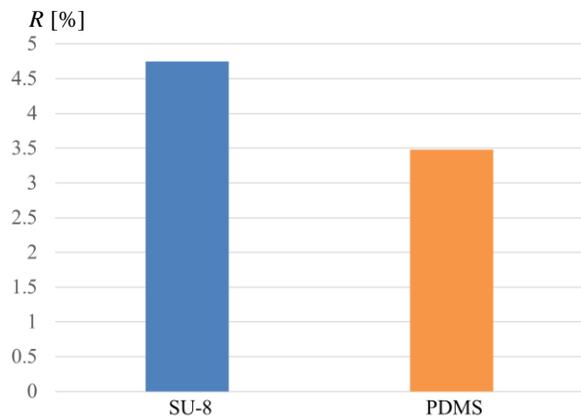


Fig. 7b: Occupation ratio of area (R) of C2C12 cultivated on micro pattern for 24 hours: SU-8 (left), PDMS (right).

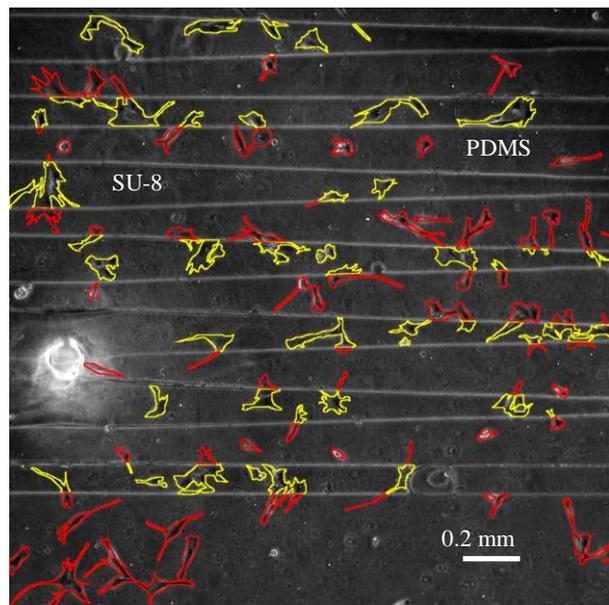


Fig. 8a: C2C12 cultivated on micro pattern for 48 hours. Dimension from left to right is 2.2 mm. Trace of contour of each cell to calculate total area of cells on each band.

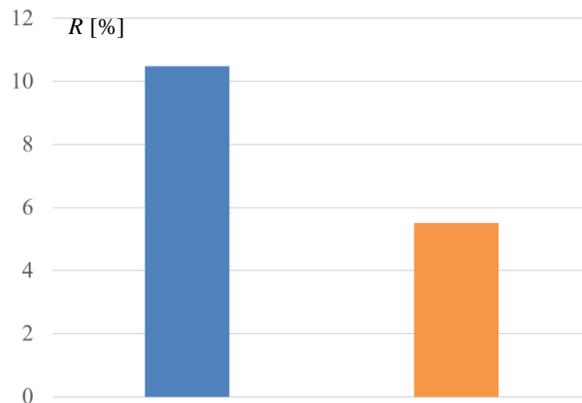


Fig. 8b: Occupation ratio of area (R) of C2C12 cultivated on micro pattern for 48 hours: SU-8 (left), PDMS (right).

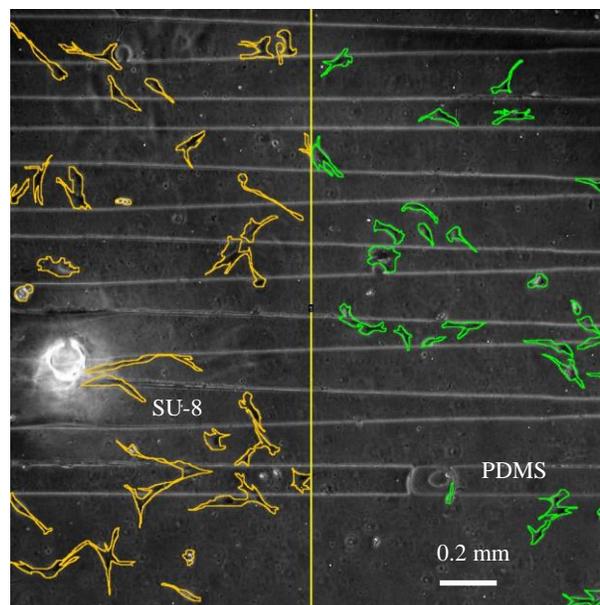


Fig. 9a: C2C12 cultivated on micro pattern for 24 hours. Dimension from left to right is 2.2 mm. Trace of contour of each cell to calculate total area of cells on each side (left (yellow) or right (green)).

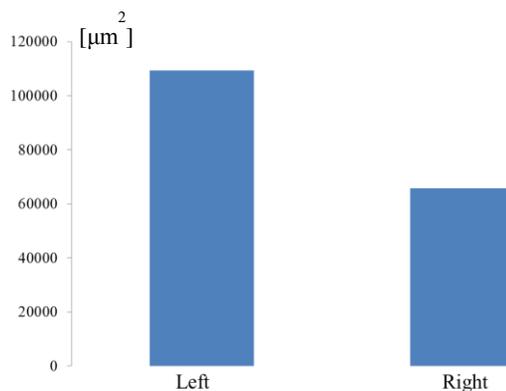


Fig. 9b: Total area of C2C12 cultivated on micro pattern for 24 hours: left side, right side (Fig. 9a).

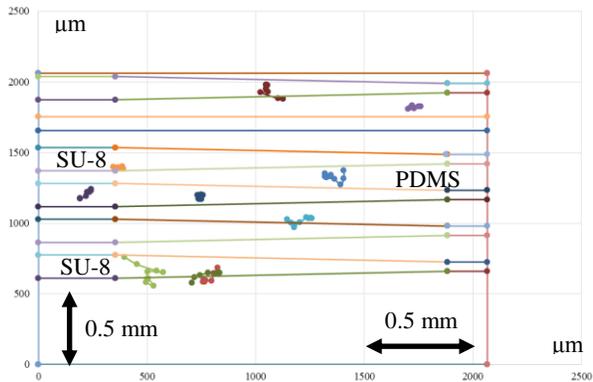


Fig. 10: Trappings of centroid of each C2C12 at every three hours for 24 hours: unit, μm .

4. DISCUSSION

In the previous studies, several methods [30-34] to control the behavior of the biological cell were investigated on the variety of aspects: the electric field [30, 31], the magnetic field [32], the flow field [12-14, 33], and mechanical field. The rigidity of the scaffold is one of the aspects, which have been considered to be sensitive to the cell.

The photolithography technique has been useful for micromachining [35]. In the present study, the taper-striped pattern with variation of mechanical property has been successfully manufactured on the scaffold surface by the photolithography technique. The introduction of another material of liquid phase by the capillary effect into the space of groove of the surface, which is sandwiched between surfaces of the base and the cap molds, is effective technique to make variations of mechanical property according to the surface micro pattern.

In the present study, the tracings on the surface of the scaffold by the stylus show steps at the border between two kinds of bands (Fig. 6). The effect of the height of micro ridges on the orientation of C2C12 was studied in the previous study [2]. Because few cells show orientation at the border between bands, the height of the step might be lower than 0.001 mm. The gap of the step between bands might be small enough for the random migration of each cell. The value, which the stylus shows, might be related to the difference of compliance (rigidity) of the surface between two bands. The groove of the tracing shows that the compliance of the band of PDMS is higher than that of SU-8 [36].

In the present study, cells are seeded at the low density on the scaffold to trace the response of sparsely populated single cell. In the confluent state, interaction between cells governs the behavior of cells [1].

5. CONCLUSION

The flat scaffold with the taper-striped pattern has been designed to observe durotactic migration of a biological cell. The surface of the scaffold with the micro taper-striped (0.05 mm < width < 0.2 mm) pattern has successfully been made by the photolithography technique. The variation of hardness of

the surface of the band has been made of the materials alternately: polydimethylsiloxane (PDMS), and epoxy based negative photoresist material (SU-8). The experimental result shows that each cell tends to migrate to the area of the band with SU-8 in 24 hours. The experimental system is effective to investigate durotaxis of cells *in vitro*.

6. ACKNOWLEDGMENT

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