

Effect of Mechanical Property of Scaffold Surface with Micro Hybrid Striped Pattern on Cell Migration

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ABSTRACT

The effect of the mechanical property of the surface of the scaffold with the micro hybrid striped pattern on the migration of cells has been studied *in vitro*. The surface of the scaffold with the micro stripe pattern of bands (the width around 0.1 mm) 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). Three kinds of cells were used in the experiment: Neuro-2a (a mouse neural crest-derived cell line), L929 (fibroblast connective tissue of C3H mouse), and C2C12 (mouse myoblast). Cells were seeded on the micro pattern, and incubated for 24 hours in the medium. The total areas of cells at each band were evaluated at the microscopic images. The experimental result shows that most of cells migrate to the area of the band with SU-8, and that the tendency is considerable on L929. The experimental system is effective to investigate durotaxis of cells.

Keywords: Biomedical Engineering, Neuro-2a, L929, C2C12, Durotaxis and Photolithography.

1. INTRODUCTION

A biological cell adheres, migrates, rotates, and deforms on the scaffold. These behaviors of cell depend on the micro morphology of the scaffold [1-5]. The cell might be sensitive to the morphology of the similar dimension to itself at the scaffold [1-4]. The cell migration might also be guided by the rigidity gradients on the scaffold (durotaxis) [6-16]. The photolithography technique is available to make the micro patterns on the scaffold of the cell culture. The previous study showed that the orientation of myoblast depends on the height of the micro ridges [1].

The acceleration technique for proliferation, orientation and differentiation of cells has been studied to make tissue *in vivo* or *in vitro*. The behavior of a cell depends on several factors: mechanical [17-19], electrical [20], and magnetic stimulations [21]. The cell migration might govern the subsequent orientation of cells. The effect of stimulations on the cell varies with the kind of cells. Control methodology for proliferation, orientation and differentiation of cells would be applied to the regenerative medicine.

In the present study, the effect of the mechanical property of the surface of scaffold with the micro pattern on the migration of cells has been studied *in vitro*.

2. METHODS

Micro Hybrid Striped Pattern

The hybrid striped pattern of scaffold has been designed on a plate of polydimethylsiloxane (PDMS) for a scaffold by the photolithography technique. The surface of the scaffold consists of parallel bands made of two alternate materials: PDMS, and SU-8 (epoxy based negative photoresist material). To distinguish two kinds of materials of bands, the variation is made on the width of each kind of band: 0.11 mm for PDMS, and 0.09 mm for SU-8. The pattern was controlled by a photomask (Fig. 1).

Mold

The borosilicate glass (Tempax) disk is used for 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 cm³/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 plate with 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.

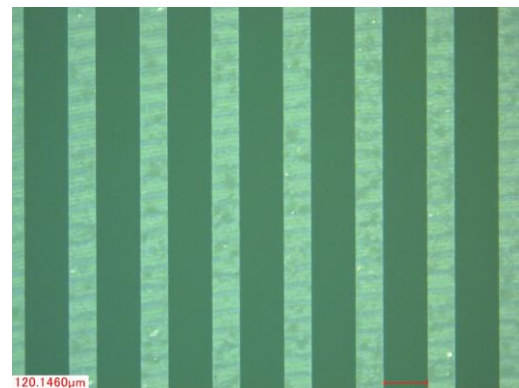


Fig. 1: Laser microscopic image of photomask for stripe pattern. Dimension from left to right is 1.5 mm.

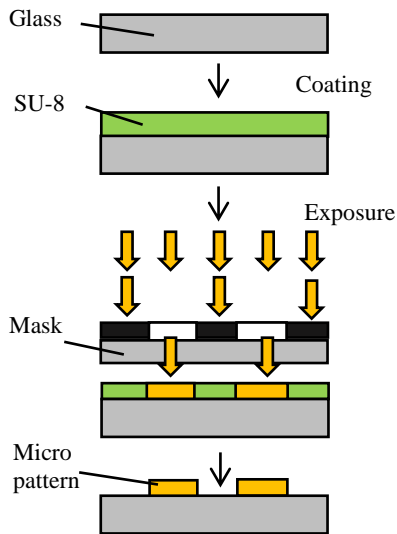


Fig. 2: Photolithography process for mold.

The photomask with the 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) (Figs. 3, 5, 8, 15, & 16).

Scaffold with 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 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 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 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 without micro pattern (Fig. 4). After the disks with SU-8 2 was baked at 393 K for five hours in the oven, the PDMS disk without micro pattern was exfoliated, and discarded.

The pattern of the surface of the scaffold was observed by the laser microscope (VK-X200, Keyence Corporation, Osaka,

Japan) (Fig. 6). The morphology of the surface of the scaffold was observed by a scanning electron microscope (SEM, JSM6380LD, JEOL Ltd., Tokyo, Japan) (Fig. 7).

Before the cell culture, 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

Three kinds of cells were used in the experiment: Neuro-2a (a mouse neural crest-derived cell line), L929 (fibroblast connective tissue of C3H mouse), and C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse).

Neuro-2a of the passage between 7 and 9 was cultured in D-MEM (Dulbecco's Modified Eagle Medium) containing 10% FBS (fetal bovine serum) and 1% penicillin/ streptomycin.

L929 of the passage between 6 and 9 was cultured in D-MEM (Dulbecco's Modified Eagle Medium) containing 10% FBS (fetal bovine serum) and 1% penicillin/ streptomycin.

C2C12 of the passage between 7 and 8 was cultured in D-MEM (Dulbecco's Modified Eagle Medium) containing 10% FBS (fetal bovine serum) and 1% penicillin/ streptomycin.

In each test, the single kind of cells was 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 for 24 hours in the incubator to maintain both the temperature of 310 K and the carbon dioxide partial pressure of 5 percent.

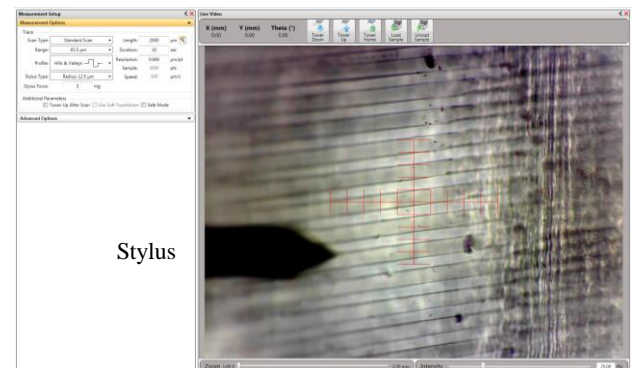


Fig. 3: Measurement of morphology of surface of mold across bands by stylus (left).

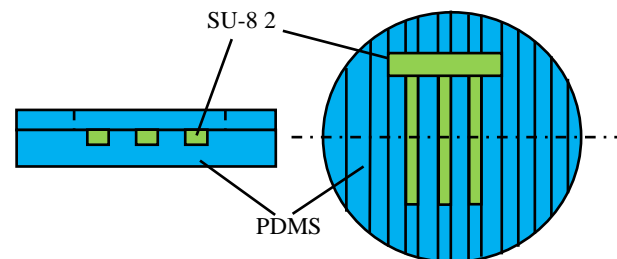


Fig. 4: Injection of SU-8 2 between two disks of PDMS: cross sectional view (left), plane view (right).

The migration tendency of each cell was checked by the time laps image of the phase contrast microscope (IX71, Olympus, Tokyo) during the cell culture. The following figures show images of cells at 24 hours after seeding. On the microscopic image, the contour of each cell was traced (Figs. 9, 11, 13), and the projected area of each cell (A_k) was calculated. In the two dimensional image, the total area of cells was evaluated at each band of the micro pattern by the occupation ratio of 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 sample microscopic image. R becomes 100 % at the confluent state in each kind of band. Five sample images of each kind of cell were selected for calculation. 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

The tracings of the surface of the mold measured by the stylus of the contact profilometer shows 0.016 mm height, and 0.2 mm pitch (Fig. 5). Fig. 6 shows the laser microscopic image of the stripe pattern of SU-8 and PDMS for the scaffold of cell culture. The image shows the band patterns with the alternate width: 0.11 mm of SU-8, and 0.09 mm of PDMS. Fig. 7 shows SEM image of the stripe of the surface of the scaffold. Fig. 8 shows the tracing measured by the stylus on the surface across the stripe. The figure shows 0.002 mm height of the step at the border between bands, and 0.2 mm pitch. The step looks very small on the SEM image.

Fig. 9 shows image of Neuro-2a by the phase contrast microscope after the incubation for 24 hours. The cells adhered on each band of the scaffold are traced, and the area surrounded by the line of contour is calculated (Fig. 10). The total area of cells at SU-8 is larger than that at PDMS. In each sample area, the occupation ratio of area (R) is higher in SU-8 than in PDMS. The ratios of R of SU-8 per R of PDMS are in the range between 2.1 and 6.2 (mean = 3.9) at Neuro-2a.

Fig. 11 shows image of L929 by the phase contrast microscope after the incubation for 24 hours. The cells adhered on each band of the scaffold are traced, and the area surrounded by the line of contour is calculated (Fig. 12). The total area of cells at PDMS is much smaller than that at SU-8. In each sample area, the occupation ratio of area (R) is higher in SU-8 than in PDMS. The ratios of R of SU-8 per R of PDMS are in the range between 12 and 103 (mean = 44) at L929.

Fig. 13 shows image of C2C12 by the phase contrast microscope after the incubation for 24 hours. The cells adhered on each band of the scaffold are traced, and the area surrounded by the line of contour is calculated (Fig. 14). The total area of cells at SU-8 is larger than that at PDMS. In each sample area, the occupation ratio of area (R) is higher in SU-8 than in PDMS. The ratios of R of SU-8 per R of PDMS are in the range between 3.3 and 15.3 (mean = 9.3) at C2C12.

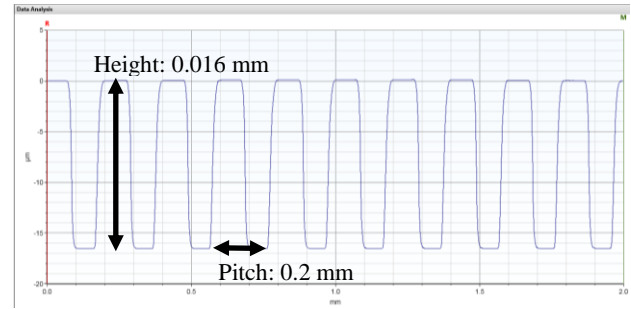


Fig. 5: Tracing (height, μm ; distance, mm) measured by stylus on surface of the mold.

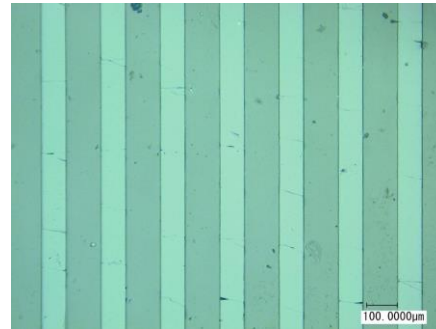


Fig. 6: Laser microscopic image of hybrid stripe pattern of SU-8 and PDMS for scaffold of cell culture. Dimension from left to right is 1.5 mm.

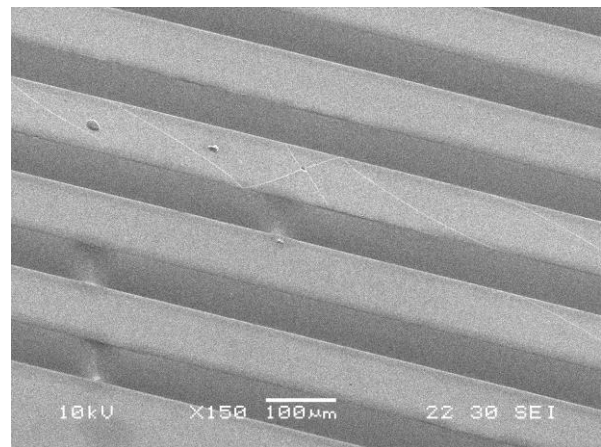


Fig. 7: SEM image of stripe of surface of scaffold. Dimension from left to right is 0.9 mm.

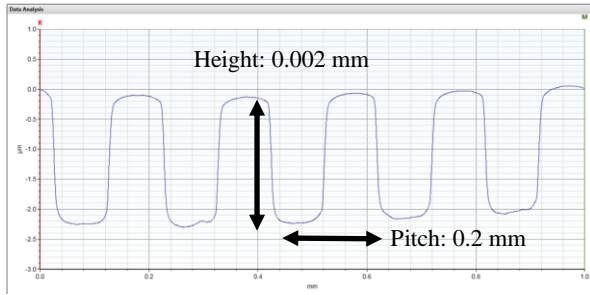


Fig. 8: Tracing (height [μm] vs. distance [mm]) measured by stylus on scaffold surface across the stripe with indentation load of 3×10^{-5} N.

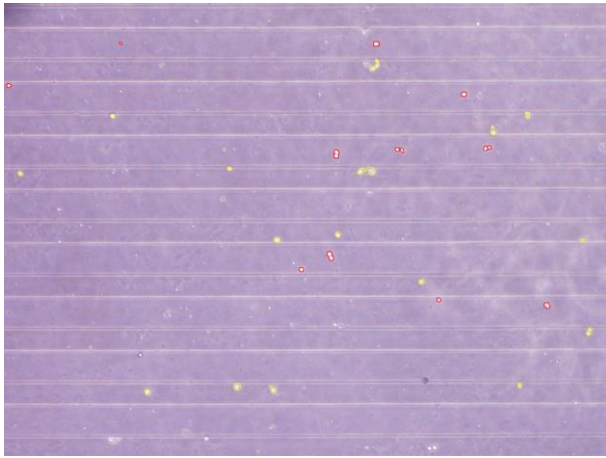


Fig. 9: Neuro-2a cultured on micro pattern for 24 hours. Dimension from left to right is 2.2 mm. Trace of contour of each cell to calculate total area of cells on each band.

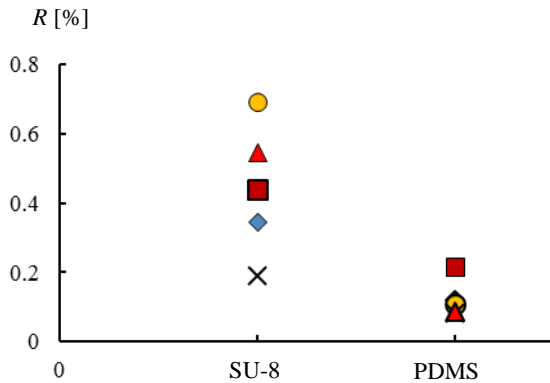


Fig. 10: Ratio of total area of Neuro-2a (%) cultured on micro pattern for 24 hours: SU-8 (left), PDMS (right): same marks show data in the same sample area.

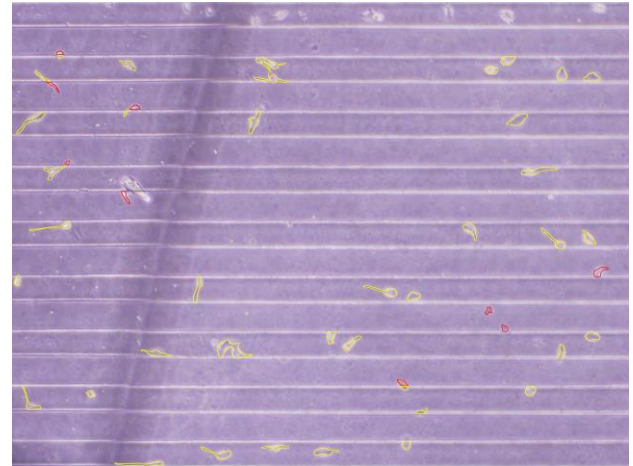


Fig. 11: L929 cultured on micro pattern for 24 hours. Dimension from left to right is 2.2 mm. Trace of contour of each cell to calculate total area of cells on each band.

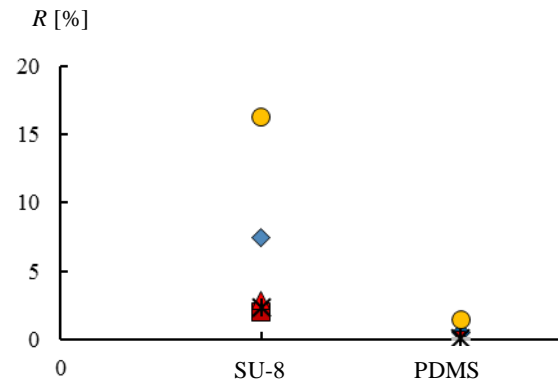


Fig. 12: Ratio of total area of L929 (%) cultured on micro pattern for 24 hours: SU-8 (left), PDMS (right): same marks show data in the same sample area.

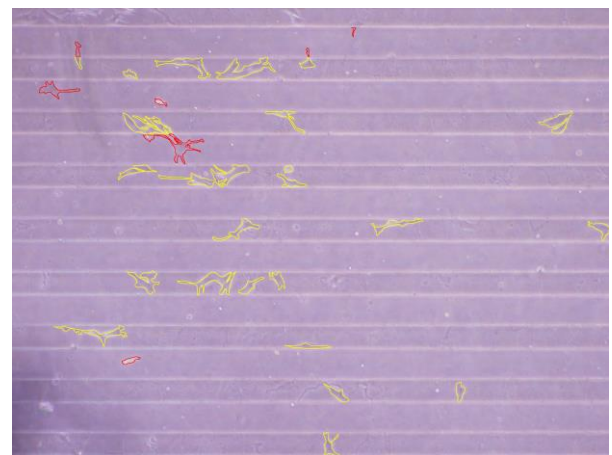


Fig. 13: C2C12 cultured on micro pattern for 24 hours. Dimension from left to right is 2.2 mm. Trace of contour of each cell to calculate total area of cells on each band.

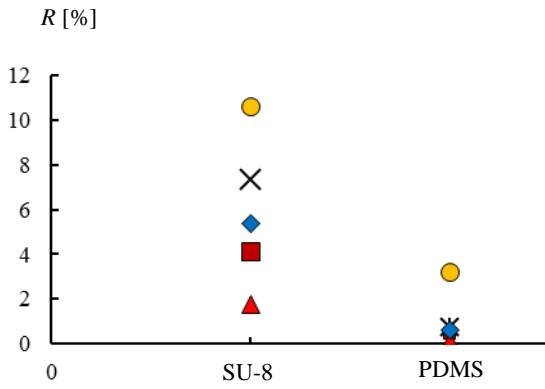


Fig. 14: Ratio of total area of C2C12 (%) cultured on micro pattern for 24 hours: SU-8 (left), PDMS (right): same marks show data in the same sample area.

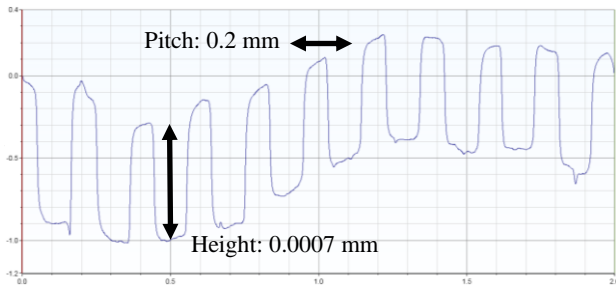


Fig. 15: Tracing (height [μm] vs. distance [mm]) measured by stylus on scaffold surface across stripe with indentation load of 10^{-5} N.

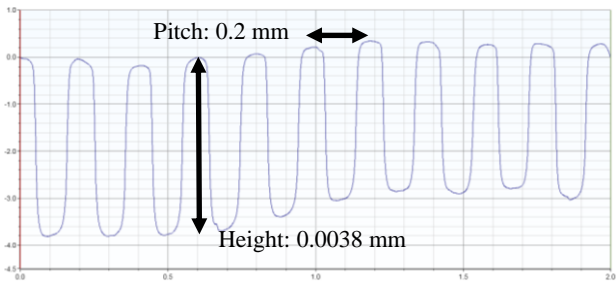


Fig. 16: Tracing (height [μm] vs. distance [mm]) measured by stylus on scaffold surface across stripe with indentation load of 1.5×10^{-4} N.

The ascending order of the ratios of R of SU-8 per R of PDMS is Neuro-2a, C2C12, and L929. At every kind of cells, the time lapse images show the migration of cells from the band of PDMS to the band of SU-8.

4. DISCUSSION

In the present study, the scaffold surface with variation of mechanical property at micro hybrid striped pattern has been successfully manufactured 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 mold, is effective technique to make variation of mechanical property according to the surface micro pattern.

In the present study, the tracing on the surface of the scaffold by the stylus shows steps at the border between two kinds of bands (Fig. 8). The effect of the height of micro ridges on the orientation of C2C12 was studied in the previous study [1]. Because few cells show orientation at the border between bands, the height of the step might be lower than 0.001 mm. 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 the compliance of the band of PDMS higher than that of SU-8. According to the indentation load of the stylus, the tracing varies (Figs. 15&16). When the indentation load is 10^{-5} N or 1.5×10^{-4} N, the difference of the height between two neighbor bands is 0.0007 mm (Fig. 15) or 0.0038 mm (Fig. 16), respectively. The difference between two cases show the deformation of 0.0031 mm by 1.4×10^{-4} N, which is related to the compliance of PDMS.

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 [17, 19].

In the present experiment, the tendency of migration guided by the rigidity on the scaffold depends on the kind of cells. The difference can be detected in 24 hours of cultivation. The tendency of migration from PDMS to SU-8 is remarkably at L929. The migration speed of the cell might be related to the gradient of compliance of the surface of the scaffold. Three kind of cells (Neuro-2a, L929, and C2C12) tend to migrate to SU-8, which has higher rigidity of the surface than PDMS.

5. CONCLUSION

The effect of the hardness of the surface of the scaffold with the micro pattern on the migration of cells has been studied *in vitro*. The surface of the scaffold with the micro stripe pattern of bands (the width around 0.1 mm) 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). The experimental result shows that most of cells migrate in 24 hours to the area of the band with SU-8, and that the tendency is considerable on L929. The experimental system is effective to investigate durotaxis of cells.

6. ACKNOWLEDGMENT

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