Effect of Flow on Cultured Cell at Micro-pattern of Ridge Lines

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ABSTRACT

A flow channel with a micro-pattern of ridge lines of a scaffold has been designed to study quantitatively the effect of flow on an oriented cell *in vitro*. The lines of parallel micro ridges (0.001 mm height, 0.003 mm width, and 0.003 mm interval) are made by the lithography technique on the lower surface of the channel as the scaffold to make orientation of each cell. Variation is made about the angle between the longitudinal direction of the ridge line and the direction of the flow: zero, 0.79 and 1.6 rad. The suspension of C2C12 (mouse myoblast cell line) was injected to the channel, and incubated for two hours on the micro ridges before the flow test for four hours. The flow rate of < 36 cm^3/hour is controlled by a syringe pump to make variation of the wall shear stress of < 3 Pa. The action of each cell adhered on the micro pattern was analyzed at the time lapse images. The experimental results show that both the migration and the deformation of each myoblast along the micro ridge are restricted by the wall shear stress higher than 3 Pa.

Keywords: Biomedical Engineering, Flow Channel, Cell Culture, Micro-pattern, Myoblast and Shear stress.

1. INTRODUCTION

Biological cells are exposed to mechanical stimulation *in vivo*. The shear stress is one of the mechanical stimulations. The endothelial cells, for example, are exposed to the shear stress in the blood flow at the wall of the blood vessels [1-3]. Some other cells are exposed to the shear stress among the deformation of the tissue.

A cell adheres on the scaffold, migrates, deforms, proliferates, and differentiates. The mechanical stress might affect the action of the cell. The biological cell takes action not only passively but also actively. The cell is moved to downstream by the flow. The cell migrates, on the other hand, to the upstream against the flow. The flow deforms the cell along the stream line. The cell changes the shape, on the other hand, to minimize the internal stress.

The 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 tissue *in vivo* or *in vitro* [4]. Control methodology for orientation of cells would be applied to the regenerative tissue technology.

The action of cell depends on the micro morphology of the scaffold [4-6]. The cell might be sensitive to the morphology of the similar dimension to itself at the scaffold.

The photolithography technique [4-7] is available to make the micro patterns on the surface. The previous study showed that the orientation of myoblast follows the longitudinal directions of the micro ridges [4-6]. Several types of flow systems have been applied to study the response of cells to the shear stress in the flow *in vitro* [8-16].

In the present study, a flow channel with a micro-patterned scaffold has been designed to study quantitatively the effect of flow on the oriented cell *in vitro*.

2. METHODS

Flow Channel

A flow channel has been designed to observe behavior of cells adhered on the micro-pattern in the flow during the cell culture *in vitro*. The flow channel consists of two disks of 50 mm diameter: the upper disk, and the lower disk (Fig. 1). The borosilicate glass (Tempax) disk is used for the lower disk for the scaffold of the cell culture. The upper surface of the lower disk has the micro-pattern. The upper disk is made of polydimethylsiloxane (PDMS), which has concave pattern at the lower surface for the flow path.

Lower Disk with Micro-pattern

The micro-pattern is fabricated by the photolithography technique at the central part of the upper surface of the lower disk for the scaffold of the cell culture. Several parallel lines of micro ridges (between micro grooves) have been made at the lower disk (Fig. 2). The height (H) (the depth of the groove), the width (W), and the interval (I) of the rectangular ridges are 0.001 mm, 0.003 mm, and 0.003 mm, respectively. Variation has been made on the angle (θ) between the longitudinal direction of the groove and the flow direction: 0 rad (parallel), 0.79 rad and 1.6 rad (perpendicular). Each pattern is drawn in the rectangle area of 1.6 mm × 0.4 mm, which is parallel position each other.

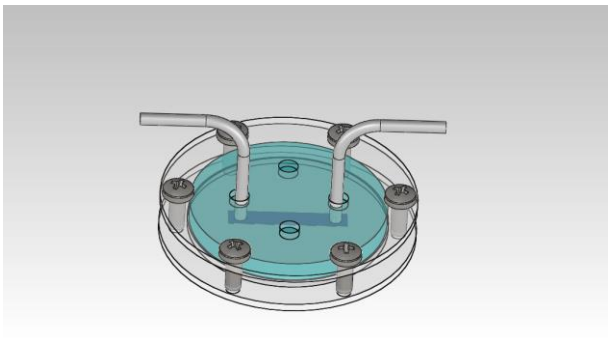

Fig. 1a: Flow channel.

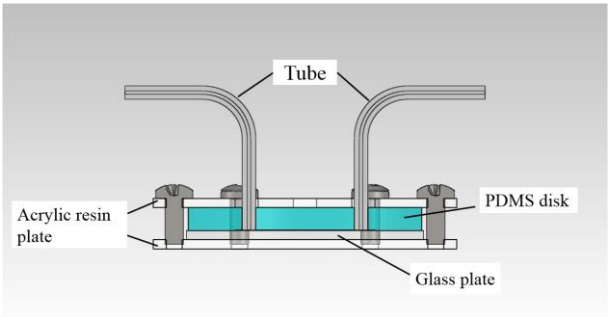

Fig. 1b: Cross section of flow channel.

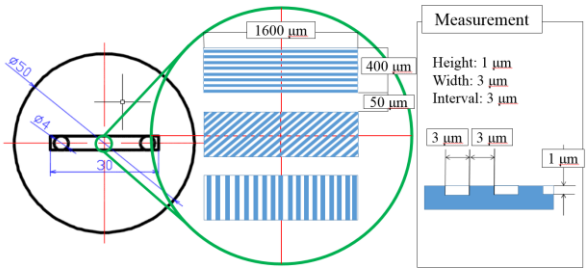

Fig. 2: Micro-pattern at flow channel.

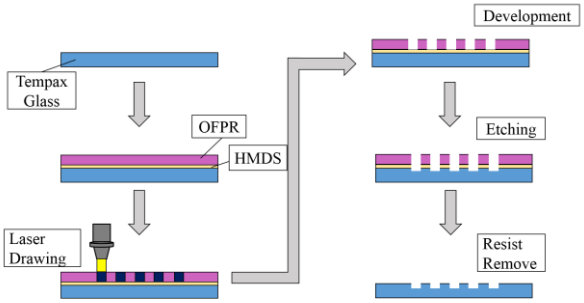

Fig. 3: Photolithography process.

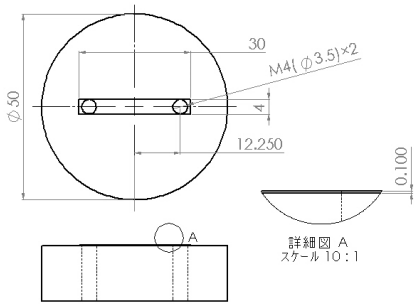

Fig. 4: Dimension (mm) of mold for upper disk of flow channel.

The borosilicate glass disk was used for the base of the lower disk in a photo lithography process (Fig. 3). The diameter and the thickness of the disk are 50 mm and 1.1 mm, respectively. The surface of the glass disk was cleaned by the oxygen (0.1 Pa, 30 cm^3/min) plasma ashing (100 W, for ten minutes) in a reactive ion etching system (FA-1, Samco Inc., Kyoto, Japan).

To improve the affinity between the glass and the photo-resist material (OFPR-800LB (Tokyo Ohka Kogyo Co., Ltd., Tokyo, Japan)), hexamethyldisilazane (HMDS) was coated on the glass at 3000 rpm for thirty seconds with a spin coater (IH-DX2, Mikasa Co., Ltd., Tokyo, Japan). The photoresist material of OFPR-800LB was coated on HMDS at 3000 rpm for twenty seconds with the spin coater. The photoresist was baked at the hotplate at 373 K for three minutes.

The pattern for the micro grooves was drawn on the photoresist material with a laser drawing system (DDB-201K-KH, Neoark Corporation, Hachioji, Japan). To control the dimension of the pattern with the laser drawing system, the parameters were selected as follows: the voltage of 3.2 V, the velocity of 0.1 mm/s, the acceleration of 0.350 mm/s^2. The pattern was baked at the hotplate at 393 K for five minutes. The photo-resist was developed with tetra-methyl-ammonium hydroxide (NMD-3, Tokyo Ohka Kogyo Co., Ltd., Kawasaki, Japan) for two minutes. The disk was rinsed two times with the ultrapure water for three minutes, and dried by the spin-dryer (SF-250, Japan Create Co., Ltd., Tokorozawa, Japan).

The glass was etched with the plasma gas using a reactive ion etching system (RIE-10NR, Samco Inc., Kyoto, Japan) to make lines of the micro grooves of 0.001 mm depth. For etching, the gas of CF_4 (30 cm^3/min at 1013 hPa) was applied at 100 W at 2 Pa for thirty minutes. To exfoliate the residual photo-resist material from the surface, the disk was exposed to the oxygen gas of 30 milliliter per minute at the power of 100 W for ten minutes using a compact etcher (FA-1): the oxygen plasma ashing.

The morphology of the micro grooves was observed by a scanning electron microscope (SEM, JSM6360LA, JEOL Ltd., Tokyo, Japan) (Fig. 9). The contact angles of the purified water on the scaffold without the micro patterns was measured by the contact angle analyzer (Phoenix-300, Meiwafosis Co., Ltd., Tokyo, Japan), before and after the oxygen plasma ashing treatment.

Upper Disk

The mold for the upper disk is made of aluminum. The mold has a convex rectangular pattern with the following dimension (Fig. 4): the width of 4 mm, the height of 0.1 mm, and the length of 30 mm. Each end of the rectangular pattern has a hole with the female screw of M4. The dimension of the convex part was measured with a laser microscope (VK-X200, Keyence Corporation, Osaka, Japan). The morphology along the transverse line of convex part was traced (Fig. 10). The mold was cleaned in the ultrasonic cleaning machine. In each hole with the female screw, one end of the bolt with the male screw was inserted. The other end of each bolt was inserted into the silicone tube (internal diameter 4 mm, external diameter 6 mm).

After the mold was enclosed with a peripheral wall of polyimide tape, 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. After degassing, PDMS was baked at 373 K for one hour in an oven (DX401, Yamato Scientific Co., Ltd). The baked disk of PDMS (9 mm thickness) with tubes was exfoliated from the mold to be used as the upper disk of the channel.

Assembly of Disks

Both the upper and the lower disks were exposed to the oxygen gas of 30 milliliter per minute at the power of 50 W for thirty seconds in the reactive ion etching system (FA-1), before the flow test to be characterized as hydrophilic (oxygen plasma ashing). Immediately after ashing, the upper disk adheres (plasma bonding) to the lower disk to make the flow path between them. The assembly of disks was baked in the oven at 348 K for five minutes. Before cell culture, the flow channel was sterilized in an autoclave at 394 K for forty minutes. After sterilization, the channel was dried in the oven. In the clean bench, the channel was sandwiched between the transparent disks (diameter of 65 mm, thickness of 2 mm) of poly-methyl-methacrylate with six bolt/nuts as the supporter to adjust the fixation of the assembly of the flow channel (Fig. 5).

One of the silicone tubes connects the flow channel with the syringe pump. The other silicone tube connects the flow channel with the reservoir of the suspension of the cells, or with the reservoir of the medium (Fig. 6). The channel is placed on the stage of an inverted phase contrast microscope (IX71, Olympus, Tokyo) to observe behavior of cells adhered on the micro pattern on the disk under the flow (Fig. 5). The CO_2 gas is blown into the reservoir of the medium and into the incubator on the stage of the microscope to maintain the carbon dioxide partial pressure at five percent in the medium. The reservoir is placed in the thermostatic bath to maintain the temperature at 310 K (Fig. 7).

Wall Shear Stress

In the present experiment, the shear rate on the wall of the scaffold is estimated with a parabolic velocity profile between the parallel walls [19]. The shear rate (γ, [s^{-1}]) on the wall of the plate is calculated by Eq. 1.

$$\gamma = 6\, q / (b\, d^{\,2}) \qquad (1)$$

In Eq. 1, q is the flow rate [$m^3\ s^{-1}$], b is the width of the channel [m] and d is distance [m] between two parallel walls. In the present study, d is 0.1 mm, and b is 4 mm. When q = 12 cm^3/hour, γ= 500 s^{-1}. The shear stress τ [Pa] is the product of the viscosity η [Pa s] of the fluid and the shear rate γ [s^{-1}] of the flow (Eq. 2).

$$\tau = \eta\, \gamma \qquad (2)$$

When γ = 500 s^{-1} and η = 0.002 Pa s (at 310 K) [11], τ = 1 Pa. Variation was made on the wall shear stress τ lower than 3 Pa by adjusting the flow rate q.

Cell Culture with Flow

C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse) of the passage between fourth and tenth was used in the tests. D-MEM (Dulbecco's Modified Eagle Medium) containing 10% FBS (Fetal Bovine Serum) and 1% penicillin/ streptomycin was used for the medium. After the medium was prefilled in the flow channel through the inlet tube by the syringe pump, the suspension (50000 cells/cm^3) of C2C12 in D-MEM was introduced into the channel through the inlet tube by the syringe pump (Fig. 6).

After several cells adhered on the micro-pattern in the flow channel in two hours, the wall shear stress was applied on the cells by the flow pulled with the syringe pump for four hours. After flow stimulation for four hours, the microscopic observation was continued for another successive four hours. To trace the same cell, the time-lapse pictures were taken with the constant interval of five minutes during the microscopic observation (Fig. 7). As the control test, cells seeded on the micro pattern without flow stimulation were also observed by the time-lapse microscopic images.

At the microscopic image, the outline of each cell was traced by "Image J", and the contour of each cell was approximated to an ellipsoid (Fig. 8). As to the ellipsoid, the coordinates of the centroid, the length of the major axis (a), and the minor axis (b) were measured. To trace the migration of each cell by the coordinates, the x axis is corrected to the direction of the flow. The ratio of axes is calculated as the deformation ratio (R) by Eq. 3.

Fig. 5a: Flow channel and tubes.

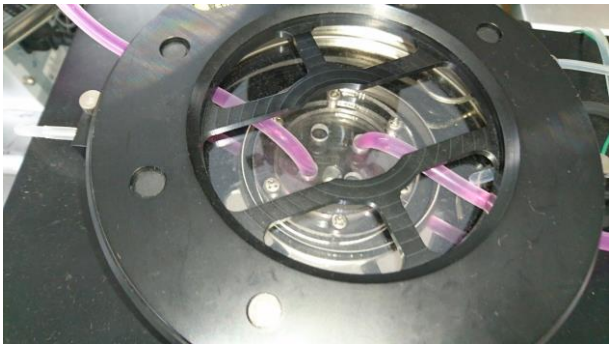

Fig. 5b: Flow channel in chamber on stage of microscope.

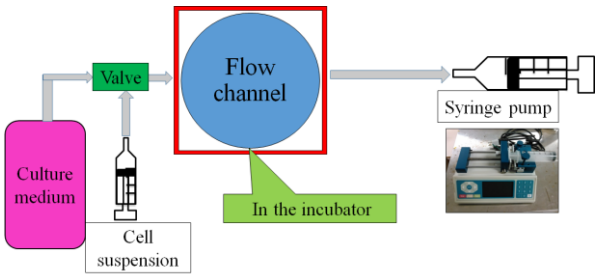

Fig. 6: Cell culture with flow.

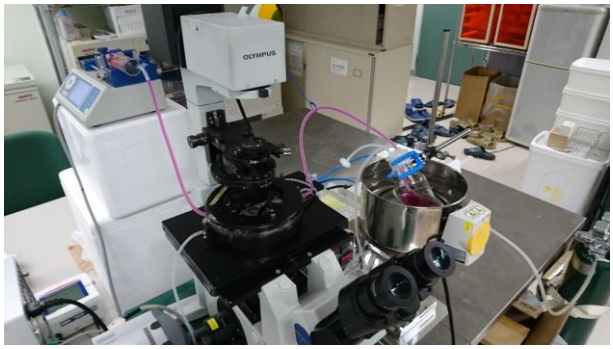

Fig. 7: Experimental system.

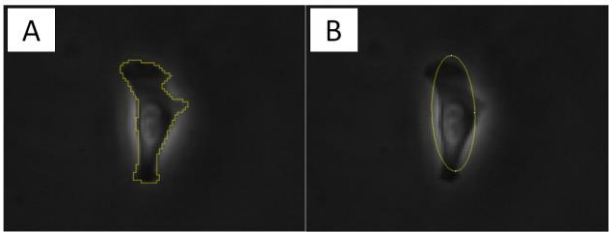

Fig. 8: Contour of each cell (A) is approximated to ellipsoid (B).

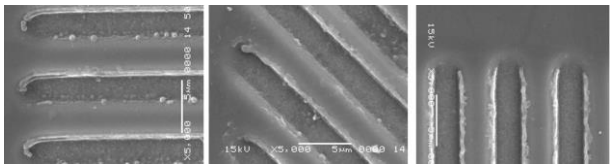

Fig. 9: SEM image of micro grooves on glass. Angle (θ) between the longitudinal direction of the groove and the flow direction: 0 rad (left), 0.79 rad (middle) and 1.6 rad (right).

$$R = 1 - b / a \qquad (3)$$

At the circle, $R = 0$. As the ellipsoid becomes flat, R approaches to unity.

3. RESULTS

Fig. 9 shows the scanning electron microscope images of grooves on the surface of the lower disk. The morphology of the rectangular groove is confirmed: the depth of 0.001 mm, the width of 0.003 mm, and the interval of 0.003 mm. The contact angle of the purified water on the scaffold without the micro patterns shows hydrophilic property of the scaffold. The contact angle approaches to zero after the oxygen ashing. The measurement by the laser microscope of the mold showed that the height (d) is 0.1 mm (Fig. 10). The experimental results show that several C2C12 cells adhere in two hours (Fig. 11). The number of cells adhered on the micro ridges between micro grooves shows that the most of cells keep adhesion on the ridges under the flow at the wall shear stress of 1 Pa for four hours (Table 1). At the wall shear stress of 3 Pa, on the other hand, more than half of cells on the ridges are exfoliated within four hours.

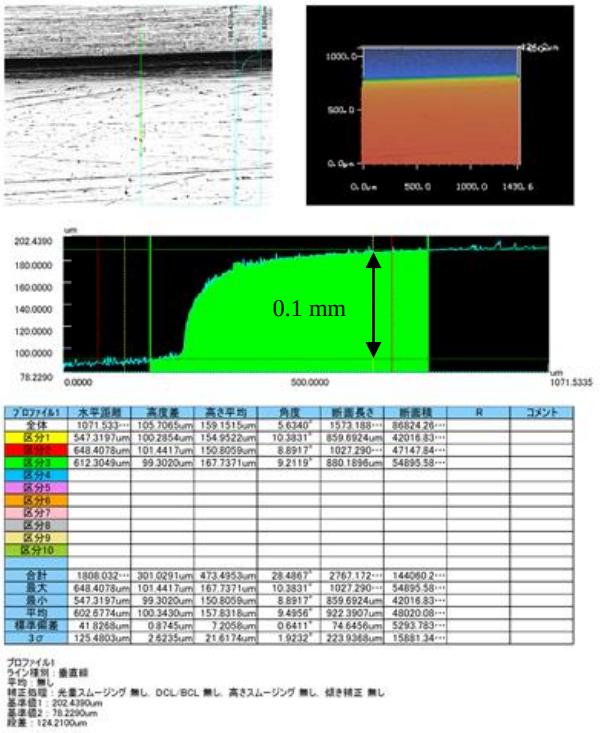

Fig. 10: Tracings of mold for upper plate.

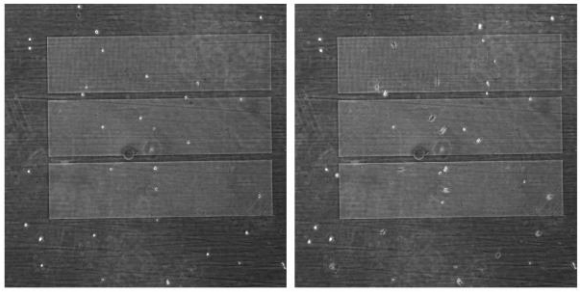

Fig. 11: Cells on micro-pattern at seeding (left), and at 2 hours without flow after seeding (right).

Table 1: Number of cells on micro ridges.

Wall shear stress	Flow stimulation		
	Before	5 min	4 hour
1 Pa	107	107	102
3 Pa	183	122	66

Fig 12 exemplifies the migration of each cell (5 cells in each figure as the sample) on the micro pattern. Each point shows the coordinates of the centroid of the cell every thirty minutes. The dotted line shows the direction of the lines of ridges in Fig. 12. The migration of each cell along the ridge decreases at the wall shear stress of 3 Pa. C2C12 on the ridge of 0.79 rad often migrates to the counter direction of the flow along the ridge line at the wall shear stress of 1 Pa.

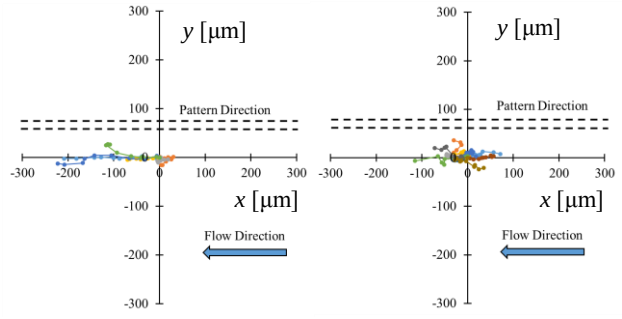

Fig. 12A: Migration of cell on ridges of 0 rad at wall shear stress of 1 Pa (left) and 3 Pa (right).

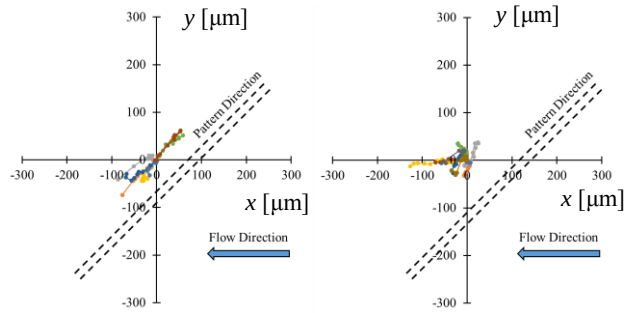

Fig. 12B: Migration of cell on ridges of 0.79 rad at wall shear stress of 1 Pa (left) and 3 Pa (right).

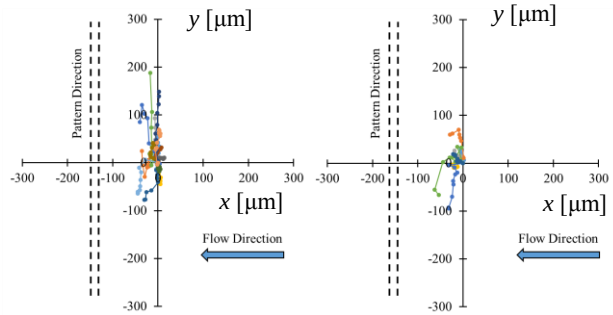

Fig. 12C: Migration of cell on ridges of 1.6 rad at wall shear stress of 1 Pa (left) and 3 Pa (right).

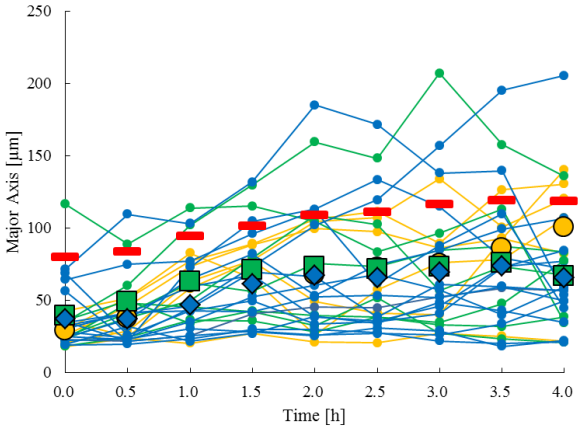

Fig. 13: Length of major axis of each cell (20 cells) on ridge (mean; circle-yellow, 0 rad; square-green, 0.79 rad; rhombus-blue, 1.6 rad) during flow stimulation at wall shear stress of 1 Pa: red bar, control without flow.

Fig.13 exemplifies the length of the major axis of each cell on the micro ridge during the flow stimulation at the wall shear stress of 1 Pa. The length of each cell repeats increase and decrease on the micro ridge during exposure to the flow. The horizontal bar shows the mean value of 20 cells, which tends to increase in four hours.

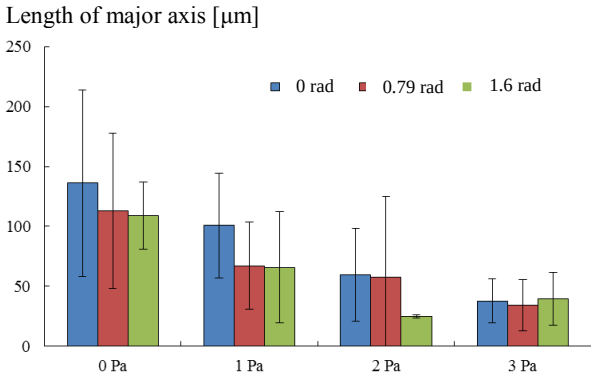

Fig. 14: Length of major axis [μm] of cell on ridge after flow stimulation for 4 hours: column, mean; bar, ±standard deviation.

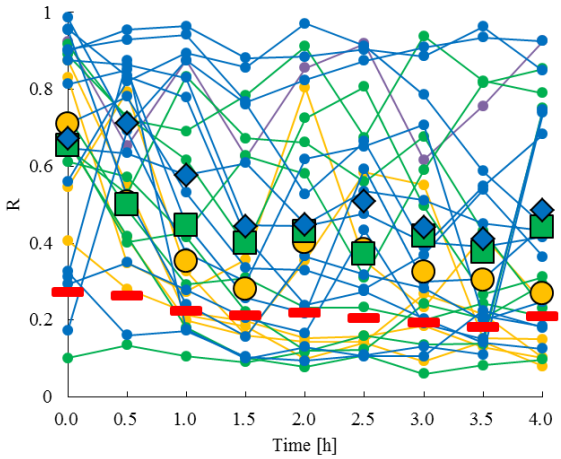

Fig. 15: Deformation ratio (*R*) of each cell (20 cells) on ridge (mean; circle, 0 rad; square, 0.79 rad; rhombus, 1.6 rad) during flow stimulation at wall shear stress of 1 Pa for four hours: red bar, control without flow.

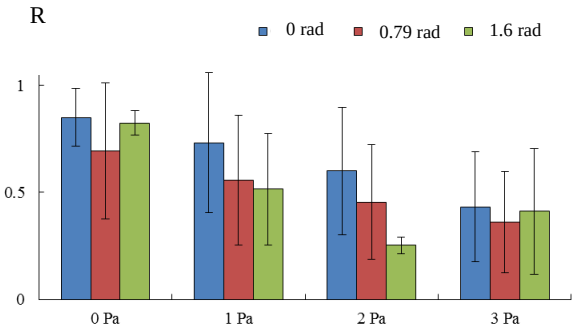

Fig. 16: Deformation ratio (*R*) of cell on ridge after flow stimulation for 4 hours: column, mean; bar, ±standard deviation.

Fig. 14 shows the mean value of the length of the major axis of cells on the ridge after the flow stimulation for four hours. At the wall shear stress of 3 Pa, the length decreases to the small value independent of the angle between the longitudinal direction of the ridge and the direction of the flow. At 1 Pa, C2C12 on 0 rad elongates more than 0.79 rad and 1.6 rad.

Fig. 15 exemplifies the deformation ratio of each cell on the micro ridge parallel to the flow during the flow stimulation at the wall shear stress of 1 Pa. The deformation ratio of each cell repeats increase and decrease on the micro ridge during exposure to the flow. The horizontal bar shows the mean value of 20 cells of control without flow.

Fig. 16 shows the mean value of the deformation ratio of cells on the ridge after the flow stimulation for four hours. At the wall shear stress of 3 Pa, the deformation ratio decreases to the small value independent of the angle between the longitudinal direction of the ridge and the direction of the flow.

4. DISCUSSION

Several types of experimental preparations were designed to evaluate the effect of stimulations on biological cells in the previous studies [1-20]. The flow is one of the stimulations on cells in the experimental systems: the donut type canal [8, 9], the cylindrical pipe [16], the channel between parallel walls [1, 2, 10, 11], the rhombus channel [12], the cross type of channel [13], and Couette type flow using the rotating disk [3, 14, 15]. For quantitative study, Couette type flow [3, 14, 15] or Poiseuille type flow is convenient [10-13, 16]. The response of the cell, which has an antisymmetric shape, depends on the direction of flow.

On the scaffold, the direction of each cell randomly scatters. To control the direction of adhesion of the cell, the micro pattern of the surface of the scaffold is available. Both the migration and the deformation of the cell depend on the micro morphology of the surface of the scaffold. The lithography technique can be applied to make the micro morphology on the surface [4-7]. In the present study, the lines of micro ridges are designed on the scaffold to make orientation of cell.

The dimension of the width of the micro groove is smaller than the diameter of the cell. The cell does not fall into the groove. The wall shear stress around 1 Pa is selected in the present study as the typical value, which affected cells in the previous study [11]. The parallel piped channel is selected, and the wall shear stress lower than 3 Pa is applied to the cell adhered on the wall of the flow channel. Three types of direction (parallel, diagonal, and perpendicular) have been tested for the orientation of cells in the flow. The micro pattern on the scaffold can also be used as markers to trace the cell. Cells keep the active action in the flow stimulation at the lower shear stress (< 3 Pa). The cell also migrates to the counter direction of the flow.

The behavior of the cell depends on the height of the ridge. In the present study, the height of the ridge is designed as 0.001 mm, which is lowest to affect orientation of cell.

The higher wall shear stress (> 3 Pa) reduces the migration of cells. A cell is deformed to a round shape at the high wall shear stress. At the mild shear stress around 1 Pa, a cell migrates actively on the scaffold to the diagonal direction. A flow can be used to apply a stress field to a cell [1-3, 8-16]. The cell directly receives the shear stress in the shear flow. The high shear flow might deform cell, peel cells off from the scaffold, and inhibit proliferation.

The mild shear flow, on the other hand, might accelerate the active deformation, migration, and proliferation. The wall shear stress of 1 Pa enhances the migration of C2C12 along the ridge. Cells restart migration after stopping the wall shear stress of 3 Pa within four hours, which shows that the cells are not damaged. The elongation of the cell is the maximum on the ridge parallel to the flow.

In the present study, the inner surface of the flow channel was treated to be hydrophilic to avoid contamination with bubbles. The hydrophilic property of the wall might affect the migration of the cell. The contact angle on the micro pattern is not measured in the present study, because the dimension of the micro pattern (0.003 mm) is much smaller than the diameter of the drop of water.

To avoid air bubbles, the level of each element of the flow system is adjusted in the test: the syringe pump, the flow channel, and the reservoir. The adjustment of the level is important to keep the inner pressure, which keeps distance between the parallel walls of the flow channel. The tubes for inlet and outlet are embedded in the PDMS disk during the processing to decrease the leakage.

Reynolds number (*Re*) is calculated by Eq. 4.

$$Re = \rho\, v\, d / \eta = \rho\, q / (b\, \eta) \qquad (4)$$

In Eq. 4, ρ is density of the fluid, and q is the flow rate. *Re* is 1, when ρ, q, b, and η are 1000 kg m^{-3}, 0.01×10^{-6} m^3/s, 0.004 m, and 0.002 Pa s, respectively. The turbulent flow may not occur in the flow of small value of Reynolds number.

The reaction might depend on the kind of cell. HUVEC (human umbilical vein endothelial cells) does not stably adhere on the ridge in the present study. The affinity between the cell and the surface of the micro pattern is not good, or the wall shear stress of 1 Pa is too high for HUVEC for four hours. In the dense state of cell (confluent), the orientation of cells might depend on the interaction between cells. The effect of flow on the single (isolated) cell has been investigated in the present study.

The cells might change the orientation at the differentiation. C2C12 made perpendicular orientation of myotubes to the flow direction in the previous study [8]. The orientation of C2C12 at diagonal direction of flow might be preparation to make perpendicular orientation of myotubes in the successive cultivation.

5. CONCLUSION

A flow channel with a micro-patterned scaffold has been designed to study the effect of flow on the action of each oriented cell *in vitro*. The lines of micro ridges are designed on the lower surface of the plate as the scaffold to make orientation of each cell. The action of each cell adhered on the micro pattern was analyzed at the time lapse images. The

experimental results show that both the migration and the deformation of each myoblast along the micro ridge are restricted by the wall shear stress higher than 3 Pa. The experimental system *in vitro* is effective to observe the effect of the flow direction on cultured cells.

6. ACKNOWLEDGMENT

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REFERENCES

[1] N. Kataoka, S. Ujita and M. Sato, "Effect of Flow Direction on the Morphological Responses of Cultured Bovine Aortic Endothelial Cells", **Medical and Biological Engineering and Computing**, Vol. 36, No. 1, 1998, pp. 122-128.

[2] M.J. Levesque and R.M. Nerem, "The Elongation and Orientation of Cultured Endothelial Cells in Response to Shear Stress", **Journal of Biomechanical Engineering**, Vol. 107, No. 4, 1985, pp. 341-347.

[3] P. Uttayarat, M. Chen, M. Li, F.D. Allen, R.J. Composto and P.I. Lelkes, "Microtopography and Flow Modulate the Direction of Endothelial Cell Migration", **American Journal of Physiology - Heart and Circulatory Physiology**, Vol. 294, No. 2, 2008, pp. H1027-H1035.

[4] K. Sugimoto, Y. Takahashi, H. Hino and S. Hashimoto, "Effect of Aspect Ratio of Checkered (Ichimatsu) Convexo-concave Micro-pattern on Orientation of Cultured Cells", **Proc. 20th World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 2, 2016, pp. 141-146.

[5] H. Sugimoto, H. Hino, Y. Takahashi and S. Hashimoto, "Effect of Surface Morphology of Scaffold with Lines of Micro Ridges on Deformation of Cells", **Proc. 20th World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 2, 2016, pp. 135-140.

[6] H. Hino, S. Hashimoto and F. Sato, "Effect of Micro Ridges on Orientation of Cultured Cell", **Journal of Systemics, Cybernetics and Informatics**, Vol. 12, No. 3, 2014, pp. 47-53.

[7] Y. Takahashi, S. Hashimoto, H. Hino and T. Azuma, "Design of Slit between Micro Cylindrical Pillars for Cell Sorting", **Journal of Systemics, Cybernetics and Informatics**, Vol. 14, No. 6, 2016, pp. 8-14.

[8] S. Hashimoto and M. Okada, "Orientation of Cells Cultured in Vortex Flow with Swinging Plate in Vitro", **Journal of Systemics Cybernetics and Informatics**, Vol. 9, No. 3, 2011, pp. 1-7.

[9] M. Ochiai, S. Hashimoto and Y. Takahashi, "Effect of Flow Stimulation on Cultured Osteoblast", **Proc. 18th World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 2, 2014, pp. 156-161.

[10] S. Hashimoto, F. Sato, H. Hino, H. Fujie, H. Iwata and Y. Sakatani, "Responses of Cells to Flow in Vitro", **Journal of Systemics Cybernetics and Informatics**, Vol. 11, No. 5, 2013, pp. 20-27.

[11] H. Hino, M. Ochiai, S. Hashimoto, K. Kimura, Y. Takahashi and T. Yasuda, "Effect of Wall Shear Stress in Flow on Myoblast", **Proc. 19th World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 2, 2015, pp. 246-251.

[12] F. Sato, S. Hashimoto, T. Yasuda and H. Fujie, "Observation of Biological Cells in Rhombus Parallelepiped Flow Channel", **Proc. 17th World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 1, 2013, pp. 25-30.

[13] H. Hino, S. Hashimoto, Y. Takahashi and S. Nakano, "Design of Cross Type of Flow Channel to Control Orientation of Cell", **Proc. 20th World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 2, 2016, pp. 117-122.

[14] H. Hino, S. Hashimoto, Y. Takahashi and M. Ochiai, "Effect of Shear Stress in Flow on Cultured Cell: Using Rotating Disk at Microscope", **Journal of Systemics, Cybernetics and Informatics**, Vol. 14, No. 4, 2016, pp. 6-12.

[15] S. Hashimoto, "Detect of Sublethal Damage with Cyclic Deformation of Erythrocyte in Shear Flow", **Journal of Systemics, Cybernetics and Informatics**, Vol. 12, No. 3, 2014, pp. 41-46.

[16] S. Hashimoto, H. Oku, N. Komoto, Y. Murashige, S. Manabe, K. Ikegami and C. Miyamoto, "Effect of Pulsatile Shear Flow on Migration of Endothelial Cells Cultured on Tube", **Proc. 6th World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 2, 2002, pp. 296-300.

[17] H. Hino, S. Hashimoto, Y. Takahashi and H. Nakajima, "Effect of Ultrasonic Vibration on Proliferation and Differentiation of Cells", **Journal of Systemics, Cybernetics and Informatics**, Vol. 14, No. 6, 2016, pp. 1-7.

[18] S. Hashimoto, H. Hino, and T. Iwagawa, "Effect of Excess Gravitational Force on Cultured Myotubes in Vitro", **Journal of Systemics, Cybernetics and Informatics**, Vol. 11, No. 3, 2013, pp. 50-57.

[19] S. Hashimoto, F. Sato, R. Uemura and A. Nakajima, "Effect of Pulsatile Electric Field on Cultured Muscle Cells in Vitro", **Journal of Systemics Cybernetics and Informatics**, Vol. 10, No. 1, 2012, pp. 1-6.

[20] S. Hashimoto and K. Tachibana, "Effect of Magnetic Field on Adhesion of Muscle Cells to Culture Plate", **Journal of Systemics Cybernetics and Informatics**, Vol. 11, No. 4, 2013, pp. 7-12.