Cell Migration in Shear Field: Comparative Study between MC3T3-E1 and 3T3-L1

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ABSTRACT

Migration of a cultured cell exposed to Couette type of shear flow has been observed at the incubator microscope *in vitro*. The shear flow was made between a rotating disk and a stationary disk, which are in parallel position each other. Each cell adhered on the disk was observed under the steady shear flow by an inverted phase contrast microscope (shear stress lower than 2 Pa). The migration of cells was evaluated at the time lapse images (every five minutes for 24 hours). Two kinds of cells were used in the test: MC3T3-E1 (mouse osteoblast precursor cell line), 3T3-L1 (mouse fat precursor cells). The experiments show the following results. Under the shear stress of 2 Pa, 3T3-L1 tends to be rounded and migrate to the downstream. MC3T3-E1, on the other hand, migrates to every direction.

Keywords: Biomedical Engineering, Shear Stress, MC3T3-E1, 3T3-L1, Couette Flow and Migration.

1. INTRODUCTION

A biological cell makes several responses in the force field [1]. The effect of the cyclic stretching on alignment and proliferation of osteoblasts has been studied in the previous study [2]. The flow can be used to apply a stress field to a specimen. The cells exposed to the flow directly receive the shear stress in the shear flow. Endothelial cells are exposed to the wall shear stress by blood flow *in vivo*. The flow direction affects orientation of endothelial cells [3]. The effect of shear stress on the response of endothelial cells were studied in the previous studies: deformation [4], orientation [5], proliferation [6], and tissue formation [7]. The high shear flow might deform a cell, peel off a cell from the scaffold, and inhibit proliferation as well as tissue formation. The mild shear flow under the threshold of the wall shear stress, on the other hand, might accelerate migration, proliferation, and orientation. These mechanotransduction was analyzed *in vitro* by microfluidics in the previous study [8].

In the previous study, cells were exposed to the shear flow in a donut-shaped open channel [9], and the effect of flow stimulation on cultured cells has been studied *in vitro*. When the flow has a free surface, it is difficult to estimate the shear stress in the fluid. Couette type of flow, on the other hand, is convenient to realize the uniform shear field [10-15]. A cone-plate type is one of the famous Couette flow devices [10-11].

In the present study, an experimental system of the Couette type flow with a rotating disk has been designed to apply wall shear stress quantitatively on the cell during incubation at the microscopic observation *in vitro*, and the effect of the shear flow on the migration of the cell has been studied.

2. METHODS

Rotating Parallel Disk System
A rotating parallel disk system is used to make Couette type of flow. The fluid is sheared between a rotating disk and a stationary disk. The stationary disk is the bottom of the culture dish (diameter 60 mm, Iwaki, Japan) (Fig. 1a).

In the system, the shear rate (γ) is calculated by Eq. 1.

$$\gamma = r\,\omega / d \tag{1}$$

In Eq. 1, ω is the angular velocity [rad s^{-1}], and d is the distance [m] between the moving wall and the stationary wall (Fig. 1b). In the rotating parallel disk system, the shear rate (γ [s^{-1}]) increases in proportion to the distance (r [m]) from the rotating axis.

The shear rate varies with the rotating speed, which is controlled by the stepping motor, which makes variation of angular velocity ω between 7 rad/s and 33 rad/s. The position for the observation has the variation on r (the distance from the rotating axis) between 12 mm and 18 mm.

The distance d (0.5 mm < d < 0.6 mm) is estimated from the positions of the focus of the walls at the microscope. The shear rate (γ) generates the shear stress (τ [Pa]) in a viscous fluid.

$$\tau = \eta\,\gamma \tag{2}$$

In Eq. 2, η is the viscosity of the fluid [Pa s]. The fluid is the medium of cell culture in the present study. When the viscosity of the fluid η is 0.002 Pa s (at 310 K), the shear stress τ varies between 0.5 Pa and 2 Pa.

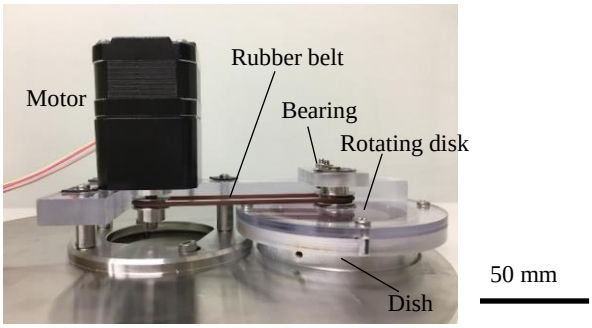

Fig. 1a: Cell culture device with rotating disk.

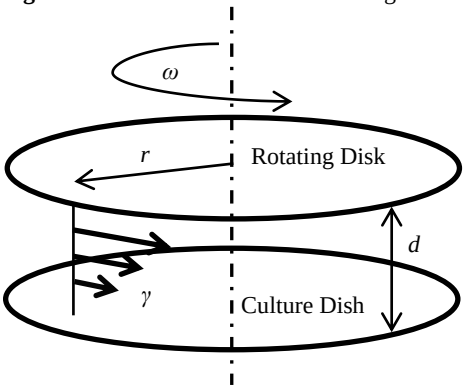

Fig. 1b: Couette flow between rotating disk and stationary disk (culture dish).

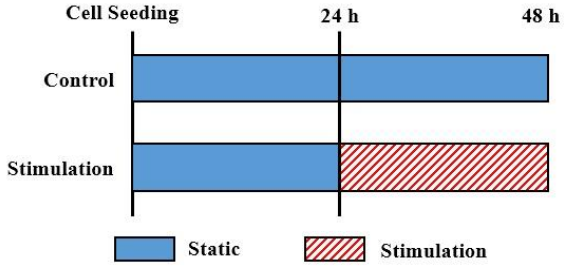

Fig. 2: Experimental protocol: resting (static), and flow (stimulation).

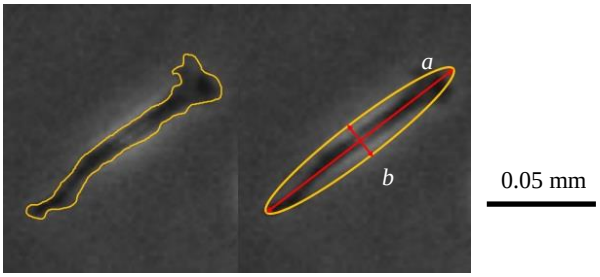

Fig. 3: Contour of cell (yellow, left) is approximated to ellipsoid (right), and major (*a*) and minor (*b*) axes are calculated (red).

The rotating disk system is mounted on the stage of the inverted phase contrast microscope mounted in the incubator (LCV-110SK, Olympus Co., Ltd., Tokyo). In the incubator, both the temperature and the partial pressure of carbon dioxide are maintained at 310 K and 5 percent, respectively. The behavior of cells adhered on the stationary wall under shear stress is observed with the microscope. The system allows observation of cells during exposure to the shear flow.

Cell Culture

Two kinds of cells were used in the experiment: MC3T3 (osteoblast precursor cell line derived from Mus musculus (mouse) calvaria; passage between fourth and ninth) -E1, and 3T3-L1 (mouse fat precursor cells, a cell line derived from cells of mouse 3T3; passage between third and ninth). D-MEM (Dulbecco's Modified Eagle Medium) containing 10% decomplemented FBS (Fetal Bovine Serum) and 1% penicillin/ streptomycin was used for the medium.

To make adhesion of cells to the bottom of the dish, the cells were cultured for 24 hours in the incubator without flow stimulation (without rotation of the disk). After the incubation for 24 hours, the cells were sheared with the rotating disk for 24 hours in the incubator (Fig. 2).

Microscopic Observation

The time-lapse image was taken every five minutes during the test. The direction of the flow adjacent to the culture plate was traced by the floating particle in the medium at the video image, and defined as the *x* axis in Figs. 5 & 6.

At the microscopic image, the contour of each cell was traced, and the apparent adhesion area (*S*) of each cell to the scaffold was calculated. The centroid of each cell image was used to trace the migration of the cell.

The contour of each cell was approximated to the ellipsoid (Fig. 3), and the shape index (*R*) was calculated by Eq. 3.

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

In Eq. 3, *b* is length of the minor axis, and *a* is length of the major axis of the ellipsoid. *R* is zero at the circle ($a = b$). *R* approaches to unity, when the major axis (*a*) increases as the elongation of the cell. At each cell, the angle (θ) between the major axis and the flow direction was measured. The angle (θ) is zero degree, when the major axis is parallel to the flow direction.

3. RESULTS

Fig. 4 exemplifies cells, which are adhered on the bottom surface of the culture dish, exposed to Couette type of the shear flow at the shear stress of 2.0 Pa: MC3T3-E1 (a), and 3T3-L1 (b). The arrow shows the direction of the flow. The left image shows cells at the beginning of exposure to the shear flow, and the right image shows cells after 24 hours exposure to the shear flow. The most of cells of 3T3-L1 are exfoliated after 24 hours of exposure to the shear flow, while the most of cells of MC3T3-E1 remains adherence on the plate.

Figs. 5 & 6 exemplify migration of twenty cells for 24 hours. In Figs. 5 & 6, the direction of the flow is from left to right along the *x* axis. The *y* axis shows the direction of the center of the rotation. The wall shear stress is proportional to the wall shear rate, so that the wall shear stress decreases with *y* cordinate. The original position of each cell is adjusted to the origin of the coordinate. To tarce the migration of each cell, the image of cell every one hour has been selected from the time lapse image. Each polygonal line, which consists of the

connected line segments, shows the locus of coordinates of the centroid of each cell traced every hour (Figs. 5 & 6). Without flow, each cell migrates on the culture plate (on the scaffold) to random direction (Figs. 5a & 6a). MC3T3-E1 migrates at random including to the perpendicular direction against the flow even at 2 Pa (Fig. 5). 3T3-L1, on the other hand, migrates to the downstream direction under the shear stress higher than 1 Pa (Figs. 6c-6e). Especially at 2 Pa, the larger migration of 3T3-L1 occurs to the downstream direction.

Fig. 7 exemplifies tracings of two parameters of a cell (3T3-L1) every hour: the area (Fig. 7a) and the migration (Fig 7b), simultaneously. When the area increases, the migration tends to decrease.

Fig. 8 shows the accumulated migration of each cell related to the shear stress: MC3T3-E1 (Fig. 8a), and 3T3-E1 (Fig. 8b). The mean value of accumulated migration of each cell is approximately 0.5 mm regardless of the shear stress condition (< 2 Pa). Only the value of accumulated migration of 3T3-L1 at 2 Pa is significantly higher.

Fig. 9 shows the area (S) of each cell related to the shear stress. Each datum shows the value, which is calculated as the mean value from data every hour for 24 hours (Figs. 9-11). The mean value is highest at 1.5 Pa at both kinds of cells: MC3T3-E1 (Fig. 9a), and 3T3-L1 (Fig. 9b).

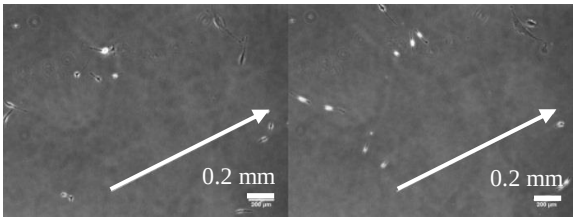

Fig. 4a: MC3T3-E1 at 2 Pa; 0 h (left), 24 h (right), flow direction (arrow), bar 0.2 mm.

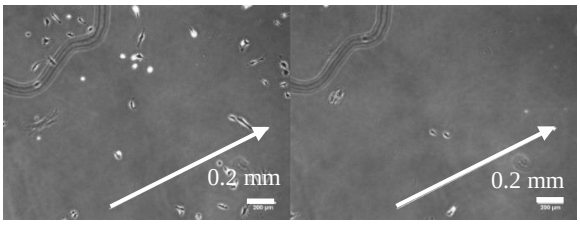

Fig. 4b: 3T3-L1 at 2 Pa; 0 h (left), 24 h (right), flow direction (arrow), bar 0.2 mm.

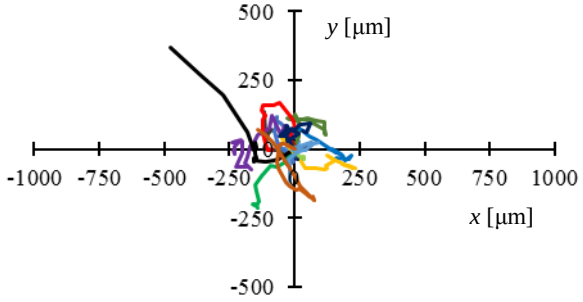

Fig. 5a: Migration of twenty cells (MC3T3-E1) without flow.

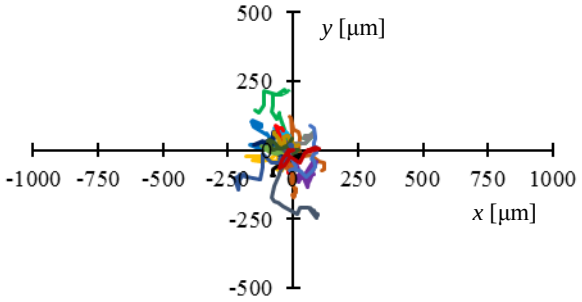

Fig. 5b: Migration of twenty cells (MC3T3-E1). (0.48 Pa < τ < 0.51 Pa)

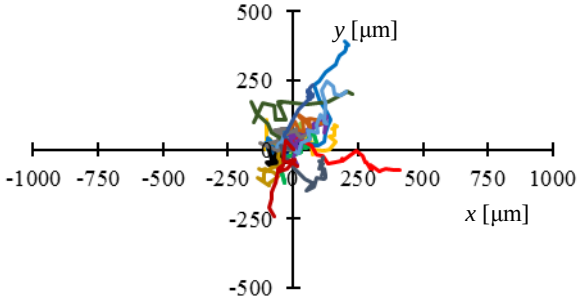

Fig. 5c: Migration of twenty cells (MC3T3-E1). (1.04 Pa < τ < 1.10 Pa)

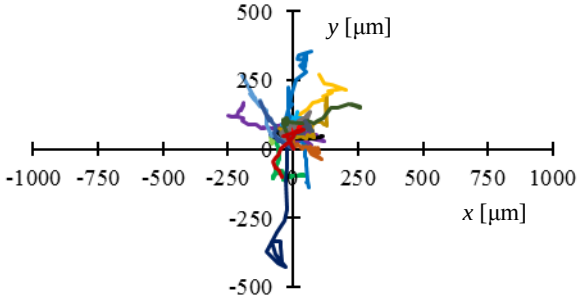

Fig. 5d: Migration of ten cells (MC3T3-E1). (1.41 Pa < τ < 1.58 Pa)

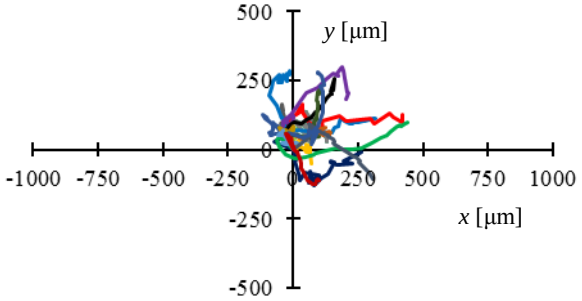

Fig. 5e: Migration of ten cells (MC3T3-E1). (1.92 Pa < τ < 2.07 Pa)

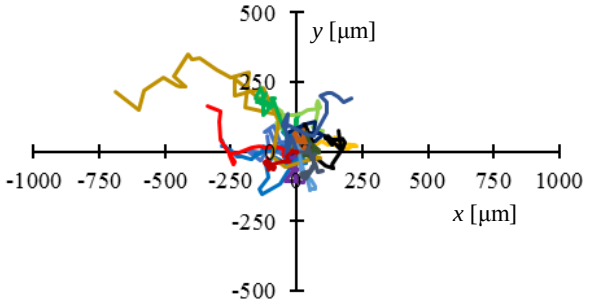

Fig. 6a: Migration of ten cells (3T3-L1) without flow.

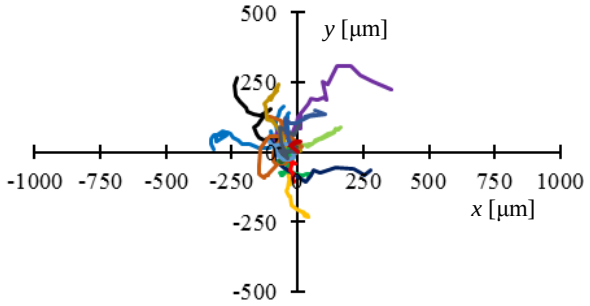

Fig. 6b: Migration of ten cells (3T3-L1). (0.48 Pa < τ < 0.52 Pa)

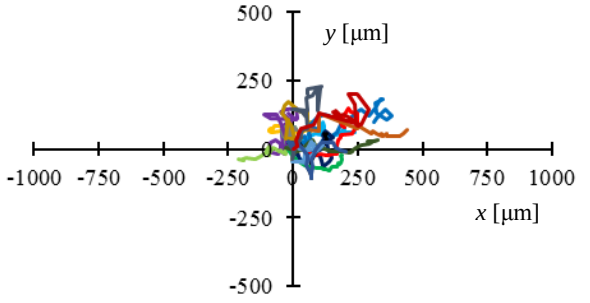

Fig. 6c: Migration of ten cells (3T3-L1). (0.97 Pa < τ < 1.03 Pa)

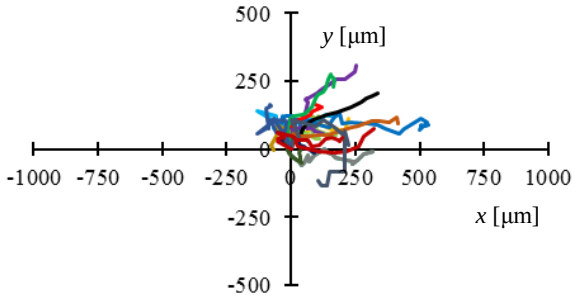

Fig. 6d: Migration of ten cells (3T3-L1). (1.42 Pa < τ < 1.57 Pa)

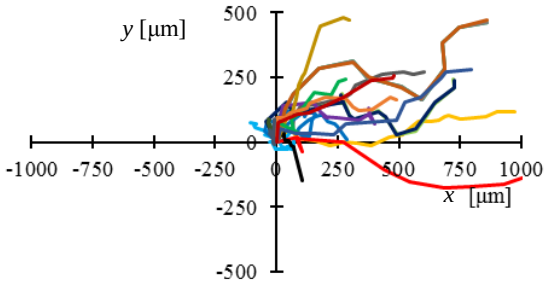

Fig. 6e: Migration of ten cells (3T3-L1). (1.93 Pa < τ < 2.10 Pa)

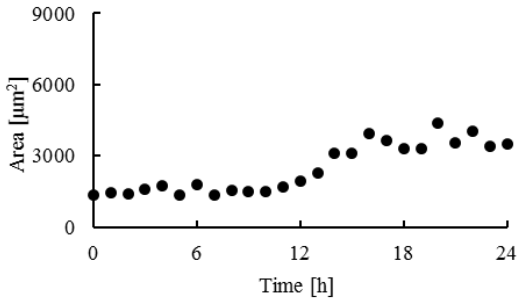

Fig. 7a: Area of 3T3- L1.

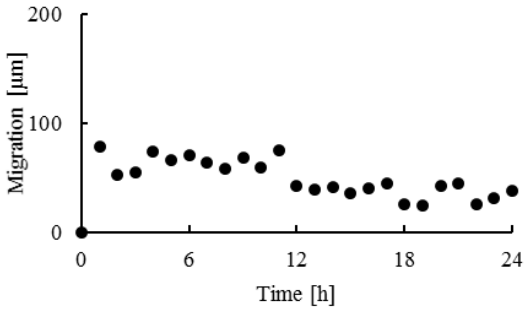

Fig. 7b: Migration of 3T3-L1.

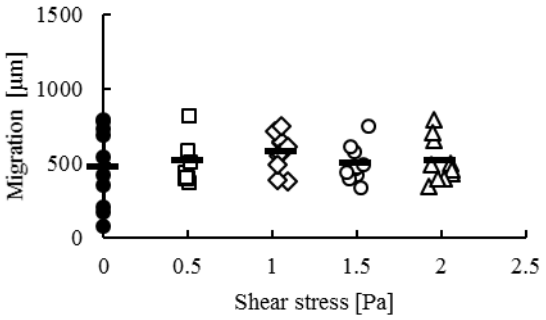

Fig. 8a: Accumulated migration of each cell (MC3T3-E1) related to shear stress: each bar shows mean value.

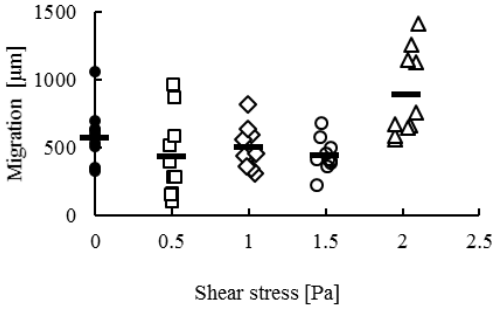

Fig. 8b: Accumulated migration of each cell (3T3-L1) related to shear stress: each bar shows mean value.

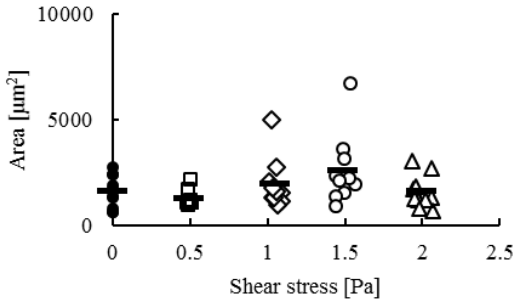

Fig. 9a: Area of each cell (MC3T3-E1) related to shear stress: each bar shows mean value.

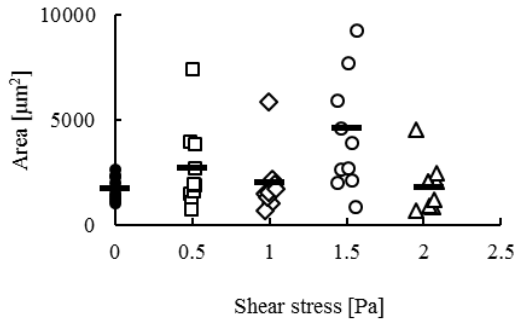

Fig. 9b: Area of each cell (3T3-L1) related to shear stress: each bar shows mean value..

Fig. 10 shows the shape index (R) of each cell related to the shear stress: MC3T3-E1 (Fig. 10a), and 3T3-E1 (Fig. 10b). The shape index of 3T3-L1 slightly decreases under the shear stress (Fig. 10b), which corresponds to get rounded.

Fig. 11 shows the angle (θ) between the major axis of each cell and the flow direction related to the shear stress: MC3T3-E1 (Fig. 10a), and 3T3-E1 (Fig. 10b). On MC3T3-E1, the dispersion of θ increases under shear flow, which shows that the major axis of MC3T3-E1 tilts to the random direction regardless of the flow direction. On 3T3-L1, on the other hand, the angle θ concentrates to zero degree, which shows that the major axis of 3T3-L1 tilts to the flow direction.

4. DISCUSSION

The Couette type of flow is convenient to estimate the shear stress in the flow with the uniform shear rate between the moving wall and the stationary wall, which is also available to non-Newtonian fluid [10-15]. Many kinds of the devices including pulsatile type flow [12, 13] were designed for quantitative experiments of biological fluid in the previous studies.

The "cone and plate type" device has the uniform shear field in the entire space between the rotating cone and the stationary plate [10, 11]. The clot formation was quantitatively studied between the rotating cone and the stationary plate [10], and between the rotating concave cone and the stationary convex cone [12]. The erythrocyte destruction was studied between the rotating concave cone and the stationary convex cone [13].

A parallel disks system does not have the uniform shear field in the entire space between rotating disk and the stationary disk. The parallel-disks system, on the other hand, has several advantages: stability of the rotating motion of the disk, stability of the optical path for the microscopic observation, morphologic preciseness of the plane of the disks, and simultaneous observation over the range of variation of shear rate proportional to the radius from the rotational axis [15]. The erythrocyte deformation was observed between counter rotating parallel discs [14]. In the present study, the rotating parallel disk system is selected to make Couette type of flow instead of the cone and plate system. The range of the shear stress lower than 2 Pa has been selected in the present study. That is a typical value for the wall shear stress of the blood vessels *in vivo* estimated by the vessel diameter and the flow rate in Poiseuille flow.

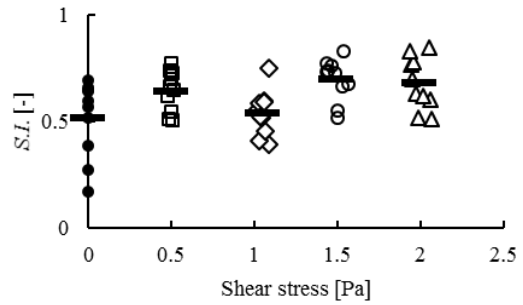

Fig. 10a: Shape index of each cell (MC3T3-E1) related to shear stress: each bar shows mean value.

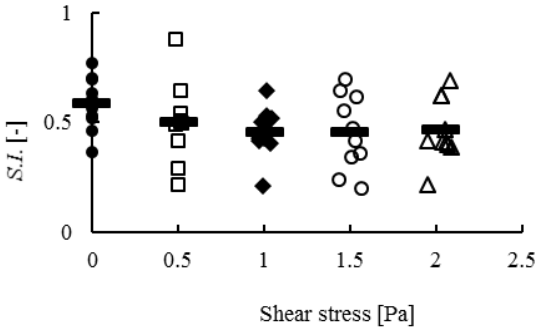

Fig. 10b: Shape index of each cell (3T3-L1) related to shear stress: each bar shows mean value.

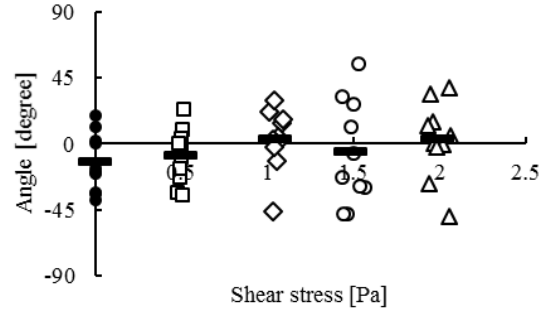

Fig. 11a: Angle of each cell (MC3T3-E1) related to shear stress: each bar shows mean value.

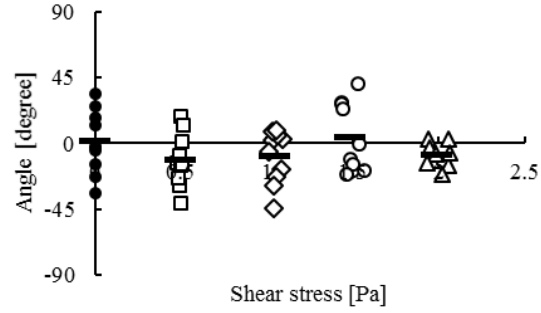

Fig. 11b: Angle of each cell (3T3-L1) related to shear stress: each bar shows mean value.

At the constant angular velocity of 31 rad s^{-1} (d = 0.5 mm), the shear rate (γ) increases from 0.73×10^3 s^{-1} to 1.12×10^3 s^{-1}, when the distance from the axis (r) increases from 12 mm to 18 mm in the observation area (Eq. 1). The gradient of shear stress (0.6 Pa / 0.006 m = 10^2 Pa m^{-1}) enables the simultaneous

observation of the behavior of cells related to variation of the shear stress (1.1 Pa < τ < 1.7 Pa) in the same view [15]. In the present study, the migration speed has been slower than 1 mm / 24 hour = 10^{-8} m/s. The steady actual flow direction adjacent to the scaffold surface of cell culture has been confirmed by the stream line traced by the direction of exfoliation of the cell and of the moving particle adjacent to the surface.

A biological cell shows passive and active behaviors in an environment. While the flow might enhance the cell migration to the downstream, a cell migrates to adapt the shear field. While the strong stimulation above the threshold damages the cell, the stimulation below the threshold remains in the cell as a memory for the response in the next step [1, 2]. The hysteresis effect governs the active behavior of the cell. The interaction between cells also governs the behaviou of each cell [16]. The seeding density is selected not so high to trace the image of each cell in the present study. The migration of the cell might also depend on the morphology and the mechanical property of the scaffold [17].

In the present study, each cell migrates independently to every direction includes the counter direction of the flow. The effect of shear flow on migration of the cell depends on the kind of cells, which might be applied to the cell sorting technology. In the present comparative study, MC3T3-E1 and 3T3-L1 are selected for the test to observe the response of the cell to the shear flow. The response of 3T3-L1 adhesion on the scaffold with the micro-pattern was different from that of the other types of cells in the previous study [18]. MC3T3-E1, on the other hand, is one of the typical adhesive cells, which are exposed to the mechanical force field *in vivo*.

5. CONCLUSION

Migration of a cultured cell exposed to Couette type of the shear flow has been observed at the incubator microscope *in vitro*. Migration might decrease, when affinity between the cell and the scaffold increases. The shear flow has been made between a rotating disk and a stationary disk, which are in parallel position each other. The migration of cells has been evaluated at the time lapse images for 24 hours. Two kinds of cells has been used in the test: MC3T3-E1 (mouse osteoblast precursor cell line), 3T3-L1 (mouse fat precursor cells). Under the shear stress of 2 Pa, 3T3-L1 tends to be rounded, to tilt to the flow direction, and to migrate downstream. MC3T3-E1, on the other hand, migrates to every direction.

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