

Effect of Wall Shear Stress in Flow on Myoblast

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ABSTRACT

An effect of wall shear stress on adhered myoblast has been studied *in vitro*. A flow channel was designed to observe the response of cells to the fluid shear stress under a microscope. A Polydimethylsiloxane disk with a concaved part is placed on the inner bottom of a culture dish to form a parallelepiped flow channel of 25 mm length \times 5 mm width \times 0.1 mm depth. The flow channel is placed in a chamber, where temperature and CO₂ content are kept 310 K and 5 percent, respectively. The chamber is placed on the stage of an inverted phase contrast microscope to observe the behavior of cells adhered on the disk under a flow. The shear stress on the wall is calculated with an estimated parabolic velocity profile between the parallel disks. After several cells adhered to the disk, the shear stress was applied on the cells in the medium flow with a syringe pump for 1 hour. The variation of the flow rate produces the wall shear stress between 0.1 Pa and 3 Pa. C2C12 (mouse myoblast cell line) was exposed to the wall shear stress with the methodology. The experimental results show that the myoblast deforms most frequently at the wall shear stress of 1 Pa.

Keywords: Biomedical Engineering, Cell Culture, Myoblast, Shear stress, Migration and Flow.

1. INTRODUCTION

Cells are exposed to mechanical stimulation *in vivo*. The shear stress is one of the mechanical stimulations. The endothelial cells are exposed to the shear stress in the blood flow at the wall of the blood vessels. The other cells are also exposed to the shear stress among the deformation of the tissue.

Cells adhere, migrate, deform, proliferate, and differentiate. The mechanical stress might affect the behavior of cells.

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 tissue *in vivo* or *in vitro* [1-5]. Control methodology for orientation and

proliferation of cells would be applied to the regenerative tissue technology.

A flow can be used to apply a stress field to a cell [6-15]. The cell directly receives the shear stress in the shear flow. The high shear flow might deform cell, peel cells off the scaffold, and inhibit proliferation. The mild shear flow, on the other hand, might accelerate the active deformation, migration, and proliferation.

In the present study, the effect of wall shear stress on adhered myoblast has been studied *in vitro*.

2. METHODS

Flow Channel

A flow channel was designed to observe the response of cells to the fluid shear stress under a microscope. The flow channel consists of a polystyrene dish of 35 mm diameter, a disk of polydimethylsiloxane (PDMS), three glass capillaries, and three polyvinylchloride tubes.

For PDMS disk, a die of carbon steel with a central rectangular convex part was machined by a flat-surface grinding machine (Fig. 1). The dimension of the convex part is as follows: 25 mm length, 5 mm width, 0.1 mm height. The dimension of the convex part was measured with a laser microscope (Fig. 2).

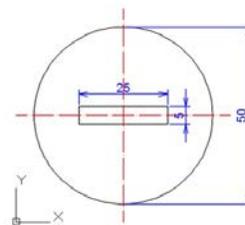


Fig. 1: Dimension (mm) of die with a central rectangular convex.

After the die was enclosed with a peripheral wall of polyimide, PDMS (Dow Corning Corporation, MI, USA) was poured with the curing agent on the die. PDMS was baked at 333 K for one hour in an oven. The PDMS disk was cut at diameter of 30 mm with the concaved center part. Three holes were punched at the ends of the concaved part of the PDMS disk. Two of them are for the inlet, and the other is for the outlet.

The PDMS disk, which has a concaved part at the center, was placed on the inner bottom of a culture dish to form a parallelepiped flow channel of 25 mm length \times 5 mm width \times 0.1 mm depth (Fig. 3). Three glass capillaries connected to the polyvinylchloride tube were inserted to the hole of the PDMS disk. The liquid of PDMS was pasted on the junction of elements, and baked at 333 K for 90 minutes in an oven (Fig. 4).



Fig. 2: Die of steel with a central rectangular convex.

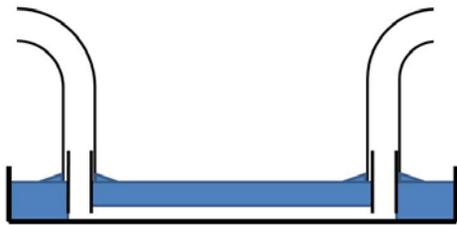


Fig. 3: Cross section of flow channel.

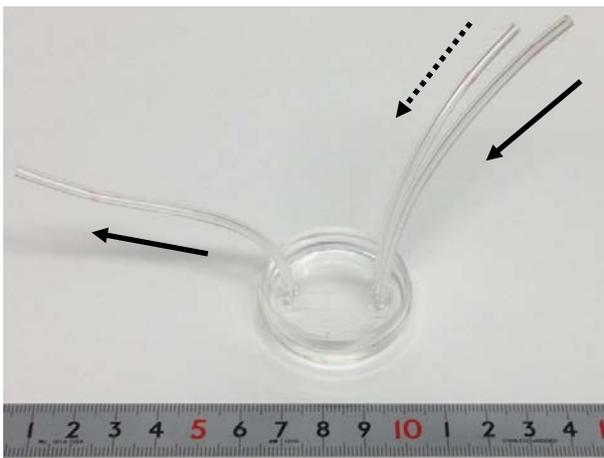


Fig. 4: Flow channel. Arrow shows flow direction.

The shear rate on the wall is calculated with an estimated parabolic velocity profile between the parallel walls [8]. The shear stress is the product of the viscosity of the medium and the wall shear rate. The shear rate (G , $[s^{-1}]$) on the wall of the plate is calculated by Eq. 1, in which a parabolic velocity profile between parallel plates is hypothesized.

$$G = 6q / (b D^2) \quad (1)$$

In Eq. 1, q is the flow rate [$m^3 s^{-1}$], b is the width of the canal [m] and D is distance [m] between two parallel walls. In the present study, D is 0.1 mm, and b is 5 mm.

The shear stress T [Pa] is the product of viscosity N [Pa s] of the fluid and the shear rate G [s^{-1}] of the flow (Eq. 2).

$$T = N G \quad (2)$$

The viscosity of the medium was measured with the cone and plate type of a viscometer (TVE-22L, Toki-Sangyo Co., Ltd. Tokyo) at 310 K.

Cell

C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse) of the passage between fourth and ninth 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 drawn into the flow channel through the first inlet tube by the syringe pump (dotted arrow in Fig. 4, Fig. 5), the suspension of C2C12 in D-MEM was introduced into the channel through the second inlet tube by the syringe pump (solid arrow in Fig. 4, Fig. 6).

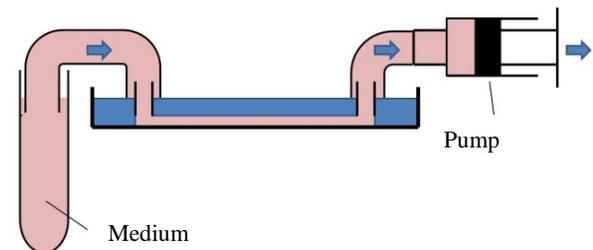


Fig. 5A: Channel is prefilled with medium.



Fig. 5B: Medium is introduced into channel with syringe pump.

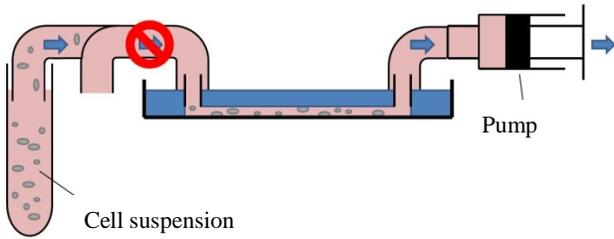


Fig. 6: Suspension of cells is introduced through another inlet into channel with syringe pump.

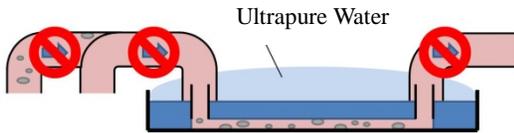


Fig. 7: Cells are incubated in channel.

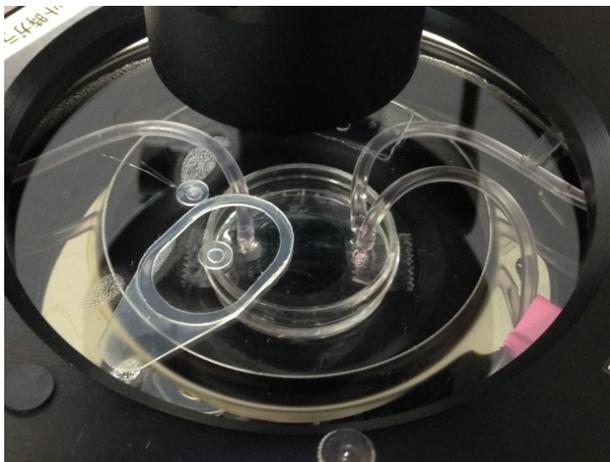


Fig. 8: Channel is placed in incubator on microscope.

After every tube was occluded (Fig. 7), the channel was placed in an incubator, where both the temperature of 310 K and the carbon dioxide partial pressure of 5 percent are maintained. The ultrapure water was poured over the dish to cover the chamber to avoid evaporation of medium.

After incubation without flow stimulation, the flow channel is move from the incubator to the microscope system.

The microscope system consists of a chamber of incubation, a microscope, and a camera (Fig. 8). The channel is placed in the chamber, where temperature and CO₂ content are kept 310 K and 5 percent, respectively. The chamber is placed on the stage of the inverted phase contrast microscope (IX71, Olympus, Tokyo) to observe the behavior of cells adhered on the dish under the flow.

After several cells adhered to the disk, the shear stress was applied on the cells in the medium flow pulled with the syringe pump for 1 hour (Fig. 9). Variation was made on the flow rate. The constant flow rate between 1.3 cm³ hour⁻¹ and 39 cm³ hour⁻¹ produces the constant wall shear stress between 0.1 Pa and 3 Pa.

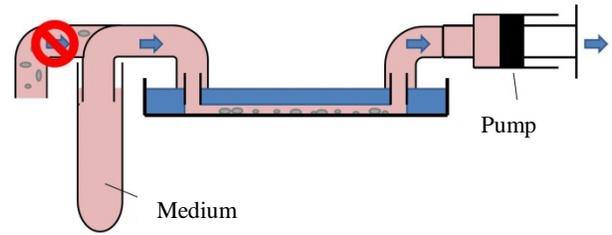


Fig. 9A: Flow stimulation on cells in channel.

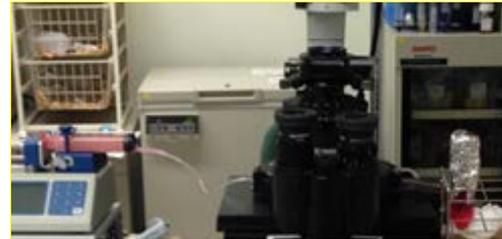


Fig. 9B: Flow stimulation on cells in channel placed in incubator on microscope.

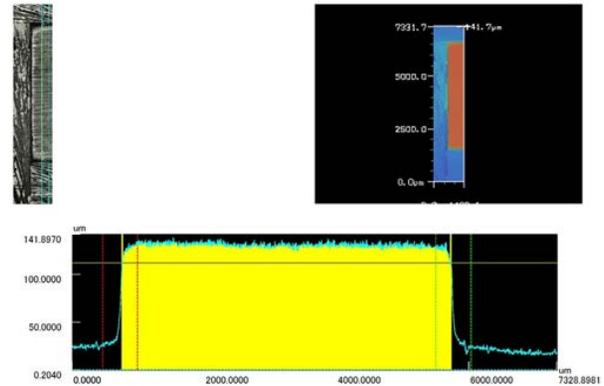


Fig. 10: Dimension of the convex part measured with a laser microscope.

The observation area was selected in the downstream side of the channel to apart from turbulent flow. To trace the same cell, the observation area was marked on the channel with the circle, and time-lapse pictures were taken with the constant interval of five minutes.

3. RESULTS

Fig. 10 shows the tracings of the surface morphology of the convex part of the machined die. The height of the convex part varies between 0.096 mm and 0.106 mm. The width varies between 4.96 mm and 5.00 mm. The length is 25.0 mm. Mean roughness is 0.0018 mm.

The viscosity of the medium measured with the cone and plate type of a viscometer was 0.002 Pa s at the shear rate of 40 s⁻¹.

Fig. 11 shows cells for one hour without flow, after incubation for 48 hours. A few cells migrate in short distance, and deform slightly for one hour.

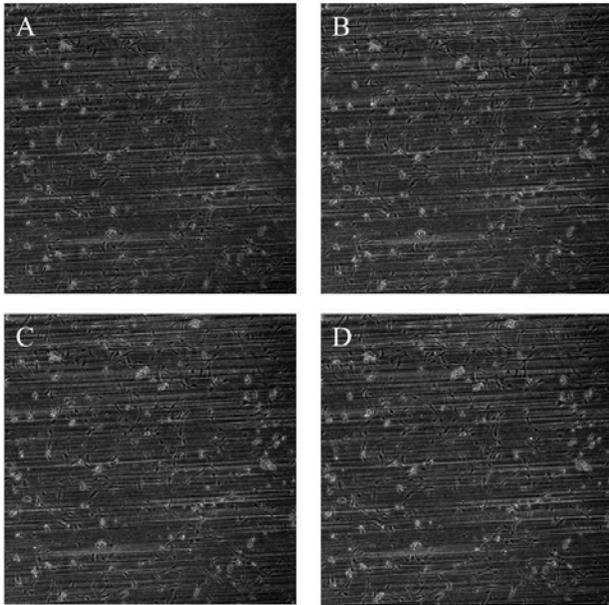


Fig. 11: Cells before (A), and after 20 minutes (B), 40 minutes (C), 60 minutes (D) of cultivation without flow. Dimension from left to right is 2 mm.

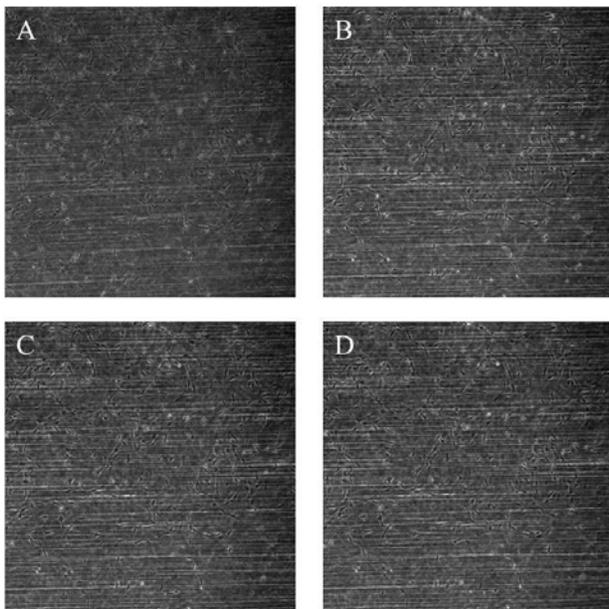


Fig. 12: Cells before (A), and after 20 minutes (B), 40 minutes (C), 60 minutes (D) of cultivation with flow (wall shear stress: 0.2 Pa). Direction of flow is from right to left. Dimension from left to right is 2 mm.

In the first flow test, cells were exposed to the wall shear stress of 0.1 Pa for one hour, after incubation for 48 hours. Most of cells keep the shape, and stay in the same position. One cell exfoliates within one hour of exposure to the flow.

Fig. 12 shows cells exposed to the wall shear stress of 0.2 Pa for one hour, after incubation for 48 hours. One cell extends the pseudo to the direction of the flow. One cell deforms, and extends perpendicular to the direction of the flow.

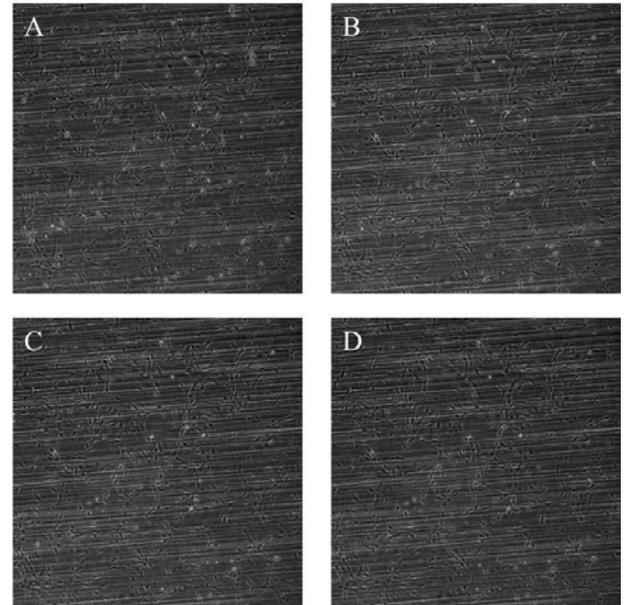


Fig. 13: Cells before (A), and after 20 minutes (B), 40 minutes (C), 60 minutes (D) of cultivation with flow (wall shear stress: 0.3 Pa). Direction of flow is from right to left. Dimension from left to right is 2 mm.

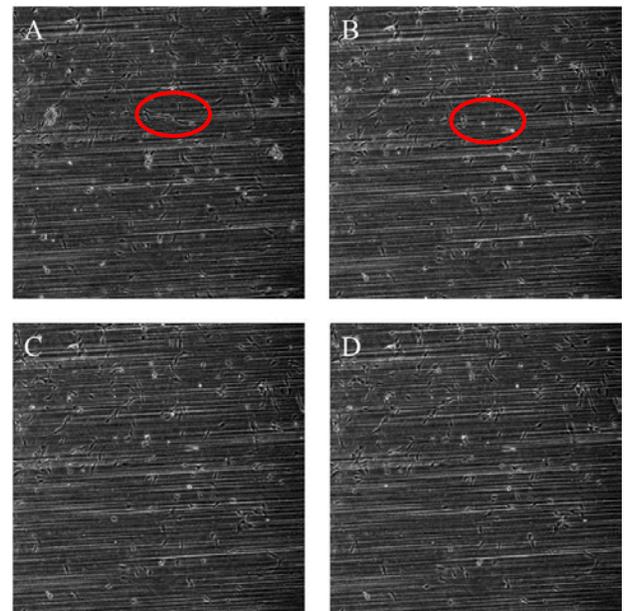


Fig. 14: Cells before (A), and after 20 minutes (B), 40 minutes (C), 60 minutes (D) of cultivation with flow (wall shear stress: 1 Pa). Direction of flow is from right to left. Dimension from left to right is 2 mm.

Fig. 13 shows cells exposed to the wall shear stress of 0.3 Pa for one hour, after incubation for 48 hours. One cell migrates and re-adheres. Several cells deform, and extend to the random direction regardless of that of flow.

Fig. 14 shows cells exposed to the wall shear stress of 1 Pa for one hour, after incubation for 24 hours. The number of

exfoliated cells is higher after incubation of 24 hours than after that of 48 hours. The cell, which extends the pseudo to the direction of flow, shortens the pseudo, and deforms to the sphere in one hour (in the circle in Fig.14). One cell migrates to the upstream direction of the flow.

Fig. 15 shows cells exposed to the wall shear stress of 3 Pa for one hour, after incubation for 24 hours. A lot of cells keep adhesion to the wall. The number of cells of deformation is lower at 3 Pa than that at 1 Pa.

The myoblast (C2C12) deforms regardless of the direction of the flow at the wall shear stress lower than 0.2 Pa. The myoblast deforms to the direction of flow at the wall shear stress higher than 0.3 Pa. The myoblast, which extends the pseudo in parallel to the direction of flow, shortens the pseudo, and deforms to the sphere at the wall shear stress of 1 Pa in one hour. More than 90 percent of myoblasts keep their adhesion, and keep their shape at the wall shear stress of 3 Pa. Some cells proliferate at the wall shear stress of 3 Pa (in the circle in Fig. 15). The cell does not exfoliate during proliferation.

4. DISCUSSION

The flow channel system of the parallel disks has been used in the previous experiments to study the effect of the shear stress on the cells [8, 9]. Although the sandwiched elastic film between the disks is convenient to avoid air leakage, the shape of the film may make the geometric fluctuation. The die with a central rectangular convex part was machined for PDMS disk in the present study. The geometric accuracy of the wall of channel improves the laminar flow between the parallel walls.

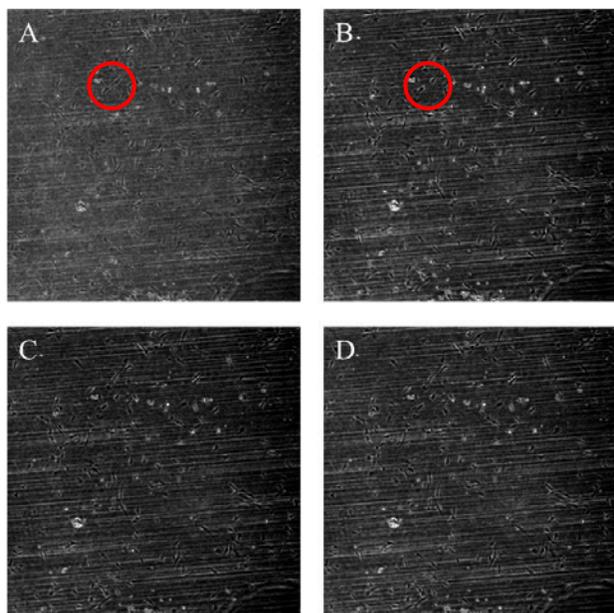


Fig. 15: Cells before (A), and after 20 minutes (B), 40 minutes (C), 60 minutes (D) of cultivation with flow (wall shear stress: 3 Pa). Direction of flow is from right to left. Dimension from left to right is 2 mm.

It is not easy to estimate the shear stress value on the wall, when the medium has the free surface [6, 7]. The parallelepiped chamber is convenient to observe the response of cells under controlled shear stress [8, 9].

To apply mechanical stimulation to the cells, centrifugal force [3] or shear flow [10] is useful.

Both acceleration of proliferation and orientation of cells are important targets in the research field of regenerative medicine on the cultured biological tissue. The behavior of biological cells depends on electric [1] and magnetic [2] fields.

The previous studies show that a mechanical field, on the other hand, governs behavior of cells. The shear flow governs the orientation of endothelial cells [6, 13].

Too strong mechanical stimulation damages cells. The moderate mechanical stimulation, on the other hand, might accelerate differentiation of cells [3]. The mechanical stress exfoliates several cells, which makes vacancy around the adhesive cell [14].

The effect of shear flow on orientation of cells depends on the kinds of cells [6]. Although HUBEC orients along the stream lines, C2C12 tilts from the stream lines to make myotubes. The previous study shows orientation of cells perpendicular to the stretch direction [5].

The cells might deform to minimize the internal stress [5]. Myoblast, of which the longitudinal axis is parallel to the flow direction, deforms to be rounded in the present experiment.

The permeability of the wall of the flow path and the temperature difference may form bubbles. The contamination of bubbles disturbs the uniform shear flow.

The short incubation time might reduce the affinity between the cell and the scaffold so that exfoliation of cells increases under the shear flow.

Although the adhesive force of a cell to a scaffold might decrease during proliferation, the myoblast (C2C12) is able to keep adhesion during proliferation at the wall shear stress of 3 Pa. The experimental results show that deformation of C2C12 occurs most frequently at the wall shear stress of 1 Pa. The deformation of a myoblast depends not only on dimension of the shear stress, but also on exposure time to the shear stress. C2C12 may form orientation at the wall shear stress of 1 Pa for several hours.

5. CONCLUSION

The effect of the wall shear stress on the adhered myoblast has been studied *in vitro*. A flow channel was designed to observe the response of cells to the fluid shear stress under observation with a microscope. The experimental results show that the myoblast deforms most frequently at the wall shear stress of 1 Pa.

6. ACKNOWLEDGMENT

This work was supported by a Grant-in-Aid for Strategic

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