Effect of Culture Medium Flow on Orientation of Muscle Cells

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ABSTRACT

The effect of slope and flow on orientation of cultured muscle cells has been studied *in vitro*. A flow chamber was designed to observe behavior of adhered cells on a tilted plane in a culture medium flow under a microscope. A thin sheet of silicone rubber of 0.1 mm thick, which has a rectangular open space, was sandwiched by two plates of transparent glass to form a rectangular channel of 2 mm width × 52 mm length × 0.1 mm depth. The chamber was tilted to make a slope of 0.17 rad perpendicular to longitudinal flow direction, and placed on an inverted microscope. C2C12 (Mouse myoblast cell line) cells are suspended in the Dulbecco's Modified Eagle's Medium, and introduced to the chamber via a tube of 2 mm internal diameter with a syringe pump. After several cells adhered to the glass plate, the medium steady flow between 3 and 10 mL/hour was applied on the cells with the syringe pump, and behavior of adhered cells in the flow is observed. The flow generates wall shear stress between 0.3 and 0.8 Pa. The experimental results show that cells tend to tilt to the direction of downstream and downward.

Keywords: Biomedical Engineering, Muscle Cells, Cell Culture, Flow, Slope, Adhesion and Orientation

1. INTRODUCTION

Behavior of biological cells depends on various environmental factors, such as electric, magnetic and mechanical fields [1, 2].

Cell culture technique has been progressed and myoblasts have been clinically applied to ischaemic cardiomyopathy in the field of regenerative medicine. Acceleration technique for orientation and proliferation of cells has been studied to make muscle tissue *in vivo* and *in vitro* [3-5]. Control methodology for adhesion and proliferation of cells would be applied to regenerative tissue technology.

In the present study, the effect of slope and flow on orientation of cultured muscle cells has been studied *in vitro*.

2. METHODS

Culture Medium Flow System

A one-way flow system was designed to observe an effect of slope and flow on cells adhered to a plate. The system consists of a flow chamber, a syringe pump, tubes and a microscope (Fig. 1). TE-331S (Terumo Co., Ltd. Tokyo) was used for the syringe pump. The plastic tube of 2 mm internal diameter and of 3 mm external diameter was used for the flow channel.

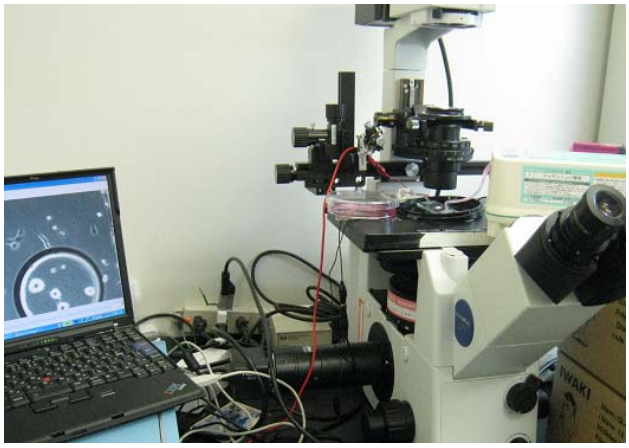

Fig. 1: Experimental system.

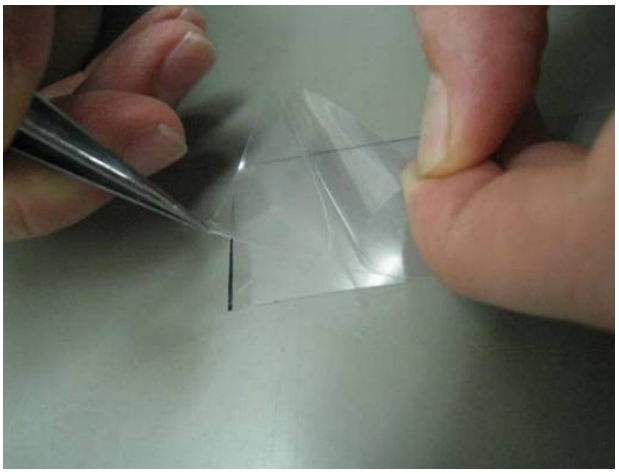

Fig. 2: Silicone sheet.

The flow chamber consists of two transparent glass plates and a thin silicone rubber sheet (Fig. 2). The dimension of two glass plates is 76 mm length, 26 mm width and 1.5 mm thick, each. A rectangular open space of 2 mm × 52 mm is cut off in a thin sheet of silicone rubber of 0.1 mm thick, and sandwiched between supporting plastic plates (Fig. 3). After being exfoliated from the supporting plastic plates, the sheet is sandwiched between two plates of glass to form a rectangular channel of 2 mm width × 52 mm length × 0.1 mm depth. The

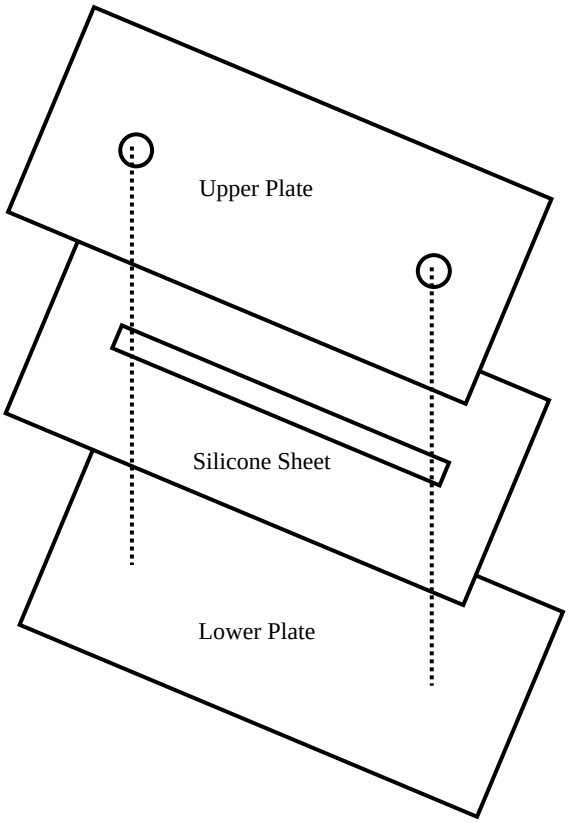

Fig. 3: Flow chamber of three plates; upper plates of glass, silicone sheet, lower plates of glass.

Fig. 4: Flow chamber.

three plates stick together without bond because of their surface affinity. At the upper glass plate, two holes of 2.5 mm diameter is machined by a grinder, and adhered to the plastic tube by a bond of polyurethane resin (Fig. 4).

The tube is connected to the plastic syringe pump. The chamber is placed on the tilted plate to make slope of 0.17 rad perpendicular to longitudinal flow direction (Fig. 5). The tilted plate is heated at 37 degrees Celsius to maintain temperature of the medium. The chamber is placed on the

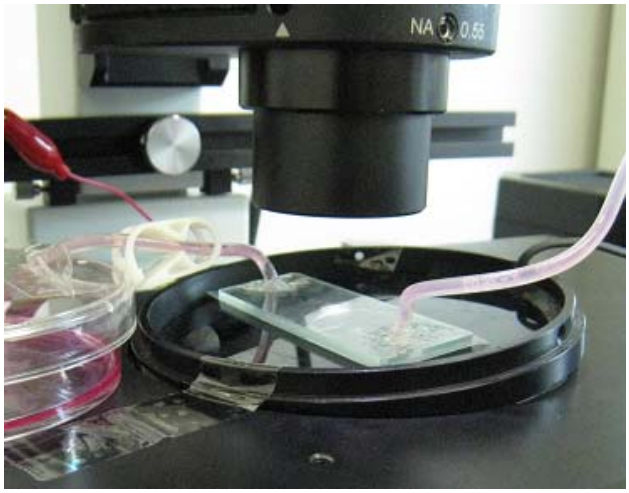

Fig. 5: Chamber placed on the tilted heating plate.

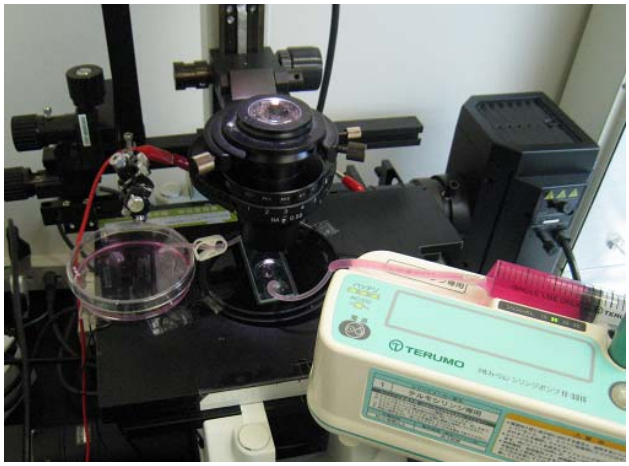

Fig. 6: Flow test with syringe pump and with microscope.

inverted phase-contrast microscope (IX71, Olympus Co., Ltd., Tokyo).

Cell Culture

C2C12 (Mouse myoblast cell line originated with cross-striated muscle of C3H mouse) were cultured on a dish with the Dulbecco's Modified Eagle's Medium (D-MEM) in an incubator for one week. Then, Cells were exfoliated from the plate of the culture dish with trypsin, and suspended in the D-MEM. The suspension was introduced to the chamber and cultured for 18 hours to make cells adhere to the glass plate of the chamber.

Flow Test

The constant flow of the medium was applied to adhered cells with the syringe pump (Fig. 6). The behavior of cells on the plate of the chamber was observed with the microscope. The photo of cells was taken every 15 minutes during the flow test for 12 hours. Variation was made in flow rate between 3 and 10 mL/hour.

Shear Rate at Wall

The shear rate (G) at the glass plate is calculated by Eq. 1, assuming a parabolic velocity profile between parallel plates.

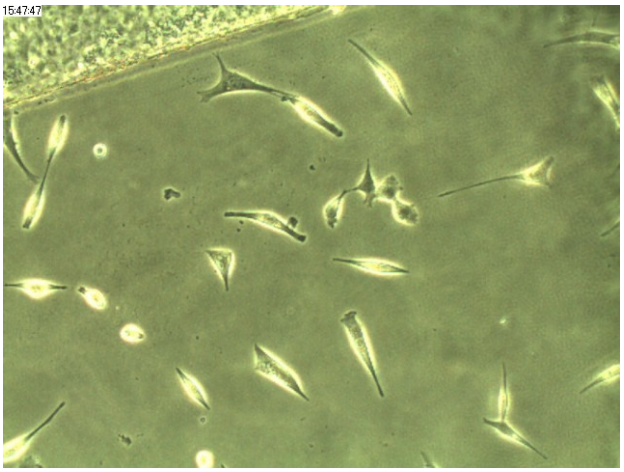

Fig. 7: Adhered cells after 18 hours.

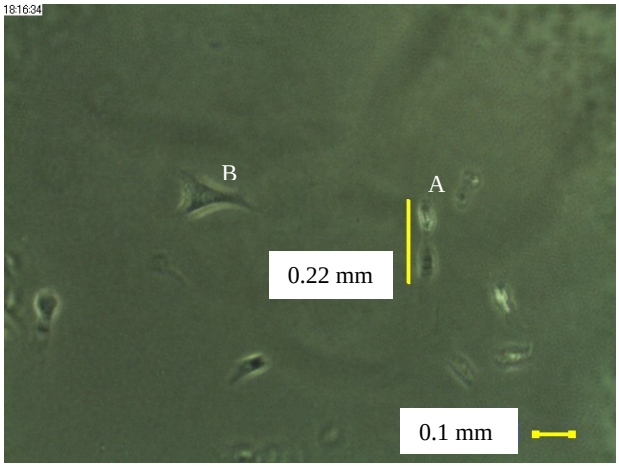

Fig. 8(a): Cells under flow of 4 mL/hour. Distance between left to right is 2 mm in Figs. 8(a)-(c).

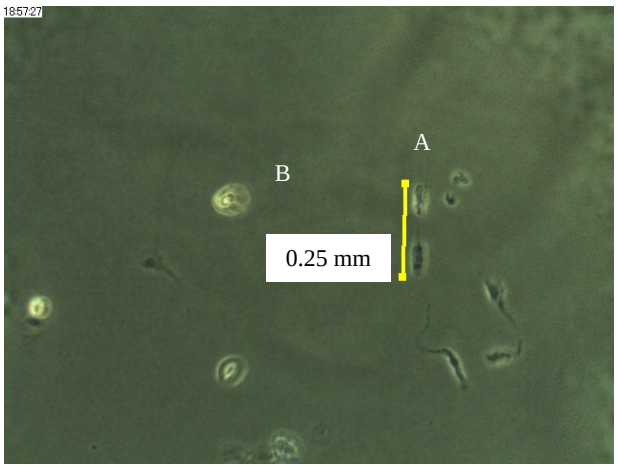

Fig. 8(b): Cells under flow of 4 mL/hour at 30 minutes after Fig. 8(a).

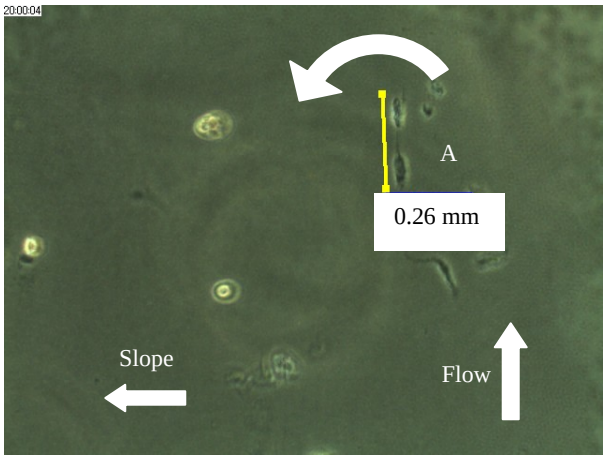

Fig. 8(c): Cells under flow of 4 mL/hour at 60 minutes after Fig. 8(a).

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

In Eq. 1, q is flow rate, b is width of the cannel (2 mm) and D is distance (0.1 mm) between two parallel walls.

The shear stress is product of viscosity (N) of the fluid and the shear rate (G) of flow by Eq. 2.

$$T = N\,G \qquad (2)$$

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

3. RESULTS

The result of measurement with the viscometer shows that the viscosity of the medium is 0.001 Pa s at 37 degrees Celsius at the shear rate of 600 sec^{-1}. The calculated shear rate at the glass wall of the flow chamber by Eq. 1 varies between 250 and 830 sec^{-1}, when the flow rate varies between 3 and 10 mL/hour. The calculated shear stress, thus, varies between 0.3 and 0.8 Pa for viscosity of 0.001 Pa s, when the shear rate varies between 250 and 830 sec^{-1}.

Cells adhered to the plate of the chamber in 18 hours without medium flow (Fig. 7). Under steady flow of 10 mL/hour, cells were entirely exfoliated in five minutes. The calculated wall shear rate and wall shear stress are 830 sec^{-1} and 1.7 Pa, respectively.

Fig. 8 exemplifies cells under flow of 4 mL/hour. The medium flows bottom up in Fig. 8. The downward direction is right to left in Fig. 8. The marked cell “A” in Fig. 8 elongates from 0.22 mm to 0.25 mm in thirty minutes under flow of 4 mL/hour, which generate a wall shear stress of 0.4 Pa estimated by Eqs. 1 & 2. The cell “A” starts to tilt to downward in Fig. 8(c).

Several movements occur on adhered cells in the flow: deformation, tilting to downstream, tilting to downward (Fig. 8

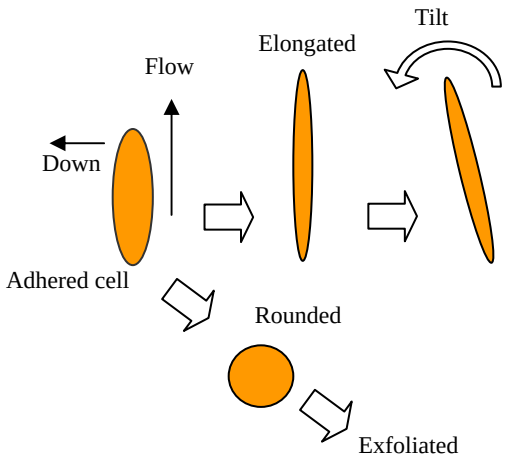

Fig. 9: Cells under flow of 4 mL/hour at 60 minutes after Fig. 8(a).

(c)), elongation along the streamline, deformation to be rounded, exfoliation, rolling to downstream (Fig. 9). Part of the cell "B" in Fig. 8(a) is exfoliated and rounded in Fig. (b).

4. DISCUSSION

Acceleration of proliferation and orientation of cells are important tasks in the research field of regenerative medicine to culture biological tissue. The previous study shows that electric stimulation enhances differentiation of muscle cells [3]. Another study shows mechanical stimulation improves tissue-engineered human skeletal muscle [6]. Another previous study shows that muscle cells can adhere and proliferate under electric stimulation with periodical pulses, and that adhesion of muscle cells can be controlled with the amplitude of pulse [7].

Bioreactors have been developed to control the environment around the cultured cell [8-10].

The previous studies show that mechanical field, on the other hand, affects on cells' behavior. Erythrocytes are very flexible, and are rolled and deformed in the shear flow [11]. The shear flow also affects on the orientation of endothelial cells [12]. The shear stress affects on the orientation of smooth muscle cells in the biological tissue [13]. The direction of mechanical field affects on fibroblast [14].

The previous study shows that the micro-grooves affects on the orientation of cell [15].

The effect of slope has been examined in the present experiment, because a tilted rotating disk was used for another study of cell culture environment [1]. The present study shows that the shear flow and slope affects on behavior of cells adhered to the plate. The combined effect of flow and slope on each cell might govern the orientation of cells in the vortex flow on the tilted rotating disk.

5. CONCLUSION

The effect of slope and flow on orientation of cultured muscle cells has been studied *in vitro*. The experimental results show that cells tend to elongate along the streamline and tilt to the direction of downstream and downward.

6. ACKNOWLEDGMENT

This work was supported by a Grant-in-Aid for Academic Frontier from the Japanese Ministry of Education, Culture, Sports and Technology.

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