Effect of Culture Medium Flow on Orientation of Muscle Cells

Saori OKUDA, Shigehiro HASHIMOTO, Kohei ONO, Masahide OKADA,
Shuichi MOCHIZUKI, Toshia FUJISATO, Hiroshi NAKAOKA, Masahiko YOSHIURA
Department of Biomedical Engineering, Osaka Institute of Technology, Osaka, 535-8585, Japan
hashimoto@bme.oit.ac.jp http://www.oit.ac.jp/bme/~hashimoto

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 0.5 and 1.7 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.

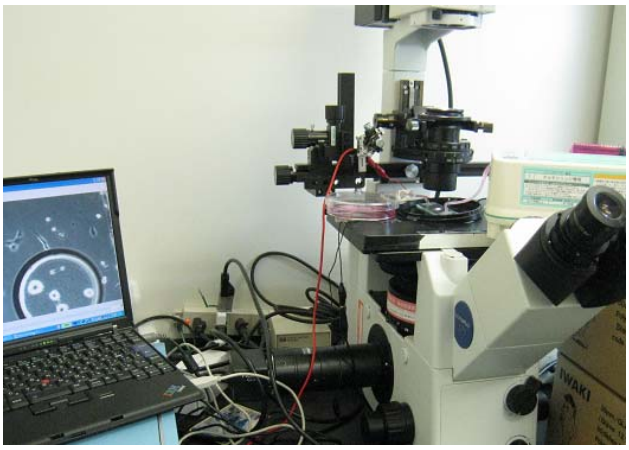

Fig. 1: Experimental system.

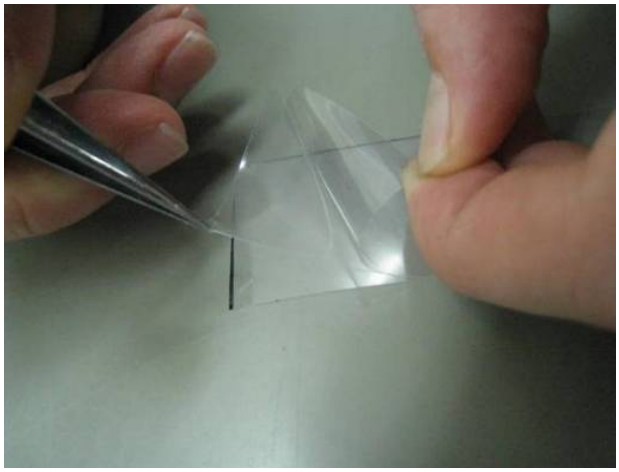

Fig. 2: Silicone sheet.

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 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

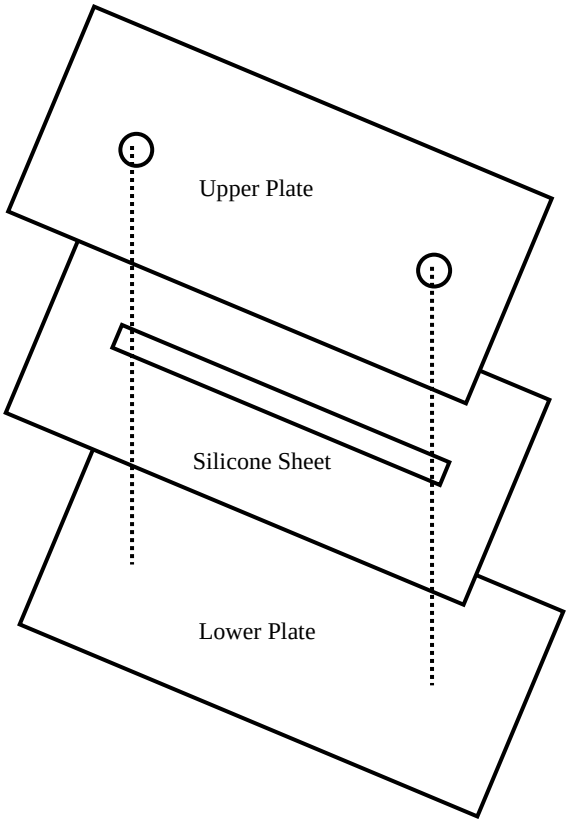

Fig. 3: Flow chamber of three plates; upper plates of glass, silicone sheet, lower plates of glass.

Fig. 4: Flow chamber.

plate is heated at 37 degrees Celsius to maintain temperature of the medium. The chamber is placed on the 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

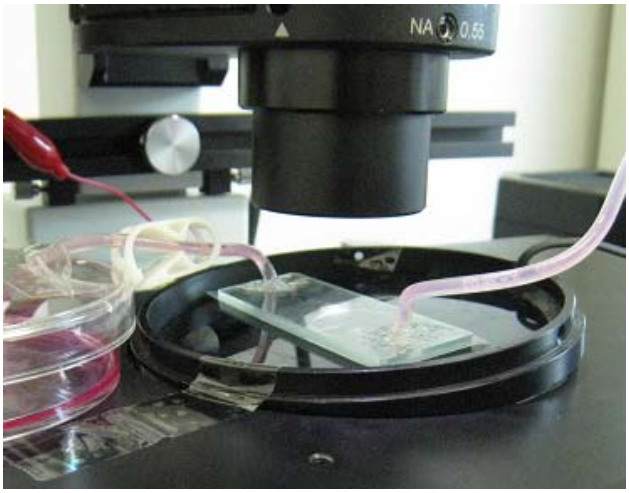

Fig. 5: Chamber placed on the tilted heating plate.

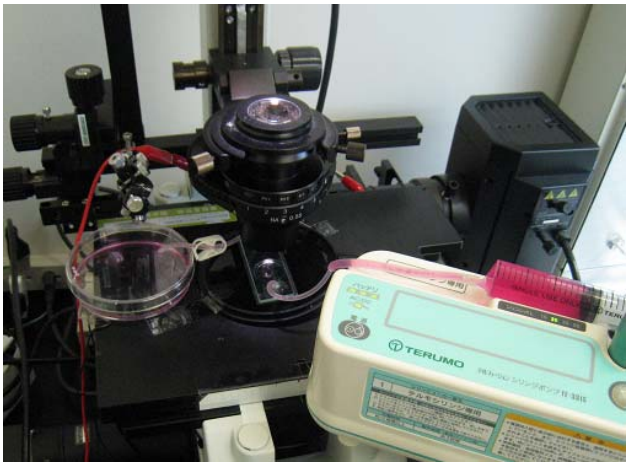

Fig. 6: Flow test with syringe pump and with microscope.

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.

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

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 shows that the viscosity of the medium is 0.002 Pa s at 37 degrees Celsius. The calculated

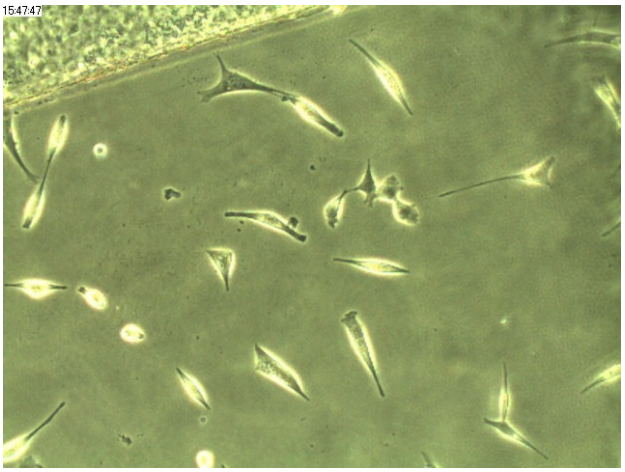

Fig. 7: Adhered cells after 18 hours.

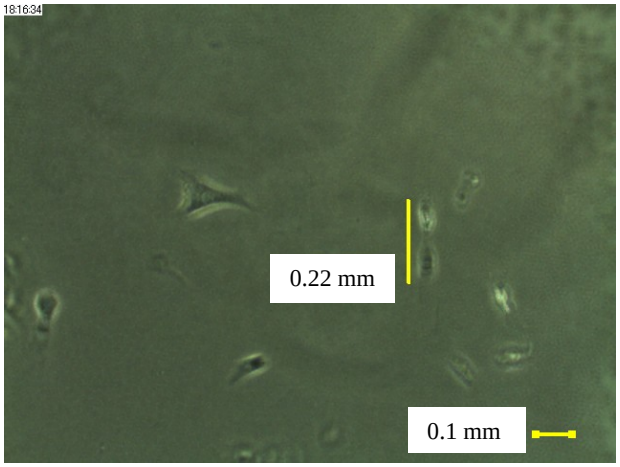

Fig. 8(a): Cells under flow of 4 mL/hour.

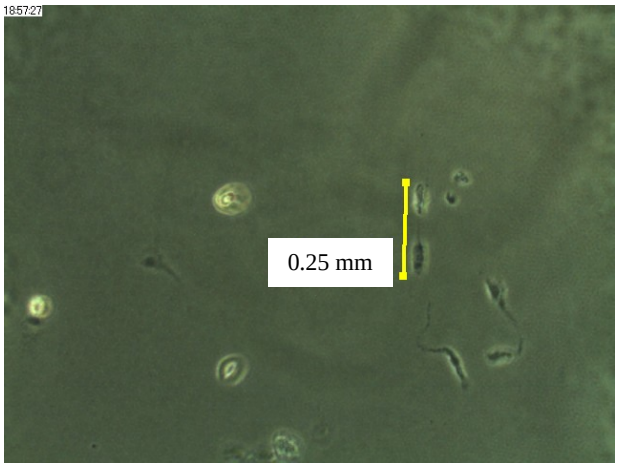

Fig. 8(b): Cells under flow of 4 mL/hour at 30 minutes after Fig. 8(a).

shear rate 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 0.5 and 1.7 Pa for viscosity of 0.002 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 marked cell 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 0.7 Pa estimated by Eqs. 1 & 2. Several movements occur on adhered cells in the flow: deformation, tilting to downstream, tilting to downward, elongation along the streamline, deformation to be rounded, exfoliation, rolling to downstream.

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.

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 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.

REFERENCES

[1] K. Yamasaki, S. Hashimoto, M. Okada, K. Ono, T. Fujisato, S. Mochizuki, M. Yoshiura, H. Tsutsui, K. Akazawa, "Design of Environment for Arrangement of Cultured Muscle Cells", **Proc. 12th World Multiconference on Systemics Cybernetics and Informatics**, Vol. 2, 2008, pp.130-134.

[2] S. Hashimoto, S. Mochizuki, Y. Morita, H. Tsutsui, M. Yoshiura, K. Akazawa, M. Ohsuga, S. Uto, H. Otani and T. Fujisato, "Environmental Design for Muscle Cell Culture

with Magnetic Field", **Proc. 2007 Inaugural IEEE International Conference on Digital Ecosystems and Technologies (IEEE-DEST 2007)**, 2007, pp. 468-472.

[3] Y. Kawahara, K. Yamaoka, M. Iwata, M. Fujimura, T. Kajiume, T. Magaki, M. Takeda, T. Ide, K. Kataoka, M. Asashima and L. Yuge, "Novel Electrical Stimulation Sets the Cultured Myoblast Contractile Function to 'on'", **Pathobiology**, Vol. 73, 2006, pp. 288-294.

[4] J. Stern-Straeter, A.D. Bach, L. Stangenberg, V.T. Foerster, R.E. Horch, et al., "Impact of Electrical Stimulation on Three-dimensional Myoblast Cultures- A Real-time RT-PCR Study", **Journal of Cellular and Molecular Medicine**, Vol. 9, No. 4, 2005, pp. 883-892.

[5] F. H. Andrade, M. B. Reid, D. G. Allen and H. Westerblad, "Effect of Hydrogen Peroxide and Dithiothreitol on Contractile Function of Single Skeletal Muscle Fibers from the Mouse", **Journal of Physiology**, Vol. 509.2, 1998, pp. 565-575.

[6] C. A. Powell, B. L. Smiley, J. Mills and H. H. Vandenburgh, "Mechanical Stimulation Improves Tissue-Engineered Human Skeletal Muscle", **American Journal of Physiology: Cell Physiology**, Vol. 283, 2001, pp. C1557-C1565.

[7] E. Yamada, S. Hashimoto, K. Tachibana, M. Okada, K. Yamasaki, H. Kondo, K. Imoto, S. Mochizuki, T. Fujisato, M. Ohsuga and H. Otani, "Effect of Electric Stimulation on Adhesion and Proliferation of Cultured Muscle Cells", **Proc. 12th World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 2, 2008, pp. 124-129.

[8] T. Sun, D. Norton, J. W. Haycock, A. J. Ryan and S. MacNeil, "Development of a Closed Bioreactor System for Culture of Tissue-Engineered Skin at an Air-Liquid Interface", **Tissue Engineering**, Vol. 11, No. 11/12, 2005, pp. 1824-1831.

[9] E. Cimetta, M. Flaibani, M. Mella, E. Serena, L. Boldrin, P. De Coppi and N. Elvassore, "Enhancement of Viability of Muscle Precursor Cells on 3D Scaffold in a Perfusion Bioreactor", **The International Journal of Artificial Organs**, Vol. 30(5), 2007, pp. 415-428.

[10] T. Sun, D. Norton, N. Vickers, S. L. McArthur, S. MacNeil, A. J. Ryan and J. W. Haycock, "Development of a Bioreactor for Evaluating Novel Nerve Conduits", **Biotechnology and Bioengineering**, Vol. 99, No. 5, 2007, pp. 1250-1260.

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

[12] M. Toda, K. Yamamoto, N. Shimizu, S. Obi, S. Kumagaya, T. Igarashi, A. Kamiya and J. Ando, "Differential Gene Responses in Endothelial Cells Exposed to a Combination of Shear Stress and Cyclic Stretch", **Journal of Biotechnology**, Vol. 133, No. 2, 2008, pp. 239-244.

[13] K. Nagayama, T. Matsumoto, "Mechanical Anisotropy of Rat Aortic Smooth Muscle Cells Decreases with Their Contraction (Possible Effect of Actin Filament Orientation)", **JSME International Journal**, Series C, Vol. 47, No. 4, 2004, pp. 985-991.

[14] J.H.-C. Wang, G. Yang, Z. Li and W. Shen, "Fibroblast Responses to Cyclic Mechanical Stretching depend on Cell Orientation to the Stretching Direction", **Journal of Biomechanics**, Vol. 37, 2004, pp. 573-576.

[15] E.T. den Braber, J.E. de Ruijter, H.T.J. Smits, L.A. Ginsel, A.F. von Recum and J.A. Jansen, "Quantitative Analysis of Cell Proliferation and Orientation on Substrata with Uniform Parallel Surface Micro-grooves", **Biomaterials**, Vol. 17 No. 11, 1996, pp. 1093-1099.