Responses of Cells to Fluid Shear Stress in Vitro

Fumihiko SATO, Shigehiro HASHIMOTO

**Biomedical Engineering, Department of Mechanical Engineering,
Kogakuin University, Tokyo, 163-8677, Japan
shashimoto@cc.kogakuin.ac.jp http://www.mech.kogakuin.ac.jp/labs/bio/**

and

Kei OYA

**Research Institute for Science and Technology,
Kogakuin University, Tokyo, Japan**

and

Hiromichi FUJIE

**Human Mechatronic Systems, Faculty of System Design,
Tokyo Metropolitan University, Tokyo, Japan**

ABSTRACT

Responses of cells to a fluid shear stress have been studied *in vitro*. A rhombus flow chamber was designed to observe the response of cells to the fluid shear stress under a microscope. A thin sheet of silicone rubber was sandwiched by two transparent polydimethylsiloxane disks to form a parallelepiped flow channel of 20 mm length × 0.1 mm depth. Variation was made on the width of the channel (from 1 mm to 3 mm) with a rhombus shape to vary the wall shear stress. Behavior of cells on the disk was observed under a flow with an inverted phase contrast microscope. 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. The constant flow rate of 18 mL/hour produces the wall shear stress between 1 Pa and 3 Pa. L929 (fibroblast-like, mouse connective tissue) was examined with the methodology. The experimental results show that the response of cells can be observed under stimulation by the fluid at the controlled wall shear stress in the flow chamber.

Keywords: Biomedical Engineering, L929, Cell Culture, Polydimethylsiloxane and Shear Stress.

1. INTRODUCTION

Biological cells respond to various environmental factors, such as electric [1-3], magnetic [4, 5] and mechanical [6-15] fields.

Cell culture technique has been developed and several methodologies have been clinically applied to regenerative medicine. Acceleration technique for orientation and proliferation of cells has been studied to make muscle tissue *in vivo* or *in vitro* [1-15]. Control methodology for orientation and proliferation of cells would be applied to regenerative tissue technology.

The mechanical stress is one of the interested points in the environment of cells, because they receive mechanical forces *in vivo*. Several methods have been designed to apply mechanical stress to cells [6-15].

A transmission point of the stress to a specimen is important. In many studies, the stress is applied to a scaffold. When fixation between the cell and the scaffold is not enough, the stress is not transmitted to the cell. A flow can be used, on the other hand, to apply a stress field to a specimen [9-15]. The specimen directly receives the shear stress in the shear flow.

A flow chamber with a laminar flow is effective to study the responses of cells to a fluid shear stress quantitatively. In the present study, responses of cells to the fluid shear stress have been studied with a parallel piped rhombus chamber *in vitro*.

2. METHODS

Rhombus Chamber

A one-way flow system was designed to observe responses of cells to a fluid shear stress *in vitro*. The system consists of a flow chamber, a syringe pump, tubes and a microscope (Fig. 1

& 2). The micro-syringe-pump (Fusion200, CXF1020, ISIS Co., Ltd., Osaka) was used for the syringe pump. A plastic tube of 3 mm internal diameter and of 5 mm external diameter was used for the connector to the flow chamber.

The flow chamber consists of two transparent polydimethylsiloxane (PDMS) disks and a thin sheet of silicone rubber (Fig. 3).

A silicon wafer was used for a surface mold for the disk (Fig. 4). The diameter and the thickness of the wafer are 50 mm and 0.30 mm, respectively. The surface of the wafer was cleaned with the isopropyl alcohol, and coated with 0.001 mm thickness of parylene in the parylene coater (PDS-2010, Speciality Coating Systems, Indianapolis). After the wafer was enclosed with a peripheral wall of polyimide, PDMS (Sylgard 184 Silicone Elastomer Base, Dow Corning Corporation) was pored with the curing agent (Dow Corning Corporation) on the wafer. After degassing (Fig. 5), PDMS was baked at 383 K for one hour in an oven (DX401, Yamato Scientific Co., Ltd) (Fig. 6).

The diameter of two PDMS plates is 50 mm. The thicknesses of the upper and the lower disks are 10 mm and 2 mm, respectively.

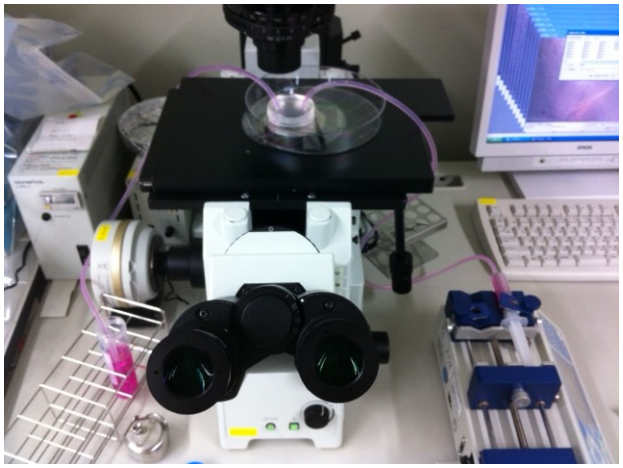

Fig. 1: Flow test system: flow chamber and microscope (middle), syringe pump (right).

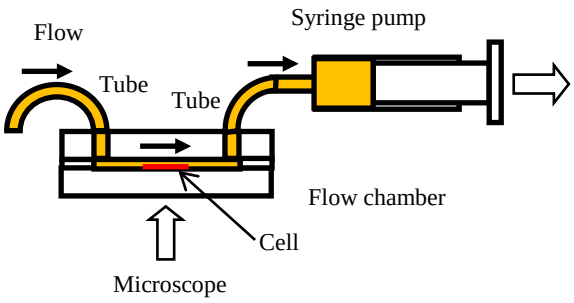

Fig. 2: Flow to syringe pump through flow chamber.

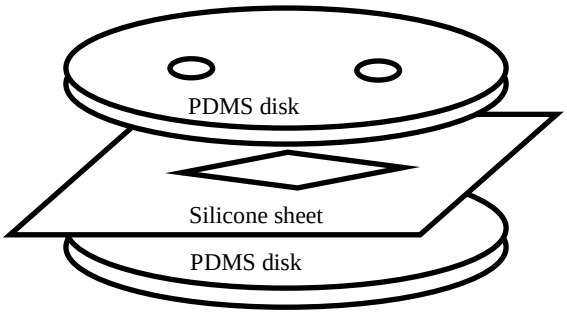

Fig. 3: The flow chamber consists of two transparent polydimethylsiloxane (PDMS) disks and a thin silicone rubber sheet.

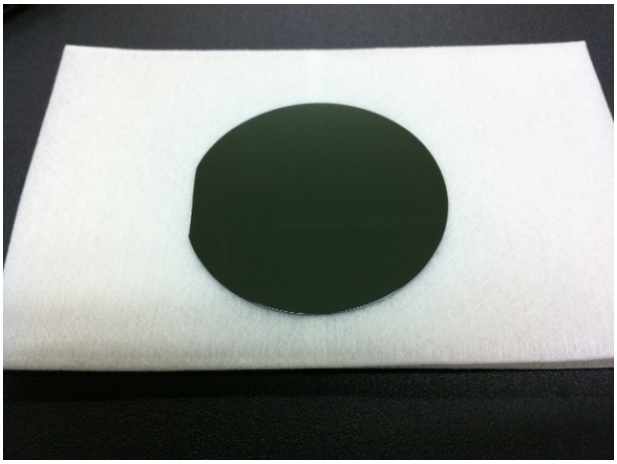

Fig. 4: Silicon wafer (diameter: 50 mm).

Fig. 5: Degassing of PDMS.

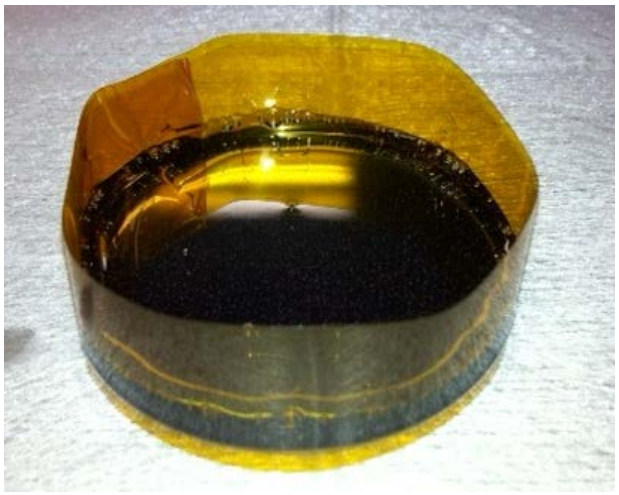

Fig. 6: PDMS baked at 383 K for one hour.

A rhombus open space of 3 mm × 20 mm is cut off in a thin sheet of silicone rubber being 0.1 mm thick, and sandwiched between the PDMS plates (Fig. 3). The open space forms a channel of 20 mm length × 0.1 mm depth, where the width varies from 1 mm to 3 mm. The three plates stick together with their surface affinity without an adhesive. At the upper plate, two holes of 5 mm diameter (Fig. 3) are machined by a punching tool. The silicone tube is stuck at the holes without an adhesive (Fig. 7). The inner surface of the chamber was exposed to the oxygen gas in a reactive ion etching system (RIE-10NR, Samco Inc., Kyoto) to be characterized as hydrophilic (oxygen plasma ashing), before assembled.

Immediately after the characterization, the flow path of the chamber was rinsed with a saline solution, and the suspension of cells was introduced, successively.

One of the tubes is connected to the plastic syringe pump (Fig. 1). The room temperature was maintained at 25 degrees Celsius. The chamber is placed on the inverted phase-contrast microscope (IX71, Olympus Co., Ltd., Tokyo).

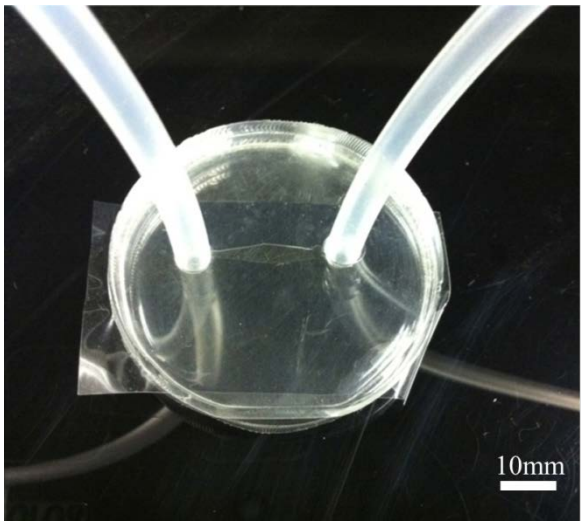

Fig. 7: The flow chamber consists of two transparent polydimethylsiloxane (PDMS) disks and the thin silicone rubber sheet.

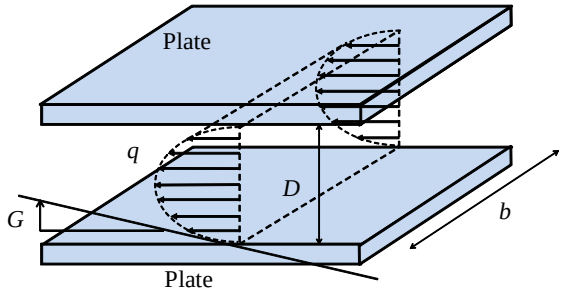

Fig. 8: Parabolic velocity profile between parallel plates.

Cell Culture

L929 (fibroblast-like, mouse connective tissue, RCB1422, Riken Bio Resource Center, Tsukuba) was used in the experiment. The cells were cultured on a dish with the E-MEM (Eagle's minimal essential medium) in an incubator for one week. Then, Cells were exfoliated from the plate of the culture dish with trypsin, and suspended in the E-MEM. The suspension was introduced to the chamber and cultured in the incubator for 3 hours to make cells adhere to the PDMS plate of the chamber before the flow test.

Flow Test

After the chamber was set on the microscope out of the incubator, the constant flow at 18 mL/hour of the medium was applied to the adhered cells with the syringe pump (Fig. 1). The flow path was carefully examined to avoid mixing of air bubbles, which might stir the medium in the flow chamber and induce exfoliation of cells. The behavior of cells on the plate of the chamber was observed with the microscope. The photos of cells were taken during the flow test for one hour.

Shear Stress on Wall

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 (Fig. 8).

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

In Eq. 1, q is the flow rate [$m^3\ s^{-1}$], b is the width of the canal [m] and D is distance [m] between two parallel walls. In the present study, D is 0.1 mm, and b ranges from 1 mm to 3 mm (Fig. 7). The rhombus chamber designed in the present study has a variation on the width of the canal. The width at the inlet of the chamber is 1 mm, and increases from 1 mm to 3 mm in proportion to the distance from the inlet. At the middle point, the width is 3 mm, and then decreases from 3 mm to 1 mm in proportion to the distance from the inlet. The width is 1 mm at the outlet. The variation of the width makes variation on the wall shear stress in the chamber, so that cells at variety of wall shear stresses can be simultaneously observed in the constant flow rate.

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).

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

The viscosity of the medium was measured with the cone and plate type of a 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 0.0010 Pa s at 25 degrees Celsius at the shear rate of 600 s^{-1}. At the flow rate of 18 mL/hour, the calculated shear rate on the PDMS wall of the flow chamber by Eq. 1 varies from 3000 s^{-1} to 1000 s^{-1}, when the width of the flow path varies from 1 mm to 3 mm. The calculated shear stress, thus, varies from 3 Pa to 1 Pa for viscosity of 0.001 Pa s, when the shear rate varies from 3000 s^{-1} to 1000 s^{-1}.

Fig. 9 shows cells under the flow of 18 mL/hour, which generates a wall shear stress between 2.9 Pa and 3 Pa estimated by Eqs. 1 & 2. The medium flows from left to right in Figs. 9-15. The distance from left to right is 0.5 mm in Figs. 9-15. The inlet of the chamber is located near left end of Figs. 9-15, and the width of the flow path linearly increases from 1.0 mm to 1.1 mm in the figures. As the increase of the width of the flow path, the wall shear stress decreases. The estimated shear stress varies from 3.0 Pa (at left-end) to 2.9 Pa (at right-end). Some cells elongate to the downstream along the streamline of the flow in 40 min (left upper A in Fig. 13). Some cells (center B in Fig. 14) exfoliate, and flow to the downstream in 43 min.

4. DISCUSSION

To estimate the wall shear rate in the chamber, a parabolic velocity profile is hypothesized as a laminar flow in the present experiment. Reynolds number (*Re*) is useful index for estimation of laminar flow.

$$Re = d\ D\ v\ /\ N \qquad (3)$$

Fig. 9: Cells after flow stimulation of 18 mL/hour for ten minutes. Dimension from left to right is 0.5 mm.

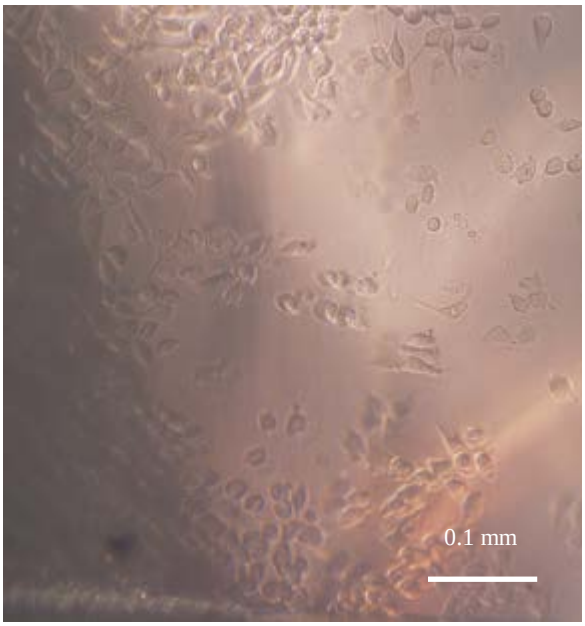

Fig. 10: Cells after flow stimulation of 18 mL/hour for 20 min. Dimension from left to right is 0.5 mm.

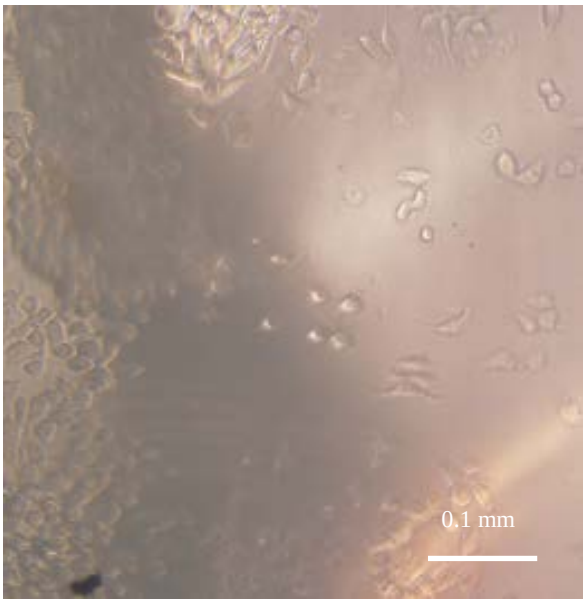

Fig. 11: Cells after flow stimulation of 18 mL/hour for 25 min. Dimension from left to right is 0.5 mm.

In Eq. 3, *d* is density of the fluid, *D* is distance [m] between two parallel walls, *N* is viscosity [Pa s] of the fluid, and *v* is the mean velocity of the flow [m s^{-1}]. The mean velocity is calculated by Eq. 4.

$$v = q\ /\ (b\ D) \qquad (4)$$

In Eq. 4, *q* is the flow rate [m^3 s^{-1}], and *b* is the width [m] of the canal.

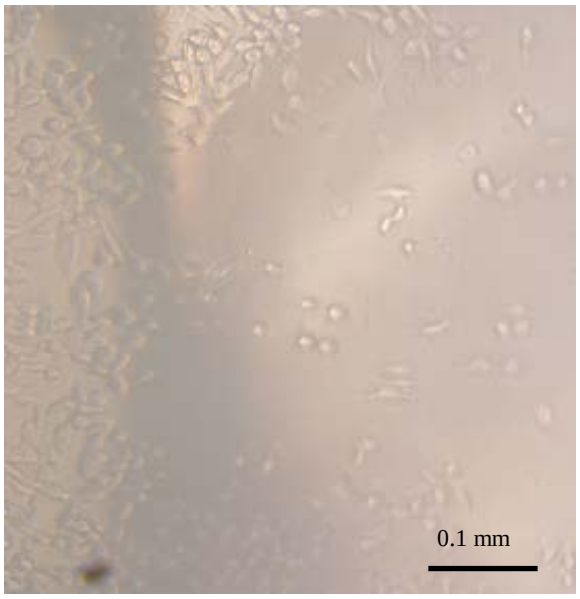

Fig. 12: Cells after flow stimulation of 18 mL/hour for 30 min. Dimension from left to right is 0.5 mm.

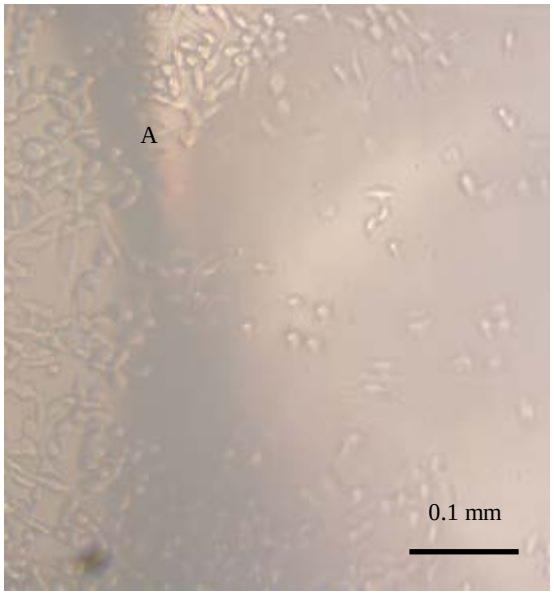

Fig. 13: Cells after flow stimulation of 18 mL/hour for 40 min. Dimension from left to right is 0.5 mm.

Re is in the range between 1.7 and 5, calculated with d (10^3 kg m^{-3}), N (10^{-3} Pa s), D (10^{-4} m), q (5×10^{-9} m^3 s^{-1}), and b (ranges from 1 mm to 3 mm) in the present experiment. The number is small enough small to estimate the laminar flow.

The parallel piped rhombus chamber designed in the present study realizes wall shear stress field, where the shear stress varies linearly along the stream line. The chamber is convenient to observe the response of cells simultaneously with the variation of the wall shear stress, while the wall shear stress is constant in the parallel piped rectangular chamber of the previous study [15].

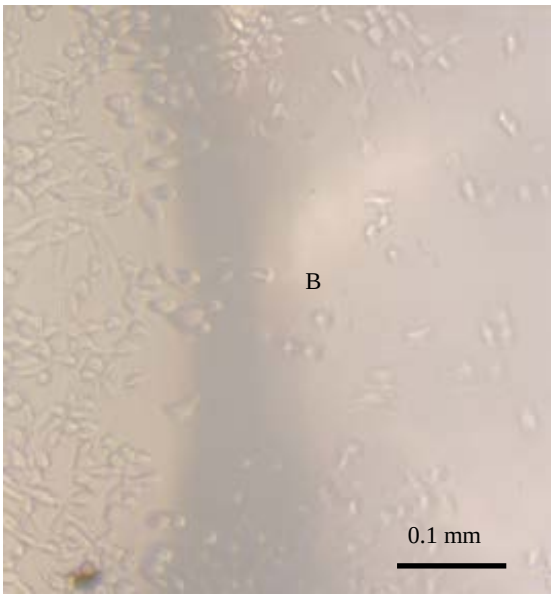

Fig. 14: Cells after flow stimulation of 18 mL/hour for 43 min. Dimension from left to right is 0.5 mm.

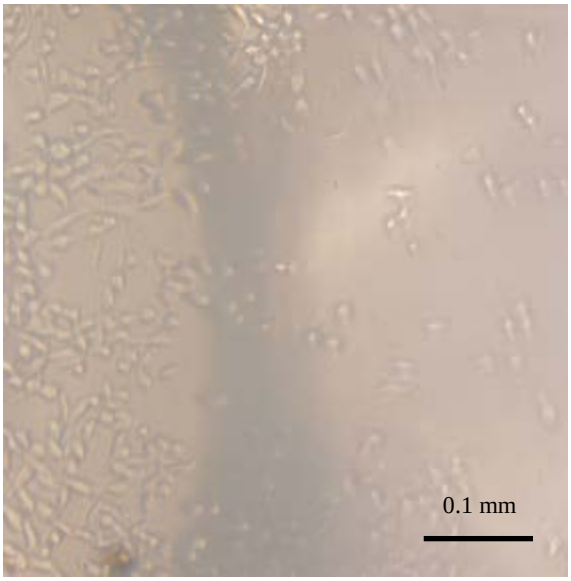

Fig. 15: Cells after flow stimulation of 18 mL/hour for 60 min. Dimension from left to right is 0.5 mm.

Both acceleration of proliferation and orientation of cells are important targets in the research field of regenerative medicine on the cultured biological tissue. The previous study shows that electric stimulation enhances differentiation of muscle cells [1]. Another study shows mechanical stimulation improves a tissue-engineered human skeletal muscle [7].

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 [9-13]. The shear stress affects on the orientation of the smooth muscle cells in the biological tissue [8]. The direction of the mechanical field affects fibroblasts [6].

The present experiment shows that the critical value of the shear stress for exfoliation of L929 from PDMS is higher than that of cells from glass, which has been studied in the previous experiment. Affinity between L929 and PDMS may be higher than that between cells and glass [15].

Too strong mechanical stimulation damages cells. The moderate mechanical stimulation, on the other hand, might accelerate differentiation of cells [9]. The mechanical stimulation decreases proliferation of cells [14]. The mechanical stress also exfoliates several cells, which makes vacancy around the adhesive cell. The differentiation might be optimization of cells to the changing environment.

5. CONCLUSION

Responses of cells to a fluid shear stress have been studied *in vitro*. The rhombus flow chamber has been designed to observe the response of cells to the fluid shear stress under a microscope. The experimental results show that both deformation and exfoliation of cells can be observed under stimulation with the fluid at the controlled wall shear stress in the flow chamber.

6. ACKNOWLEDGMENT

This work was supported by a Grant-in-Aid for Strategic Research Foundation at Private Universities (2008-2012) from the Japanese Ministry of Education, Culture, Sports and Technology.

REFERENCES

[1] 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.

[2] 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.

[3] 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.

[4] Y. Sakatani, S. Hashimoto and J. Yoriki, “Effect of Static Magnetic Field on Muscle Cells in Vitro”, **Proc. 14th World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 2, 2010, pp. 280-284.

[5] J. Yoriki, S. Hashimoto, K. Tachibana, et al., “Effect of magnetic field on adhesion of muscle cells to culture plate”, **Proc. 13th World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 2, 2009, pp. 223-228.

[6] 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.

[7] 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.

[8] K. Nagayama and 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.

[9] 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.

[10] Y. Sugaya, N. Sakamoto, T. Ohashi and M. Sato, “Elongation and Random Orientation of Bovine Endothelial Cells in Response to Hydrostatic Pressure: Comparison with Response to Shear Stress”, **JSME International Journal**, Series C, Vol. 46, No. 4, 2003, pp. 1248-1255.

[11] 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.

[12] A.M. Malek and S. Izumo, “Mechanism of Endothelial Cell Shape Change and Cytoskeletal Remodeling in Response to Fluid Shear Stress”, **Journal of Cell Science**, Vol. 109, 1996, pp. 713-726.

[13] 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”, **Am. J. Physiol. Heart Circ. Physiol.**, Vol. 294, 2008, pp. H1027-H1035.

[14] S. Hashimoto, T. Ooshima, F. Sato, Y. Sakatani and Aki Nakajima, “Effect of Vortex Flow on Cultured Cells in Vitro”, **Proc. 15th World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 2, 2011, pp. 112-117.

[15] A. Nakajima, H. Iwata, S. Hashimoto, T. Susa and F. Sato, “Effect of Shear Flow on Cultured Cartilage Cells”, **Proc. 15th World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 2, 2011, pp. 124-128.