

Simultaneous Measurement of Wall Shear Stress Distribution and Three-Dimensional Shape of Living Endothelial Cells Cultured in Microchannel*

Yasuhiko SUGII**

** The University of Tokyo,
7-3-1, Hongo, Bunkyo, Tokyo, 113-8656, Japan
E-mail: sugii@icl.t.u-tokyo.ac.jp

Abstract

In order to investigate vascular diseases such as cause of atherosclerosis and myocardial infarction, relationships of endothelial cells (ECs) covered with surface blood vessels and blood flow stimulation have been experimentally studied. In the study, in order to investigate the relationship between response of ECs and shear stress caused by blood flow, a non-intrusive measurement method for shear stress distribution and topography of living ECs with subcellular resolution was developed based on velocity distributions measured by micro PIV (Particle Image Velocimetry) technique. ECs were cultured with higher shear stress stimulation in a straight microchannel with width of 400 μm and depth of 100 μm made from polydimethylsiloxane (PDMS) microchip. By optimizing cells cultured condition such as the liquid introduction method and the surface coating for enhancement of cell attachment on the microchannel wall, a cell culture method in the microchip with continuous shear stress stimulation was developed. Height and wall shear stress distributions of ECs cultured with shear stresses of 0.1 and 1.0 Pa were measured. The developed technique is useful to study relationships between wall shear stress distribution and transient morphological response in the living cells.

Key words: Endothelial Cell, Shear Stress, Morphology, Microchannel, Micro PIV

1. Introduction

As microcirculation, whose diameter ranges from 5 to 100 μm , maintains tissue and organs by delivering oxygen and material throughout the body, a number of studies of the biophysical behavior of blood flow in the vascular network have been reported ⁽¹⁾⁻⁽³⁾. Since endothelial cells (ECs), which line blood vessels, are constantly exposed to hemodynamic forces caused by the blood flow, fluid shear stress is a critical factor in determining physiological vascular functions such as maintenance of vessel tone, prevention of thrombosis, promotion of fibrinolysis, and initiation of angiogenesis ⁽⁴⁾⁻⁽⁵⁾. It was reported that wall shear stresses on ECs in artery and in vein under normal physiological conditions range from 1 to 2 and 0.1 to 0.6 Pa, respectively ⁽⁶⁾. Response of ECs to shear stress, such as remodeling of blood vessel ⁽⁷⁾⁻⁽¹¹⁾, secretion of nitric oxide (NO) and intercellular calcium (Ca^{2+}) ⁽¹²⁾⁻⁽¹⁷⁾, using rotation disks, flat plates and tubes, have been reported. In these flow chambers, shear stress on cell is controlled by change in the inlet flow rate. It was reported

*Received 2 Sep., 2010 (No. 10-0392)
[DOI: 10.1299/jbse.5.625]

Copyright © 2010 by JSME

that not only shear stress but also cell contact to surrounding cells influence remodeling⁽¹⁸⁾⁽¹⁹⁾, and shear stress on ECs affects cell growth⁽²⁰⁾.

Wall shear stress distribution and topography of ECs in a tapered microfluidic channel were measured by the use of micro PIV (Particle Image Velocimetry) technique⁽²¹⁾. However, ECs cultured on glass cover slips without shear stress stimulation were exposed to the shear stress and were fixated to measure wall shear stress distribution and topography. Therefore, it is impossible to investigate in the real time the dynamics of transient morphological changes of the cells.

Conversely, in order to develop biochemical reactors and bioassay systems, the authors developed *in vitro* blood vessel models by cultured ECs with low shear stress stimulation of 0.007 Pa in a microchannel, in which leukocyte adhesion onto cells surface was investigated⁽²²⁾. Human fibroblasts were cultured in microchannel with shear stress stimulation of 0.02 Pa to investigate relationship between cell growth, cell morphology and perfusion rate⁽²³⁾. However, the shear stress was significantly lower than the shear stress of cells which endure under physiological conditions. In order to investigate response of ECs to shear stress under physiological conditions, a cell culture method in the microchannel with shear stress to be confluent were developed by optimizing cells cultured condition such as the liquid introduction method and the surface coating for enhancement of cell attachment on the microchannel wall⁽²⁴⁾. The results showed that cell morphology was influenced by not only shear stress but also surrounding cells.

In the study, in order to investigate the relationship between response of ECs and shear stress caused by blood flow, a non-intrusive measurement method for wall shear stress distribution and topography of living ECs surface with subcellular resolution was developed based on velocity distributions measured by micro PIV technique. The technique was applied to ECs cultured with high shear stress stimulation in polydimethylsiloxane (PDMS) microchip.

2. Experiments

2.1 Fabrication of PDMS microchip

A polydimethylsiloxane (PDMS) microchip for a blood vessel model of *in vitro* experiment to culture ECs was fabricated. The microchip consists of a single straight microchannel, which has a rectangular cross sectional shape with a width of 400 μm , depth of 100 μm and long of 2 cm and one inlet and outlet fabricated using soft-lithographic techniques as follows. A negative photoresist SU-8 was poured on a glass plate using spin-coater. After soft-bake on a hot plate, a UV-light was exposed through a photolithography mask using a mask aligner to impress the channel pattern onto the SU-8. The exposed substrate was hard-baked and developed with a developer solution to remove unexposed areas of photoresist. The PDMS slurry on the master was baked in the oven. The PDMS plate was processed to enhance the hydrophobicity by oxygen plasma surface treatment and then covered with thin glass substrate. Micro tubes with the inner diameter 0.3 mm were connected to holes punched for fluid inlet and outlet.

2.2 Cell culture technique in microchannel

A conventional cell culture technique for a culture dish was already established briefly described as follows. (1) Cells suspended in culture medium are put into a dish. (2) Cells attach and grow on surface of the dish wall. (3) Dish with cells is left stationary in 5% CO_2 incubator at 37 degree Celsius. (4) Culture medium is replaced at twice or three times in a week to prevent the lack of metabolic nutrient and oxygen. Conversely, in a microchannel, culture medium for each cell is too small due to its scale, resulting in lack of metabolic nutrient and oxygen and accumulation of cell wastes. Therefore, it is necessary that culture medium is continuously supplied in the microchannel. However, continuous flow of culture

medium will increase the risk of contamination and air bubble, which cause serious damage to the cells. Moreover, the flow may prevent cells attachment on the surface. Therefore, small flow rate was applied to culture ECs in microchannel. The wall shear stress τ of laminar flow in rectangular channel was theoretically obtained by ⁽²⁵⁾,

$$\tau = \frac{6\mu Q}{a^2 b} \quad (1)$$

where μ is viscosity of working fluid, Q the flow rate, a the depth and b the width of the microchannel.

Cell culture technique with high shear stress by optimizing cells cultured condition such as surface coating on the wall and liquid introduction method by withdrawal was applied described as follows ⁽²⁴⁾. Withdrawing the micro syringe by the pump, it is possible to avoid contamination and air bubble at around the connecting point such as needle and tube. Even if contamination and air bubble was mixed at the tube dipped into the solution, contamination and air bubble was ejected by infusion of the solution. Human umbilical vein endothelial cells (HUVECs) were cultured in a 35 mm² culture dish with an EC culture medium (EGM-2, Cambrex). HUVECs used in the experiments were fourth generation. A developed cell culture technique in a microchannel was briefly described as follows. After sterilization using ultraviolet lamp and 70 % ethanol, channel walls were coated with matrigel, which is one of an extracellular matrix gel and often used for surface coating of a dish. Solution of matrigel (0.1 mg/ml) was introduced into the microchannel. The microchip was incubated in 5% CO₂ incubator at 37 degree Celsius for one hour without flow to adsorb the matrigel and then rinsed with the culture medium. ECs suspension at a cell density of 10⁶ cells/ml was introduced at the flow rate of 5.0 μ l/min by the use of the liquid introduction method described above. The microchip was incubated in 5% CO₂ incubator at 37 degree Celsius for three hours to be attached ECs on the inner wall without flow and then the culture medium was continuously flowed in the incubator by the head pressure. It is possible that ECs were cultured until confluent for 100-150 hours with exposing steady fluid shear stress of 0.1 and 1.0 Pa.

2.3 Height and wall shear stress measurement technique

EC presents an undulating surface with elevation caused by cell nucleus. Cell surface corresponded to the position at zero velocity because of no-slip condition at cell surface. The wall shear stress τ is given by Newton's law of viscosity,

$$\tau = \mu \left. \frac{\partial u_n}{\partial n} \right|_{n=0} \approx \mu \left. \frac{\partial u}{\partial z} \right|_{n=h} \quad (2)$$

where $\partial u_n / \partial n$ is the wall normal velocity gradient. The wall normal velocity gradient $\partial u_n / \partial n$ can be approximated by the velocity gradient $\partial u / \partial z$ in depth direction z because of the small inclination of the cell shape.

In principle, shear rate was estimated using fitting curve from velocity at difference z position and cell height was also estimated as zero velocity z position shown in Fig.1. Therefore, it is possible to reconstruct wall shear stress distribution on cell surface and cell height distribution from velocity distributions at difference focal planes.

In the paper, a second order polynomial was fit to three velocities in the streamwise direction at three focal position $z = 5, 10, 15 \mu$ m obtained using micro PIV technique. Cell surface position was obtained by extrapolating the velocity profile to zero velocity position and the wall shear stress was obtained from Eq.(2) at the cell surface. Micro PIV technique is an optical and non-intrusive measurement technique for velocity distribution at micro resolution ^{(24) (25)} and was applied to microfluidic device ⁽²⁶⁾. Micro PIV system consists of

an epi-fluorescent inverted microscope with a 40 \times oil immersion objective lens (NA=1.30), a double pulsed Nd:YAG laser, wavelength $\lambda = 532$ nm, color filters and a cooled CCD double-frame camera with resolution of 1376 \times 1040 pixels and grey level of 12 bits. Culture medium seeded with the fluorescent particles with diameters of 1.0 μm which excites at 535 nm and emits at 575 nm was injected at the constant flow rate to be shear stress of 0.1 and 1.0 Pa by Eq.(1) using microsyringe pump. The observed region was 220 \times 166 μm^2 , with each pixel representing a 0.16 \times 0.16 μm^2 area. Applying the highly accurate PIV technique ⁽²⁶⁾ to the images, the time-averaged velocity distributions were obtained from 130 image pairs. Background noise due to out-of-focus particles was removed by subtracting a background intensity calculated from the series of images. An interrogation window of 57 \times 57 pixels was taken with 50% overlap, corresponding to a spatial resolution of 4.5 \times 4.5 μm^2 . Velocity distributions at three focal planes $z = 5, 10, 15$ μm were obtained. Initially, the objective lens adjusted to focus on the glass surface manually and then the lens was moved along z direction to measure the velocity distributions at three focal planes parallel to glass surface.

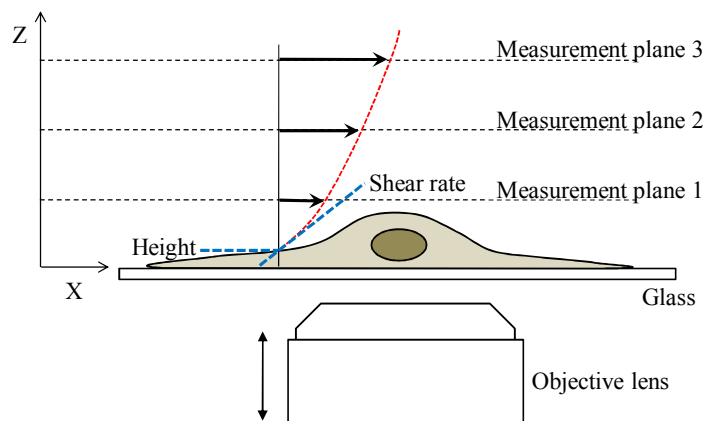


Fig.1 Height and wall shear stress of endothelial cells cultured in microchannel

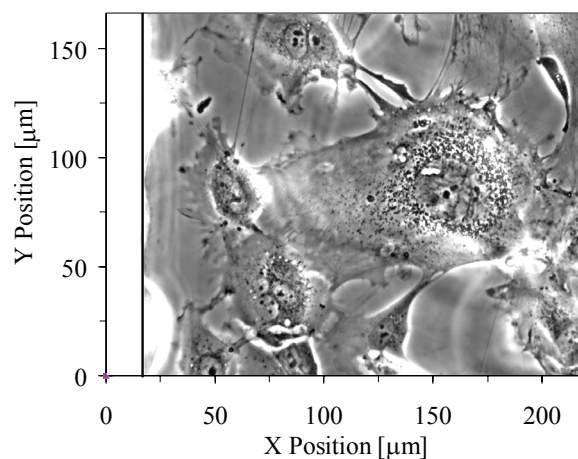
3. Results

In order to measure absolute value of cell height, the measurement technique for wall shear stress distributions and cell height distributions was applied to sub-confluent condition, which include both of glass surface and cell surface. Figure 2 shows a phase-contrast image of living ECs cultured in the microchip with (a) shear stresses of 0.1 Pa and (b) 1.0 Pa. EC monolayer in the microchip was cultured by flowing culture medium continuously to expose the shear stress. The focal plane was set at cells surface attached on the bottom wall of the microchannel. Black vertical lines in the figures represent the side wall of microchannel. In Fig.2 (a) shear stress of 0.1 Pa, large cell was observed as round shape around $X = 160$ μm , $Y = 90$ μm . ECs were randomly aligned with no preferred orientation subject to the shear flow. Around $X = 150$ - 190 μm , $Y = 0$ - 40 μm , glass surface was confirmed. In contrast, ECs in Fig.2 (b) shear stress of 1.0 Pa were observed as elliptic shape and the morphology of ECs was stretched in the flow direction. Around $X = 170$ μm , $Y = 120$ μm , glass surface was confirmed. It was confirmed that ECs cultured with shear stress of 1.0 Pa significantly aligned to flow direction.

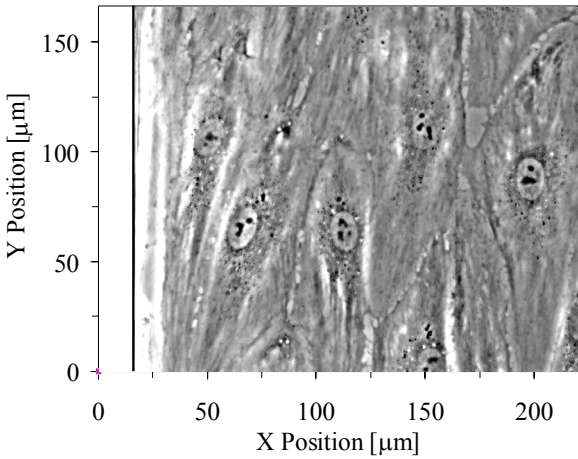
After focus on the glass surface manually, velocity distributions at three focal planes $z = 5, 10, 15$ μm were measured using micro PIV technique and cell height distributions and wall shear stress distributions were obtained. Figure 3 shows contours of surface height of ECs cultured in the microchip with (a) shear stresses of 0.1 Pa and (b) 1.0 Pa. In Fig.3 (a) shear stress of 0.1 Pa, around $X = 150$ - 190 μm , $Y = 0$ - 40 μm , height became minimum value - 4.0 μm in spite of glass surface area. The results mean that initial focal position was out of

the glass surface of the microchannel so that velocity distributions at three focal planes $z = 9, 14, 19 \mu\text{m}$ were measured instead of $z = 5, 10, 15 \mu\text{m}$. Therefore, all of obtained height was measured as $-4.0 \mu\text{m}$ smaller. At the center of nucleus around $X = 160 \mu\text{m}$, $Y = 90 \mu\text{m}$, the height of these region was not obtained because the position of measurement plane was closer to glass surface than cell surface resulting in no velocity data. The height around the edge of the nucleus obtained as maximum value $10 \mu\text{m}$, which represented correct value of height was $14 \mu\text{m}$ by taking account into difference of focal position. A sharp gradient was observed around nucleus. In Fig.3 (b) shear stress of 1.0 Pa , around $X = 170 \mu\text{m}$, $Y = 120 \mu\text{m}$, glass surface, height became minimum value $-4.5 \mu\text{m}$, which also indicated out of focus on glass surface. Therefore, all of obtained height was measured as $-4.5 \mu\text{m}$ smaller. Nucleus was observed as maximum value, $1.0 \mu\text{m}$, around $X = 110 \mu\text{m}$, $Y = 80 \mu\text{m}$, which represented correct value of height was $5.5 \mu\text{m}$. Height of all of nucleus ranged $4.5\text{--}5.5 \mu\text{m}$. The results showed that ECs with higher shear stimulation shows a flatter with a bulge in the region of the nucleus.

Figure 4 shows contours of wall shear stress distribution on ECs surface cultured in the microchip with (a) shear stresses 0.1 Pa and (b) 1.0 Pa . In Fig.4 (a) shear stress of 0.1 Pa , around $X = 150\text{--}190 \mu\text{m}$, $Y = 0\text{--}40 \mu\text{m}$, glass surface area, wall shear stress was minimum 0.1 Pa . The value corresponded to theoretical value by Eq.(1). The wall shear stress at the center of nucleus $X = 80 \mu\text{m}$, $Y = 145 \mu\text{m}$ was maximum value 0.25 Pa , whose height was $14 \mu\text{m}$ in Fig.3 (a). Although the edge of the nucleus around $X = 160 \mu\text{m}$, $Y = 90 \mu\text{m}$ shows $14 \mu\text{m}$ height same as nucleus $X = 80 \mu\text{m}$, $Y = 145 \mu\text{m}$, wall shear stress was about 0.15 Pa smaller than that of nucleus. The results showed that wall shear stress was out of proportion to its height. The maximum value of wall shear stress on the top of nucleus is about 2.5 times larger than the value of glass surface. In Fig.4 (b) shear stress of 1.0 Pa , around $X = 170 \mu\text{m}$, $Y = 120 \mu\text{m}$, glass surface area, wall shear stress was minimum 1.0 Pa , also corresponded to theoretical value by Eq.(1). Nucleus around $X = 110 \mu\text{m}$, $Y = 80 \mu\text{m}$, which has maximum height $5.5 \mu\text{m}$, was maximum wall shear stress 1.6 Pa . The wall shear stress fluctuated smaller than undulations of cell surface. The results also showed that wall shear stress was out of proportion to its height. The maximum value of wall shear stress on the top of nucleus is about 1.6 times larger than the value of glass surface.

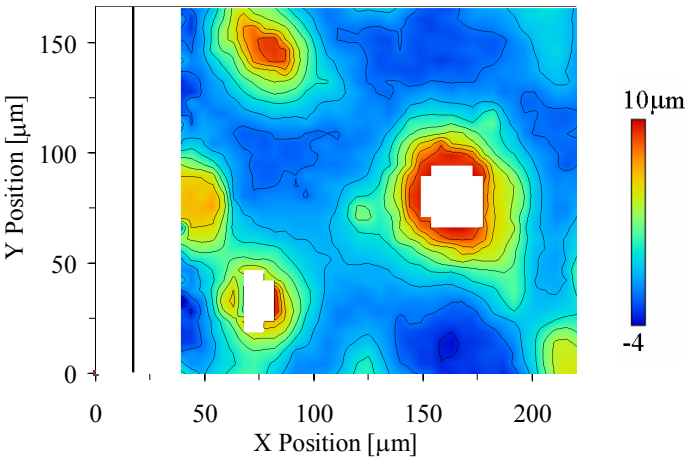


(a) Shear stress of 0.1 Pa by Eq.(1)

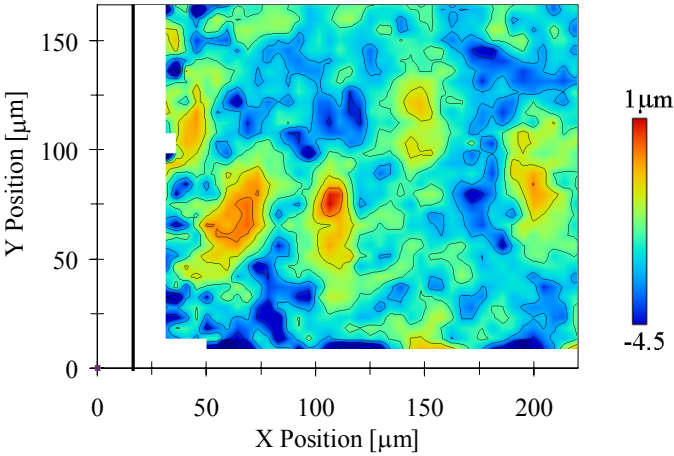


(b) Shear stress of 1.0 Pa by Eq.(1)

Fig.2 Endothelial cells cultured with shear stress stimulation in microchannel



(a) Shear stress of 0.1 Pa by Eq.(1)



(b) Shear stress of 1.0 Pa by Eq.(1)

Fig.3 Height of endothelial cells cultured with shear stress stimulation in microchannel

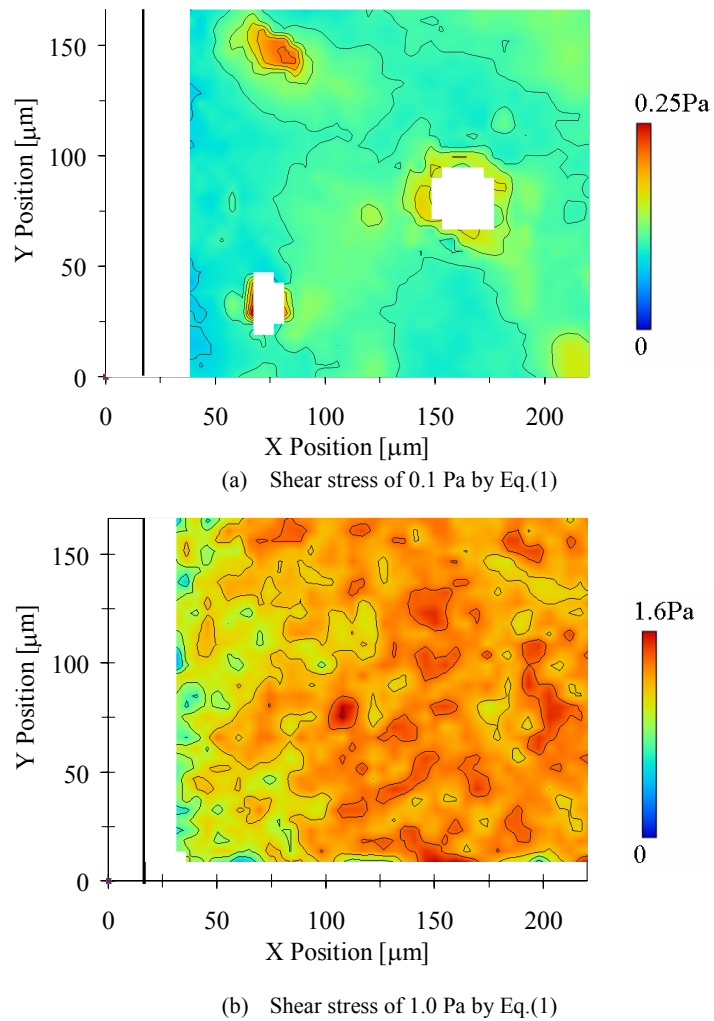


Fig.4 Wall shear stress distributions of endothelial cells cultured with shear stress stimulation in microchannel

4. Discussion

In the paper, we have developed a non-intrusive measurement method for wall shear stress distribution and topography of living ECs with subcellular resolution of $4.5 \times 4.5 \mu\text{m}^2$. The technique was applied to ECs cultured in microchannel with continuous two kinds of shear stress stimulation over 100 hours.

The error of applied PIV analysis technique for the particle images was estimated as 5 %⁽²⁹⁾. Since Brownian motion of small tracer particle induced measurement error, velocity vector was obtained by averaging spatially and temporally to reduce the error less than 1 %. The thickness of measurement plane determined the depth resolution and also measurement accuracy. An obtained velocity became a weighted average of the flow within the measurement depth⁽³⁰⁾. Since the measurement depth in the system was evaluated as 2.1 μm theoretically, the error caused by the velocity gradient in the depth direction was estimated as less than 1 %. In addition, the error of height and wall shear stress of cell caused by curvature of cell surface was less than 1 % to be neglected. Therefore, measurement error of height and wall shear stress of cell was roughly estimated as less than 10 %. Since obtained wall shear stress on the glass surface showed good agreement with theoretical value, the effectiveness of the technique has been proven. However, the immeasurable region occurred due to out of focus by manual.

The maximum heights of cells stimulated with shear stress of 0.1 and 1.0 Pa were measured as 14 and 5.5 μm , respectively. It was seemed that the difference of cell height

caused by shear stress was slightly larger compared with the previous studies. In the previous studies, not absolute cell height but the height differential between the maximum in the nucleus region and the minima in the cytoplasm was usually measured using AFM⁽¹⁰⁾. Since the change of differential in the previous studies consisted of not only the change of the nucleus but also the cytoplasm, the change of the cell height showed smaller value than the present result.

Wall shear stresses of cell surface were out of proportion to its height. Previous reports also showed that shear stress distribution on the cell surface depended on not only the surface geometry but also that of surrounding cells⁽¹¹⁾. Since during the cell growth up, the cell density increased, which mean the surface geometry of surrounding cells changed results. It was reported that single cell and cells that were not confluent were not orientated in flow direction⁽¹⁸⁾⁽¹⁹⁾. In contrast, cells with shear stress of 1.0 Pa in the experiment, that were not confluent, were observed as elliptic shape and the orientation in the flow direction. The one of the reasons of the difference of cell response was considered that continuous shear stress may affect cell growth⁽²⁰⁾.

5. Conclusions

In order to investigate the relationship between response of ECs and shear stress caused by blood flow, a non-intrusive measurement method for wall shear stress distribution and topography of living ECs with subcellular resolution was developed based on velocity distributions measured by micro PIV technique. Measured wall shear distributions and height of living ECs cultured with shear stress stimulation showed cells response to shear stress. The experimental technique is useful to study relationships between wall shear stress distribution and transient morphological response in the living cells.

Acknowledgements

This work was partially supported by KAKENHI (22360080), a Grant-in-Aid for Scientific Research (B) from the Ministry of Education, Culture, Sports, Science and Technology in Japan (MEXT).

References

- (1) Maeda, N., Erythrocyte rheology in microcirculation, *The Japanese Journal of Physiology*, Vol.46 (1996), pp.129-134.
- (2) Goldsmith, H.L., The microrheology of human blood, *Microvascular Research*, Vol.31 (1986), pp.121-142.
- (3) Sugii, Y., Nishio, S. and Okamoto, K., In vivo PIV measurement of red blood cell velocity field in microvessels considering mesentery motion, *Physiological Measurement*, Vol.23 (2002), pp.403-416.
- (4) Gouverneur, M., Berg, B.V.D., Nieuwdorp, M., Stroed, E. and Vink, H., Vasculoprotective properties of the endothelial glycocalyx: effects of fluid shear stress, *Journal of Internal Medicine*, Vol.259, No.4 (2006), pp.393-400.
- (5) Pries, A.R. and Secomb, T.W., Microvascular blood viscosity in vivo and the endothelial surface layer, *American Journal of Physiology - Heart and Circulatory Physiology*, Vol.289 (2005), pp.H2657-H2664.
- (6) Kamiya, A., Bukhari, R. and Togawa, T., Adaptive regulation of wall shear stress optimizing vascular tree function, *Bulletin of Mathematical Biology*, Vol.46, No.1 (1984), pp.127-137.
- (7) Fry, D.L., Acute vascular endothelial changes associated with increased blood velocity gradients, *Circulation Research*, Vol.22 (1968), pp.165-197.
- (8) Levesque, M.J. and Nerem, R.M., The elongation and orientation of cultured endothelial

- cells in response to shear stress, *Journal of Biomechanical Engineering*, Vol.107 (1985), pp.341-347.
- (9) Dewey, C.F.Jr., Bussolari, S.R., Gimbrone, M.A.Jr. and Davies, P.F., The dynamic response of vascular endothelial cells to fluid shear stress, *Journal of Biomechanical Engineering*, Vol.103 (1981), pp.177-185.
- (10) Barbee, K.A., Davies, P.F. and Lal, R., Shear stress-induced reorganization of the surface topography of living endothelial cells imaged by atomic force microscopy, *Circulation Research*, Vol.74 (1994), pp.163-171.
- (11) Fukushima, S., Fujioka, H. and Tanishita, K., Shear stress distribution on the surface of endothelial cells during flow-induced morphological remodeling, *JSME International Journal Series C*, Vol.46, No.4 (2003), pp.1275-1283.
- (12) Ando, J., Komatsuda, T. and Kamiya, A., Cytoplasmic calcium response to fluid shear stress in cultured vascular endothelial cells, *In Vitro Cellular and Developmental Biology - Plant*, Vol.24, No.9 (1988), pp.871-877.
- (13) Nozawa, T., Cheng, C.P., Noda, T. and Little, W.C., Effect of exercise on left ventricular mechanical efficiency in conscious dogs, *Circulation*, Vol.90 (1994), pp.3047-3054.
- (14) Berger, H.J., Prasad, S.K., Davidoff, A.J., Pimental, D., Ellingsen, O., Marsh, J.D., Smith, T.W. and Kelly, R.A., Continual electric field stimulation preserves contractile function of adult ventricular myocytes in primary culture, *American Journal of Physiology - Heart and Circulatory Physiology*, Vol.266 (1994), pp.H341-H349.
- (15) Mochizuki, S., Vink, H., Hiramatsu, O., Kajita, T., Shigeto, F., Spaan, J.A.E. and Kajiya, F., Role of hyaluronic acid glycosaminoglycans in shear-induced endothelium-derived nitric oxide release, *American Journal of Physiology - Heart and Circulatory Physiology*, Vol.285 (2003), pp.H722-H726.
- (16) Oblak, T.D.A., Root, P. and Spence, D.M., Fluorescence monitoring of ATP-stimulated, endothelium-derived nitric oxide production in channels of a Poly(dimethylsiloxane)-based microfluidic device, *Analytical Chemistry*, Vol.78, No.9 (2006), pp.3193-3197.
- (17) Sakamoto, N., Oi, M., Ohashi, T. and Sato, M., Nitric oxide production and smooth muscle cell phenotype of endothelial cell-smooth muscle cell cocultured model, *Journal of Biomechanical Science and Engineering*, Vol.1, No.1 (2006), pp.224-233.
- (18) Masuda, M. and Fujiwara, K., Morphological responses of single endothelial cells exposed to physiological levels of fluid shear stress, *Frontiers of medical and biological engineering*, Vol.5, No.2 (1993), pp.79-87.
- (19) Katakoka, N., Ujita, S., Kimura, K. and Sato, M., The morphological responses of cultured bovine aortic endothelial cells to fluid-imposed shear stress under sparse and colony conditions, *Transactions of the Japan Society of Mechanical Engineers, Series C*, Vol.63, No.607 (1997), pp.838-845.
- (20) Lin, K., Hsu, P.P., Chen, B.P., Yuan, S., Usami, S., Shyy, J.Y.-J., Li, Y.-S. and Chien, S., Molecular mechanism of endothelial growth arrest by laminar shear stress, *Proceedings of the National Academy of Sciences*, Vol.97 (2000), pp.9385-9389.
- (21) Rossi, M., Lindken, R., Hierck, B.P. and Westerweel, J., Tapered microfluidic chip for the study of biochemical and mechanical response at subcellular level of endothelial cells to shear flow, *Lab on a Chip*, Vol.9 (2009), pp.1403-1411.
- (22) Tanaka, Y., Kikukawa, Y., Sato, K., Sugii, Y. and Kitamori, T., Culture and leukocyte adhesion assay of human arterial endothelial cells in a glass microchip, *Analytical Sciences*, Vol.23, No.3 (2007), pp.261-266.
- (23) Korin, N., Bransky, A., Dinnar, U. and Levenberg, S., A parametric study of human fibroblasts culture in a microchannel bioreactor, *Lab on a Chip*, Vol.7 (2007), pp.611-617.
- (24) Sugii, Y., Development of micro fluidic device cultured endothelial cells with fluid shear stress exposure and measurement of morphological response, *Transactions of the Japan Society of Mechanical Engineers. B*, Vol.75, No.759 (2009), pp.2233-2238. (in Japanese)

- (25) Usami, S., Chen, H.-H., Zhao, Y., Chien, S. and Skalak, R., Design and construction of a linear shear stress flow chamber, *Annals of Biomedical Engineering*, Vol.21, No.1 (1993), pp.77-83.
- (26) Santiago, J.G., Wereley, S.T., Meinhart, C.D., Beebe, D.J. and Adrian, R.J., A particle image velocimetry system for microfluidics, *Experiments in Fluids*, Vol.25 (1998), pp.316-319.
- (27) Sugii, Y. and Okamoto, K., Quantitative visualization of micro-tube flow using micro-PIV, *Journal of Visualization*, Vol.7, No.1 (2004), pp.9-16.
- (28) Sugii, Y., Okamoto, K., Hibara, A., Tokeshi, M. and Kitamori, T., Effect of Korteweg stress in miscible liquid two-layer flow in a microfluidic device, *Journal of Visualization*, Vol.8, No.2 (2005), pp.117-124.
- (29) Sugii, Y., Nishio, S., Okuno, T. and Okamoto, K., A highly accurate iterative PIV technique using gradient method, *Measurement Science and Technology*, Vol.11 (2000), pp.1666-1673.
- (30) Bourdon, C.J., Olsen, M.G. and Gorby, A.D., The depth of correlation in micro-PIV for high numerical aperture and immersion objectives, *Journal of Fluids Engineering*, Vol.128 (2006), pp.883-886.