1994년도 추계학술대회 논문집 제 16 권 제 2 호 94 / 12

Three-Dimensional Morphological Changes of Human Endothelial Cell with Fluid Flow

Dong Ik Shin*, Dong Sun Lee**, Jun Keun Chang*,
Jongwon Kim°, Byoung Goo Min*°

*Dept. of Biomedical Engineering, °Inst. of Biomedical Engineering,
Seoul National University
and **Choong Wae Medical Institute

Introduction

The endothelium formed on the inner surface of the artificial graft bear the hemodynamic stress with changing the alignment of endothelial cells. Previous studies of morphological changes of endothelial cells have been focused on the molecular biology of adhered proteins in cell attachment and movement [1-4]. Micro-pipette aspiration [5, 6], centrifugation of cells [7, 8], and exposure of cells to fluid shear stress [9-14] are used to measure the morphological changes and biochemistry of endothelial cells. According to these studies, endothelial cells are oriented with the flow direction under influence of shear stress and become more elongated when exposed to higher shear stress [15, 16]

The objectives of study is to develop the real-time image processing system to investigate the morphological changes of endothelial cell with shear stress including three-dimensional measurement of morphological changes of endothelial cell.

Real-Time Image Processing System with Laminar Flow Chamber

Cell shape changes and motion were examined by a CCD camera (LK-636, TOSHIBA, Japan) attached to the microscope and connected to a image grabber (MIPS, medical image processing system, Choong Wae Medical, Seoul), IBM PC, monitor (SuperVision Pro 21", Dae Woo Electronics, Korea) and video recorder (Chromatic Series, Hitachi, Japan) [17].

The flow chamber is mounted on the stage of a inverted microscope (IMT-2, Olympus, Japan). The flow chamber is connected to the flow circuit by two 4 mm I.D. ports. Flow is generated by a roller pump (MasterFlex L/S Drive with Easy Load™ head, Cole-Pharmer Instrument Co., Chicago, IL) between two reservoirs.

Three-Dimensional Morphology Analysis

To investigate the mechanotransduction of endothelial cells, live-cell real-time imaging of endothelial cell surfaces is very important to reveal the dynamic nature of these structures when they are subjected to defined flow shear stress [18]. Three-dimensional morphological information, such as cellular height, is critical to study the morphological changes of endothelial cell with shear stress. MIPS (medical image processing system, Choong Wae Medical, Seoul) was modified to study the three-dimensional height information from two-dimensional microscopy image. MFCS (microscope focus control system, Choong Wae Medical) and stepping motor (M062-FD-335, Superior Electronics, Bristol, CT) were connected to MIPS and controlled the fine-adjust nut of the microscope (IMT-2, Olympus, Japan). Twenty sequential images of cellular morphology, taken by turning the fine-adjust nut with stepping motor, were automatically stored in the host PC and the pixel-by-pixel substractions of gray values of sequential images were accomplished in the MIPS. A schematic block diagram of MIPS and MFCS system was shown in Figure 1.

Results and Discussion

The contours of the target cell at the specific height level were collected and reconstructed to analyze the morphological parameters of cytochalasin D-treated human umbilical vein endothelial cells. Figure 2 shows the sequential images of cells acquired with MIPS and MFCS and Figure 3 shows the edge of endothelial cells from Figure 2. Figure 4 summarize the three-dimensional

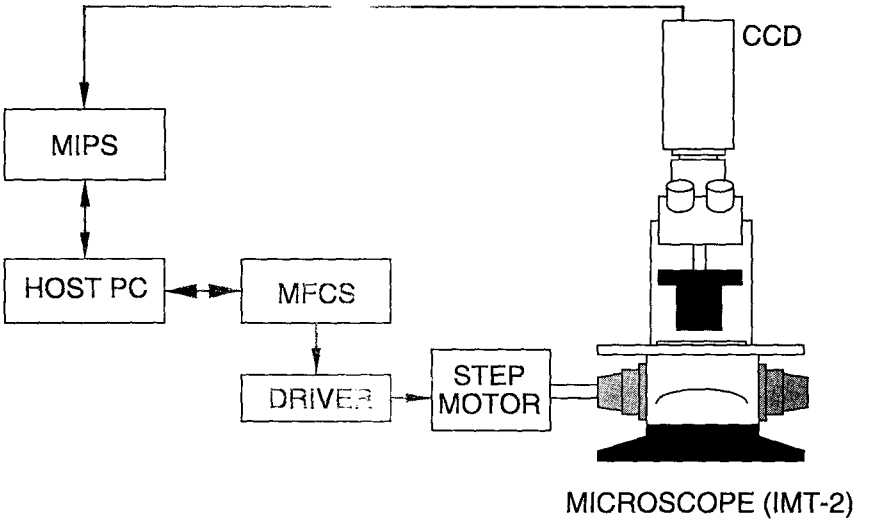

Figure 1. Schematic block diagram of MIPS and MFCS system.

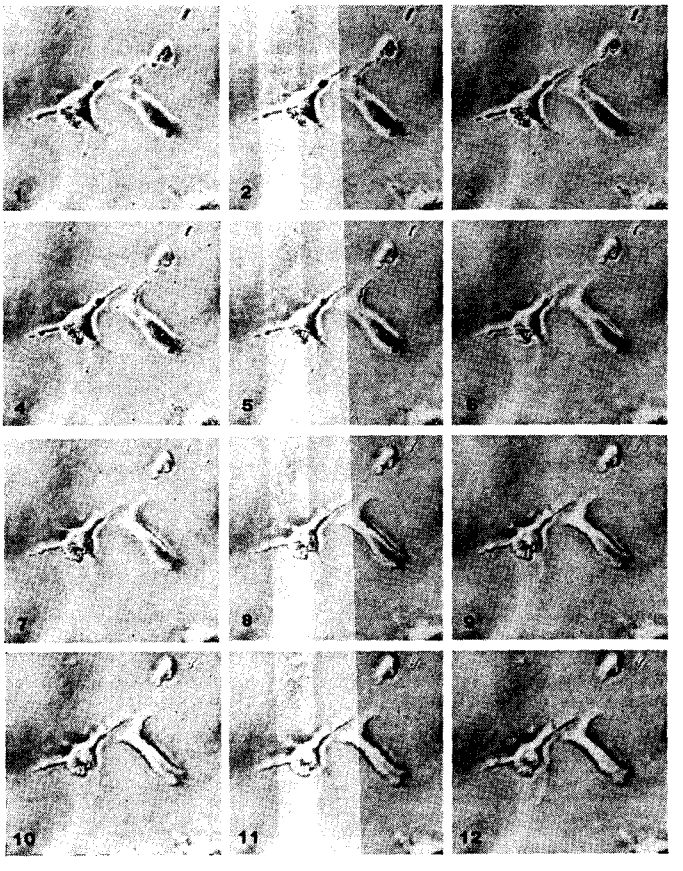

Figure 2. Sequential images of cells acquired with MIPS and MFCS.

morphology of cytochalasin D-treated endothelial cell with shear stress. Cellular height of control culture was shown in Figure 4 (A). The height of cytochalasin D-treated endothelial cell is shown in Figure 4 (B). The height of the cell after 5 minutes exposure of fluid flow and 60 minutes exposure of fluid flow is shown in Figure 4 (C) and 4 (D), respectively. The height of cytochalasin D-treated endothelial cells was increased about 3 times compared to that of the control cell.

References

1. D. A. Hammer, D. A. Lauffenburber, A dynamic model for receptor-mediated cell adhesion to surfaces, *Biophysical Journal* **52**, 475-587 (1987).
2. M. Dembo, D. C. Torney, K. Saxman, D. Hammer, The reaction-limited kinetics of membrane-to-surface adhesion and detachment, *Proceedings of Royal Society of London B* **234**, 55-83 (1988).
3. G. I. Bell, Models for the specific adhesion of cells to cells, *Science* **200**, 618-627 (1978).
4. Y. Takemoto, T. Matsuda, T. Kishimoto, M. Maekawa, T. Akutsu, Molecular understanding of cellular adhesion on artificial surfaces, *Transaction of American Society of Artificial Internal Organs* **35**, 354-356 (1989).
5. E. Evans, D. Berk, A. Leung, Detachment of agglutinin-bonded red blood cells: I. Forces to rupture molecular-point attachment, *Biophysical Journal* **59**, 838-848 (1991).
6. F. Moussy, A. W. Neumann, W. Zingg, The forces of detachment of endothelial cells from different solid surfaces, *Transaction of American Society of Artificial Internal Organs* **36**, M568-M572 (1990).
7. W. Hertl, W. S. Ramsey, E. D. Nowlan, Assement of cell-substrate adhesion by a centrifugal method, *In Vitro* **20**, 796-801 (1984).
8. T. A. Horbett, J. J. Waldburger, B. D. Ratner, A. S. Hoffman, Cell adhesion to a series of hydrophilic-

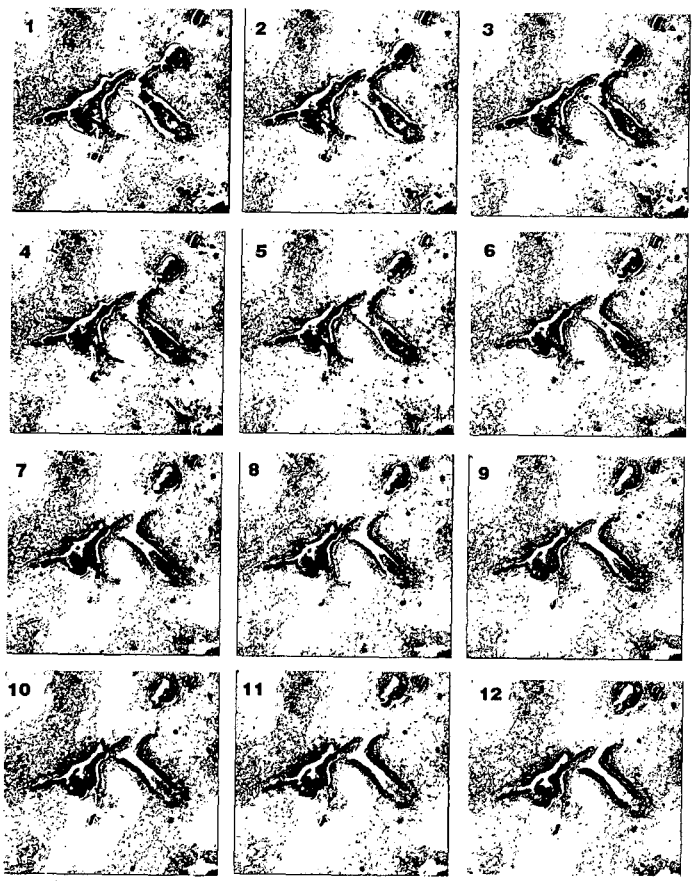

Figure 3. Focused edge of endothelial cells from Figure 2.

hydrophobic copolymers studied with a spinning disc apparatus, *Journal of Biomedical Materials Research* **22**, 383-404 (1988).

9. T. G. van Kooten, J. M. Schakenraad, H. C. Van der Mei, H. J. Busscher, Development and use of a parallel-plate flow chamber for studying cellular adhesion to solid surfaces, *Journal of Biomedical Materials Research* **26**, 725-738 (1992).

10. C. F. Dewey Jr., Effects of fluid flow on living vascular cells, *Journal of Biomechanical Engineering* **106**, 31-35 (1984).

11. J. K. Chang, J. Kim, J. H. Kim, D. C. Han, B. G. Min, Morphological changes of human endothelial cell under high Reynolds number flow, N. A. Langrana, M. H. Friedman, E. S. Groods, Eds., Bioengineering Conference (The American Society of Mechanical Engineers, Breckenridge,

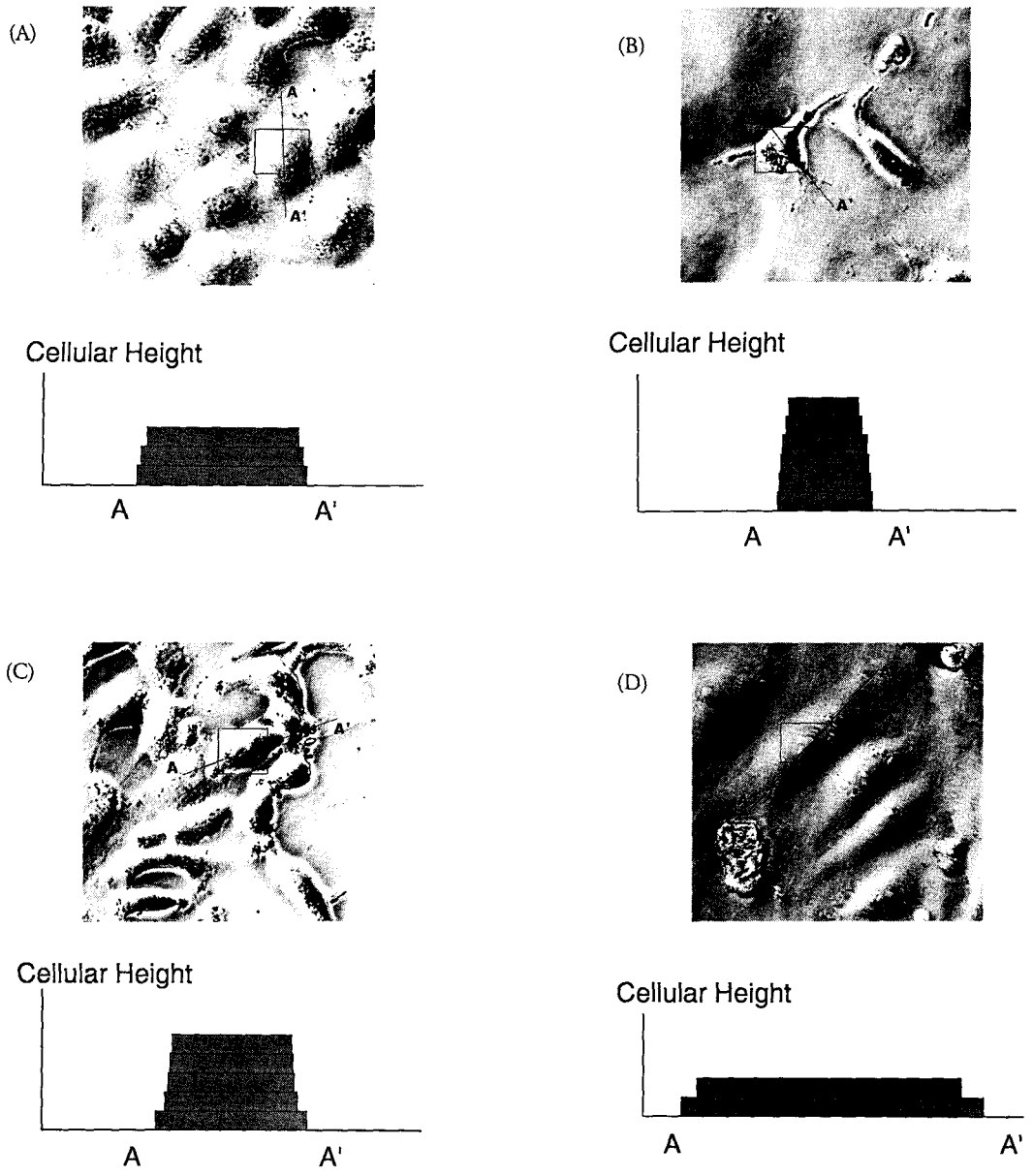

Figure 4 *Three-dimensional morphology of cytochalasin D-treated endothelial cell with shear stress. (A) control endothelial cell, (B) 1 hr cytochalasin D treatment, (C) 5 min, and (D) 60 min exposure of shear stress.*

Colorado, 1993), vol. BED-24, pp. 285-288.

12. G. A. Truskey, J. S. Pirons, The effect of fluid shear stress upon cell adhesion to fibronectin-treated surfaces, *Journal of Biomedical Materials Research* **24**, 1333-1353 (1990).

13. A. R. Koslow, et al., A flow system for the study of shear forces upon cultured endothelial cells, *Journal of Biomechanical Engineering* **108**, 338-341 (1986).

14. J. S. Budd, K. E. Allen, P. R. F. Bell, Effects of two methods of endothelial cell seeding on cell retension during blood flow, *British Journal of Surgery* **87**, 878-882 (1991).

15. R. Nerem, Cellular engineering, *Annals of Biomedical Engineering* **19**, 529-545 (1991).

16. G. Helmlinger, R. V. Geiger, S. Schreck, R. M. Nerem, Effects of pulsatile flow on cultured vascular endothelial cell morphology, *Journal of Biomechanical Engineering* **113**, 123-131 (1991).

17. D. I. Shin, et al., Morphological changes of human endothelial cell under fluid flow, E. A. Friedman, M. M. Beyers, Eds., American Society for Aritificial Internal Organs, 40th Anniversary Meeting (ASAIO Publisher, San Francisco, California, 1994), pp. 95.

18. S. Kasas, V. Gotzos, M. R. Celio, Observation of living cells using the atomic force microscope, *Biophysical Journal* **64**, 539-544 (1993).