Organization of F-Actin on Cytochalasin D treated Endothelial Cells under Shear Stress

Jun Keun Chang*, Jin Hee Kim°, Hyun A Chang°, Dong Ik Shin*,
Jongwon Kim°, Dong Chul Han**, Byoung Goo Min*°.

*Dept. of Biomedical Engineering, °Inst. of Biomedical Engineering,
and **Dept. of Mechanical Design and Production Engineering,
Seoul National University

Introduction

Shear stress induces configurational changes in endothelial cells under both *in vitro* and *in vivo* conditions. These cells become more elongated and oriented to the direction of the flow [1, 2]. When endothelial cells are in low-shear regions (≤ 3-5 dyne/cm^2) of blood vessels or under static conditions, actin microfilament mainly localize at the periphery of the cells, referred to as 'dense peripheral band' [1]. The formation and redistribution of actin microfilament bundles or stress fibers accompanies these shape changes as well as axial realignment in microtubules [3]. Studies characterizing the actin microfilaments suggested that the actin microfilaments are important in the repair of the endothelium and in the maintenance of endothelial integrity [4] and intracellular signaling [5-7].

The objectives of study is to examine the distributional changes occurring in actin microfilaments and focal contacts of endothelial cells as a consequence of shear stress. To clarify whether the cell shape or the cytoskeleton is dominant when endothelial cell recognize the shear stress or not, cytochalasin D was used to disrupt the cytoskeleton. For this investigation, subconfluent endothelial cells adhered on fibronectin-coated glass surface were used.

Flow Chamber and Experimental Setups

A new flow chamber system and image analysis system were developed to investigate the mechanotransduction of endothelial cells. Exposure of shear stress for several hours is needed to study the morphological changes and cytoskeleton organizations of cells, more accurate temperature and pH control of circulating culture medium is very important to maintain the physiological conditions of *in vitro* cell culture with fluid flow. Thus, a new flow chamber system was developed to satisfy following requirements, i) accurate temperature control of circulating culture medium, ii) pH control of circulating culture medium, iii) shear stress of physiological levels (less than 30 dyne/cm^2) which driven by the hydrostatic pressure head to eliminate the pulsatility of the recirculating roller pump, and iv) convenient sterilization of whole system to prevent the contamination of cells during flow exposure. Figure 1 shows the schematic illustration of the experimental system.

Laminar flow chamber for endothelial cell adhered on the micro slide glass [8] was designed to expose cultured cell with a known hydrostatic shear stress. The flow chamber for slide glass consists of four parts: a stainless-steel base block measuring 88 by 38 by 13 mm, two silicon rubber gaskets (Korea ShinEtsu Silicon Co., Seoul), a glass window, and a fibronectin-coated micro slide glass on which endothelial cells were cultured.

The magniture of shear stress, τ_w, developed in laminar flow chamber which driven by hydrostatic pressure head, H, was calculated from the pressure difference, ΔP, between the upper and lower reservoirs and the gab, h, between wide parallel plates (two glasses in this flow chamber). The shear stress τ_w (dyne/cm^2) generated on the endothelial cell monolayer could be calculated as follows:

$$\tau_w = \mu \frac{6Q}{wh^2} \qquad (1)$$

For 200 μm gab height flow chamber, shear stress τ_w (dyne/cm^2) could be expressed with the first-order equation:

$$\tau_w = 1.7818H \qquad (2)$$

with H the the length of hydrostatic pressure head, expressed in centimeter.

Cytoskeleton Organization Experiments with Cytochalasin D-treated Endothelial Cell

Rapid induction of cytoskeleton polymerization was investigated with the laminar flow chamber apparatus. Each cytoskeleton was disrupted with anti-cytoskeleton drugs to verify the flow-induced initial polymerization of cytoskeleton. Morphological changes of endothelial cell with shear stress were recorded with MIPS (Choong Wae Medical), the changes of cellular height were derived with MFCS (Choong Wae Medical) and MIPS.

Shear stress with physiological range was used for the cytoskeleton induction. Defined flow condition was:

$$H = 11.2 \text{ cm},\ \tau_w = 20 \text{ dyne/cm}^2,\ Re = 26.6 \quad (3)$$

with H the length of hydrostatic pressure head, τ_w wall shear stress, and Re Reynolds number. Fluid flow was exposed to cultured endothelial cell for 1-2 hours, and the temperature and pH were maintained 37 °C and 7.4 during experiments, respectively. The flow chamber was sterilized with ethylene oxide and the whole flow lines and reservoirs which contact with circulating perfusate were autoclaved before experiments.

Results and Discussion

Morphologic changes of endothelial cells adhered on 5 $\mu g/cm^2$ *fibronectin-coated glass with cytochalasin* D treatment were evaluated at 20 dyne/cm^2 shear stress. Very rapid recovery of cellular area was evoked by 2 hours fluid shear exposure, as shown in Figure 2. After the treatment of cytochalasin D, endothelial cells shrink around the nucleus with lammellapodia. The arrow in the upper left corner presents the flow directions. The number below the photograph indicate the lapsed time of fluid shear stress exposure. As time goes by, endothelial cells recover the cellular area and volume with shear stress by the new focal adhesion point at cell periphery which was served from lammellapodia. The cellular are of endothelial cells was recovered to original level just after 40-50 minutes passed from the initiation of flow. This recovery follows the rapid polymerization of G-actin which was stimulated by shear stress.

To confirm the shear-induced polymerization of actin microfilaments and the involvement of actin-binding proteins, endothelial cells were stained with rhodamine phalloidin and monoclonal mouse anti-talin antibody with the indirect double immunofluorescent staining after 1 hour flow exposure. Endothelial cells in Figure 3 were stained just after treatment of cytochalasin D for 1 hour and cells in Figure 4 were stained after 1 hour flow exposure on the cytochalasin D-treated endothelial cells. Figure 3 (A) shows the disrupted actin microfilaments with cytochalasin D treatment and Figure 3 (B) shows the localization of talin in the lammellapodia which serve as a focal adhesion site at the *shear-induced cytoskeleton recovery*. Figure 4 (A) shows the well-distributed newly polymerized F-actin to the direction of shear stress (flow moves left to right) and Figure 4 (B) represent the focal contact formation at the periphery of endothelial cell for tight binding to resist the

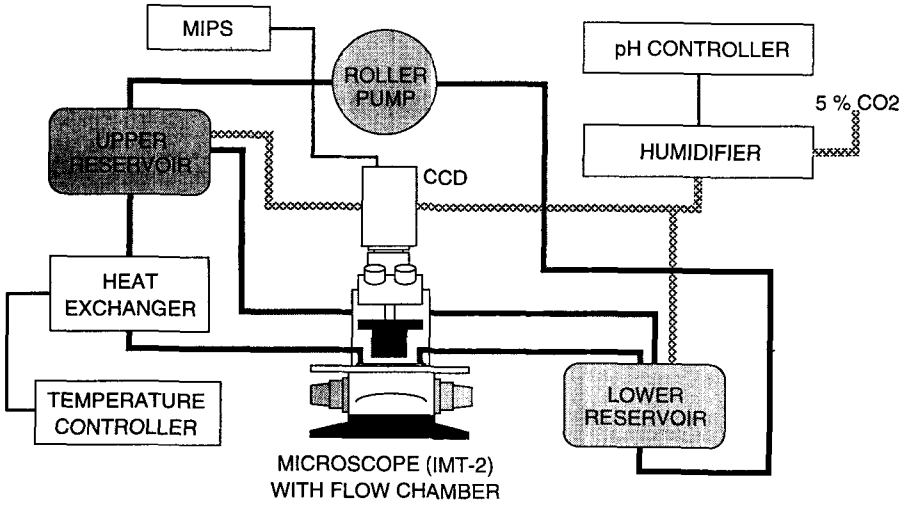


Figure 1. Schematic illustrations of experimental system.

shear stress.

The preferential location of atherosclerotic plaques at bifurcation and at the orifices of branch vessels has promoted speculation that fluid shear stress within these regions may contribute to the pathogenesis of circulatory disease like atherosclerosis and stenosis [9]. At the same time, experimental studies continue to implicate the destabilization of cytoskeleton of endothelial cell as an initiating factor in lesion formation [3]. As a conclusion, shear stress evoke the rapid induction of actin microfilament polymerization and morphology recovery in the cytoskeleton-disrupted endothelial cells.

References

1. D. W. Kim, B. L. Langille, K. K. Wong, A. Gotlieb, Patterns of endothelial microfilament distribution in the rabbit aorta in situ, *Circulation Research* **64**, 21-31 (1989).
2. R. Nerem, Cellular engineering, *Annals of Biomedical Engineering* **19**, 529-545 (1991).
3. A. Wechezak, T. N. Wight, R. F. Viggers, L. R. Sauvage, Endothelial adherence under shear stress is dependent upon microfilament reorganization, *Journal of Cellular Physiology* **139**, 136-146 (1989).
4. G. Gabbiani, F. Gabbiani, D. Lombardi, S. M. Schwartz, Organization of actin cytoskeleton in normal and regenerating arterial endothelial cells, *Proceedings of National Academy of Science* **80**, 2361-2364 (1983).
5. B. Alberts, et al., *Molecular biology of the cell.* (Garland Publishing, Inc., New York, 1994).
6. C. Vaziri, C. P. Downes, Association of a receptor and G-protein-regulated phopholipase C with the cytoskeleton,

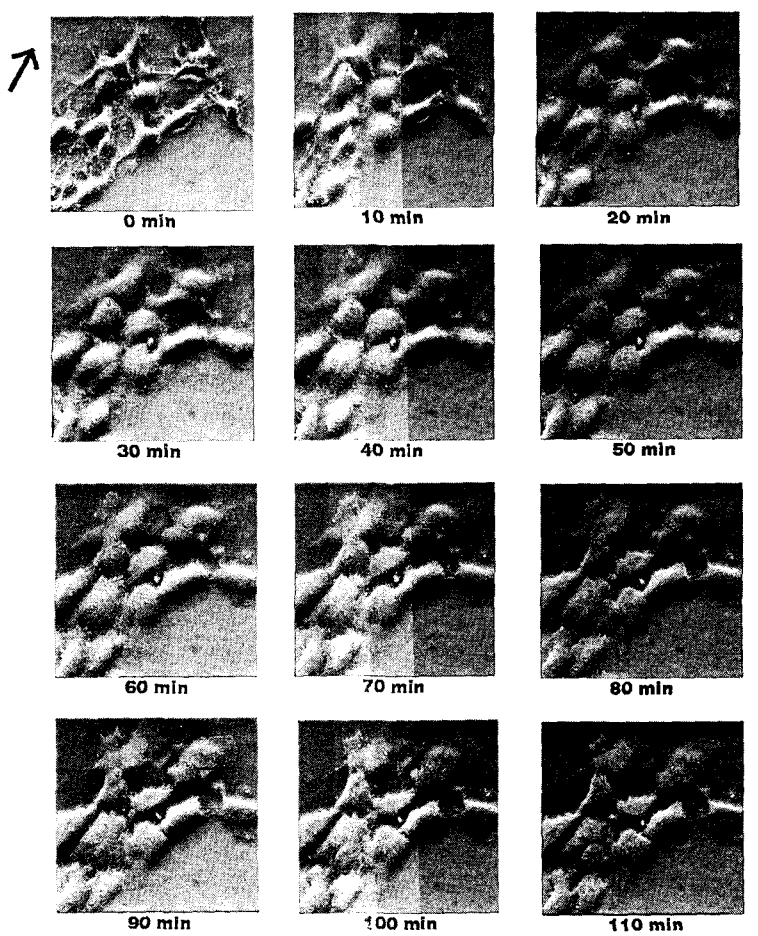


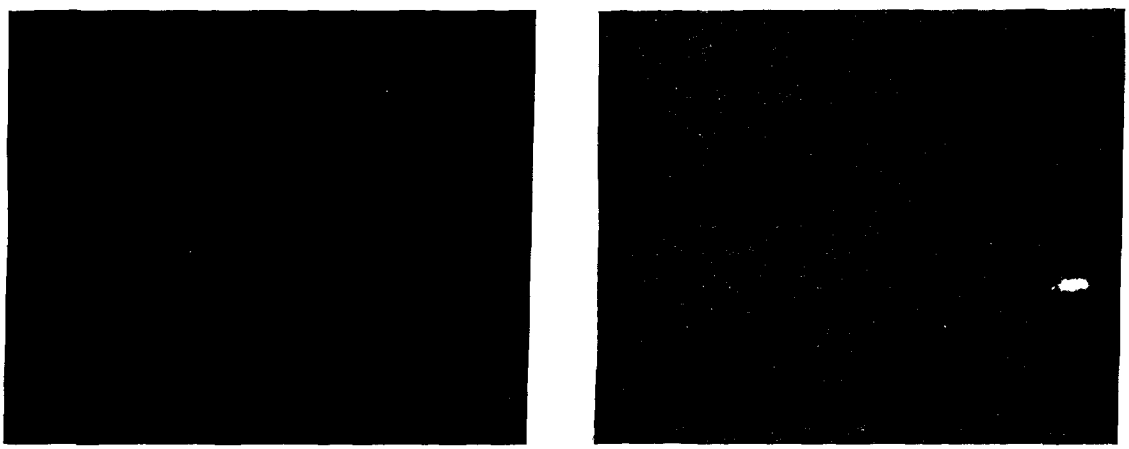
Figure 2. Micrographs of cytochalasin D-treated endothelial cell with shear stress. Rapid recovery of cellular area was evoke with 2 hour fluid shear exposure.

The Journal of Biological Chemistry **267(32)**, 22973-22981 (1992).

7. B. Payrastre, et al., Phosphoinositide kinase, diacylglycerol kinase and phospholipase C activates associated to the cytoskeleton: Effect of epidermal growth factor, *The Journal of Cell Biology* **115(1)**, 121-128 (1991).

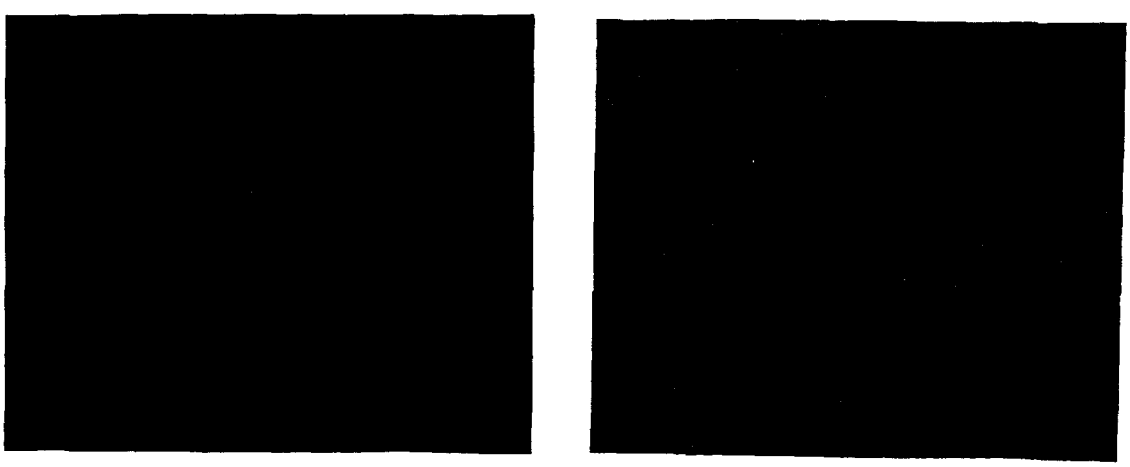
8. N. Kawagoishi, et al., In vitro evaluation of platelet/biomaterial interactions in an epifluorescent video microscopy combined with a parallel plate flow cell, *Artificial Organs* **18(8)**, 588-595 (1994).

9. R. M. Nerem, Vascular fluid mechanics, the arterial wall, and atherosclerosis, *Journal of Biomechanical Engineering* **114**, 274-282 (1992).



(A) (B)

Figure 3. Actin cytoskeleton (A) and talin (B) in cytochalasin D-treated endothelial cells.



(A) (B)

Figure 4. Acin cytoskeleton (A) and talin (B) in cytochalasin D-treated endothelial cell with 1 hour exposure of fluid shear.