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Effects Of Disturbed Flow On Endothelial Cells

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ABSTRACT

Atherosclerotic lesions tend to localize at curvatures and branches of the arterial system, where the local flow is often disturbed and irregular. The effects of such flow conditions on cultured human umbilical vein endothelial cells (HUVECs) were stuied in vitro by using a horizontal flow channel. Detailed shear stress distributions and flow structures have been computed by using the finite volume method in a general curvilinear coordinate system. HUVECs in the separation areas with low shear stresses were generally rounded in shape. In contrast, the cells under higher shear stresses were significantly elongated and aligned with the flow direction. These *in vitro* experiments have provided data for the understanding of the *in vivo* responses of endothelial cells under complex flow environments found in regions of prevalence of atherosclerotic lesions.

Key words : Atherosclerosis, Disturbed flow, Endothelial cells, Morphology, Shear stress

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INTRODUCTION

Vascular endothelium has received much attention in the study of pathogenesis of atherosclerosis [1, 2]. As an interface between the blood and the vessel wall, the endothelium occupies a unique location directly exposed to the hemodynamic forces imposed by the flow of blood. There is ample evidence that hemodynamic forces can exert significant influences on the endothelial cells in terms of their morphology [3, 4], cytoskeleton organization [5, 6], ion channel activation [7] and gene expression[8]. All these factors may be implicated in the early development of atherosclerosis. *In vivo*, the hemodynamics of blood flow is complex [9, 10], and the local flow patterns in the arteries are quite different from steady laminar Newtonian flow. Car and Kotha [11] have recently reported that the separation surface at a bifurcation is dependent on the Reynolds number and branching geometry, and that there is a marked difference between the seperarion surfaces of T and Y bifurcations, especially at higher Reynolds numbers. Flow characteristics such as boundary layer separation, eddy formation, recirculation, and secondary flow may be enhanced in regions near the arterial branches and bends, where atherosclerotic lesions are prone to develop [12].

Alignment of cells and reorientation of cytoskeletal proteins under various flow conditions have been demonstrated *in vivo* and *in vitro* [4, 5]. *In vivo* studies [13] have shown that the ECs near the apex of a flow divider, where the shear stresses on the cells are high and steady, appear to be stretched in the local flow direction. In contrast, on the lateral walls of branching sites, where the shear stresses are low and variable, the ECs tend to be round in shape.

There are a few *in vitro* studies on the effects of complex flow conditions on ECs. Dewey et al. [14] subjected vascular ECs to unsteady fluid shear stress, and emphasized that the shear stress gradient is as important as the absolute stress level in determining cell responses. Helmlinger et al. [15] have investigated the responses of cell shape, orientation, and actin microfilament localization to pulsatile flows with different types of shear stress distributions.

Though the modern research of disturbed flow on endothelial cells has switched from the morphological alternation to the differential alternation. Yet the aim of the present *in vitro* study is only to investigate the responses of cultured HUVECs to complex flow conditions induced in a horizontal flow channel (HF), in terms of alterations of cell shape, orientation. If it works, then I can do progressive experiments in this way to indentify more events in the context of atherosclerotic lesion formation. In the type of expansion flow found in the HF, eddy formation

and flow separation develop well below the critical Reynolds number that leads to turbulence. These inertial effects may create flow patterns which are similar to those found in artery branch points. In the present work, subconfluent HUVEC monolayers were tested; the results were analyzed quantitatively and compared with the detailed flow structures, which are derived by the use of a computer program based on the finite volume method in a boundary-fitted coordinate system [16].

METHODS AND PROCEDURES

Flow Channel: A horizontal flow chamber (HF) was employed to simulate some features of the flow patterns near arterial branches and bends, e.g., flow separation and reattachment. The resulting stagnation line and eddies may be locally similar to arterial flow separations *in vivo*. The HF was created by combining two parallel flow channels [17]. The glass slide and the gasket were fastened between a polycarbonate base plate and a stainless plate, using vacuum suction. In the test section, the channel width was 1 cm, the step width was 0.1 cm and the channel height was 0.05 cm. Total length and the entrance length were 4.5 cm and 1.5 cm, respectively. The flow rate was 78 ml/min. Figure 1 shows the diagram of the HF. To visualize the experimental flow patterns, red blood cells fixed with 4% paraformaldehyde in PBS were used as the maker particles.The hematocrit for this purpose was about 0.1% and the cells were clearly visible under phase microscopy.

Experimental Procedures: ECs were isolated from fresh human umbilical cords by collagenase perfusion [18]. The cell pellet was resuspended in a culture medium consisting of medium M199(Gibco, Grand Island, NY, USA) supplemented with 20% fetal bovine serum (Gibco, Grand Island, NY, USA), 30 μg/ml endothelial cell growth supplement (ECGS, Collaborative Research Inc., Bedford, MA, USA), and 1% penicillin/streptomycin (Gibico, Grand Island, NY, USA). The cell suspension was seeded onto glass slides (75 by 26 mm, Corning, Corning, NY, USA) which had been pre-coated with a layer of fibronectin (5μg/cm^2). The slide was maintained at 37°C in a incubator with a humidified mixture of 95% air and 5% CO_2, until the attainment of a subconfluent monolayer with a steady cell density of 4-6 x 10^4 cells per cm^2. The slide with the monolayer of cultured cells was mounted in the flow chamber and connected to a perfusion loop system, which was kept in a constant-temperature controlled enclosure and maintained at pH 7.4 by continuous gassing with a mixture of 5% CO_2 in air. The

osmolality of the perfusate was checked and adjusted to 285-295 mOsm/kg H_2O during the perfusion. The chamber was placed on the stage of an inverted microscope (Diaphot, Nikon, Tokyo, Japan). A CCD video camera (WV-50, Panasonic, Tokyo, Japan) was attached to the microscope, and the video image was transmitted to a video monitor and recorder (JVC, Tokyo, Japan), enabling the recording of all results in the video field. The cells were exposed to the flow for 24 hr. After flow exposure, the cells were photographed.

Computational Simulation: The detailed flow patterns and shear stress distributions were computed using a numerical technique, the finite volume method, in a general curvilinear coordinate system. The calculation procedure utilized a pressure-based algorithm, which had been employed previously in simulating internal flows [16], such as the flows in arteries and bifurcations. Detailed procedures have been described in Lee and Chiu [19]. The numerical technique was validated first by comparing the computed velocity fields with the experimental flow patterns. Good agreement (within 5%) was obtained between the experiments and the numerical predictions.

Studies on Morphology: The cell morphology in the preshearing condition and after 24 hr of exposure to flows was examined by using a phase microscopy and recorded to determine the alterations of cell shape and orientation. Photomicrographs of cell morphology in different flow areas were taken from the HF. In order to determine the projected cell area, cell perimeter, and shape index (S.I.), the cell boundaries were traced manually, and all cells in each selected area were analyzed quantatively using a NIH image software, written by Wayne Rasband of the National Institutes of Health. The accuracy of this method, as used in this study, was verified to have less than 1% error by calculating specific shapes with known theoretical areas. The S.I., which is defined as (4π area/perimeter^2), equals 1 for a circular cell and approaches zero for a highly elongated cell. Statistically, the Student's t-test was used to determine the significance of differences of the mean values between cells in the preshearing condition and after 24hr of flow. The level of statistical significance was selected as $p<0.01$.

RESULTS

Computational Results. Figure 2 shows the computed shear stress distributions on the HF. It should be noted that in HF, the shear stress varies in the flow direction primarily. Large shear stress gradients exist in the region of flow disturbance where was located in the separation area.

The shear stress distribution in the fully developed flow area was nearly constant (26 dynes/cm^2). These results are consistent with those obtained for a uniform Poiseuille flow, $\tau = 6Q\mu/(wh^2)$, where Q is the flow rate, μ is the dynamic viscosity, w is the channel width, and h is the channel height.

Morphological Findings. Figure 3 shows the morphological photograph of the subconfluent EC monolayers by exposure in the HF for 24 hr. The shape changes of the ECs after exposure to flow for 24 hr were analyzed quantitatively by assessing the S.I. of the cells at 0 hr and 24 hr, and the results are shown in Table 1. These results on cell shape are consistent with those reported by others on steady laminar flow and disturbed flow. After exposure to flow for 24 hr, the S.I. of the ECs in separation area, where the mean shear stresses were very low (<1 dyne/cm^2) was significantly higher than those in the fully developed area, where the mean shear stress was high (>25 dyne/cm^2).

DISCUSSION

The propensity for atherosclerotic lesion development in the regions near the arterial branches and curvatures suggests that hemodynamic factors play an important role in the initiation and progression of atherosclerosis [7, 14, 20]. It has been proposed that the complex flow characteristics at arterial branches and curvatures, such as flow separation, oscillating shear stress, and large shear stress gradient, could contribute to the observed pathology of the artery wall [21]. These local complex flow patterns cause changes in EC morphology [20]. In the present study using a HF, the cells in the regions with a low shear stress environment became more rounded in plane view, whereas the cells under high shear stress became elongated and aligned with the local flow direction. It was supported by the S.I. of ECs from the present data (Table 1)also.

Atherosclerosis is a multifactorial disease involving a complex array of circulating blood cells and plasma components, their interactions with the cells and matrix proteins of the arterial wall, and the effects of flow pattern on mass transfer. It has been postulated that the rounded ECs in the vicinity of flow separation in arteries *in vivo* tend to have a higher turnover rate and that the intracellular junction around mitotic cells may become leaky to allow for a local influx of low density lipoproteins (LDL). The aim of this report is only to study on the effect of disturbed flow on endothelial cell morphology by the use of a horizontal flow chamber. This

horizontal flow chamber can be useful in future experiments for examining cytoskeletal organization, LDL accumulation, cell division, gene expression or other states of the ECs under flow.

From the present study I postulate that the reorganization of cytoskeletal structure coupled with the alternation of cell morphology are important responses to the local hemodynamic environment. The rounded cells observed in the regions near the arterial branches are exposed to shear stress fluctuations and thus vulnerable to injury or death. The flow reversal and reattachment in the separation region will enhance the effects of mass transfer and blood-artery wall interaction. These may be factors in the formation and development of atherosclerosis.

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FIGURES AND TABLES

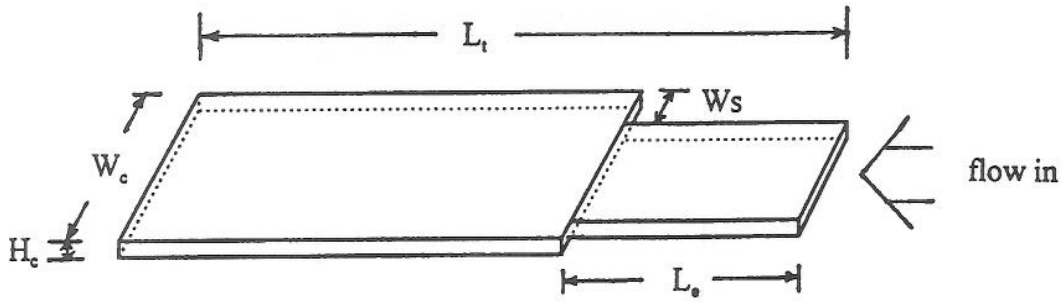

Fig. 1a. Diagrams showing the horizontal flow channel (HF). Direction of the main flow is right to left. L_t : the total length (= 4.5 cm), L_e : the entrance length(= 1.5 cm), W_c : the channel width(= 1 cm), W_s : the step width(= 0.1 cm), H_c : the channel height (= 0.05 cm).

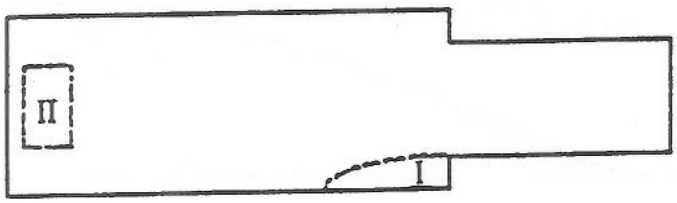

Fig. 1b. The overlooking diagram of HF. I : the separation area, II : the fully developed area.

Direction of the main flow is right to left.

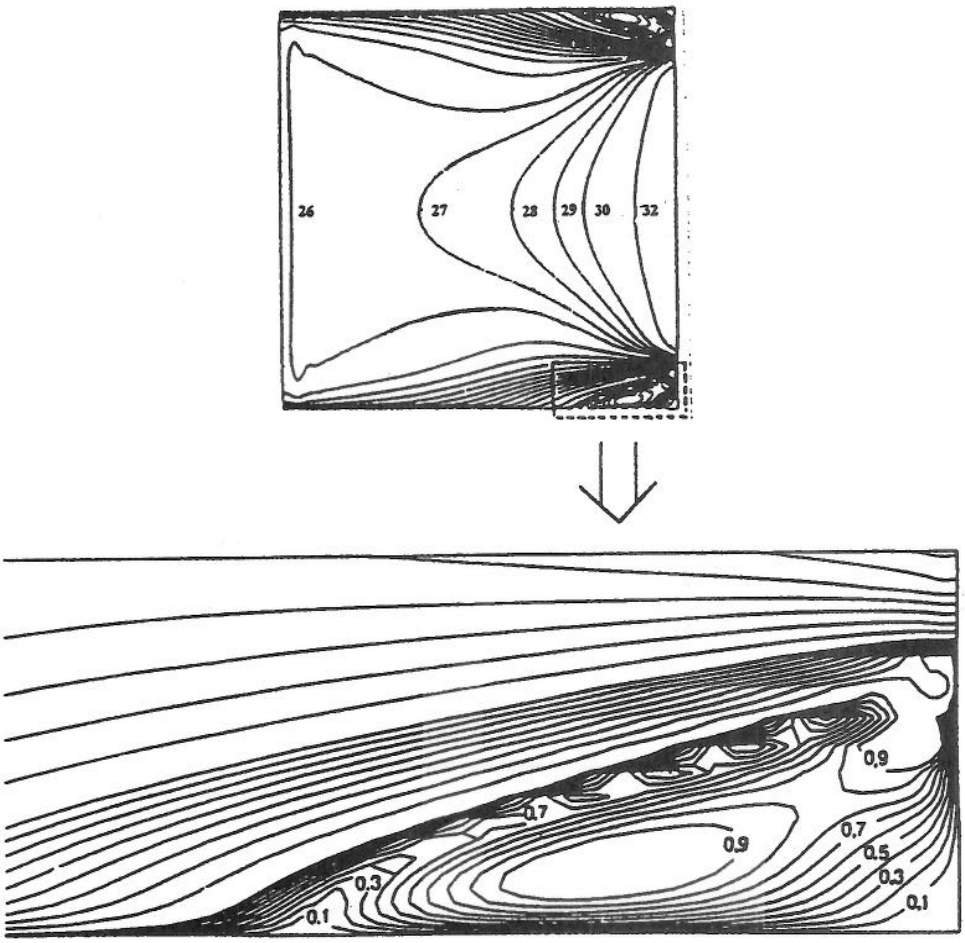

Fig. 2. Computer-simulated shear stress distributions on HF. Direction of the main flow is right to left. It is emphasized that most of the region of the lower chart is located on the separation area.

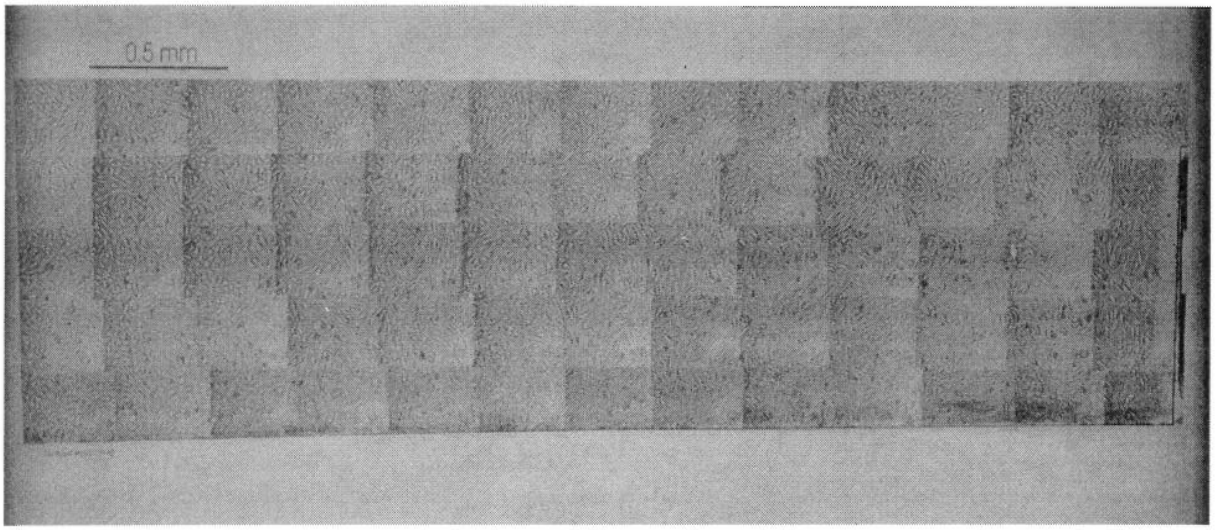

Fig. 3a. The photograph of subconfluent EC monolayers before shearing. The right lower part of this figure is located on the separation area. Direction of the main flow is right to left.

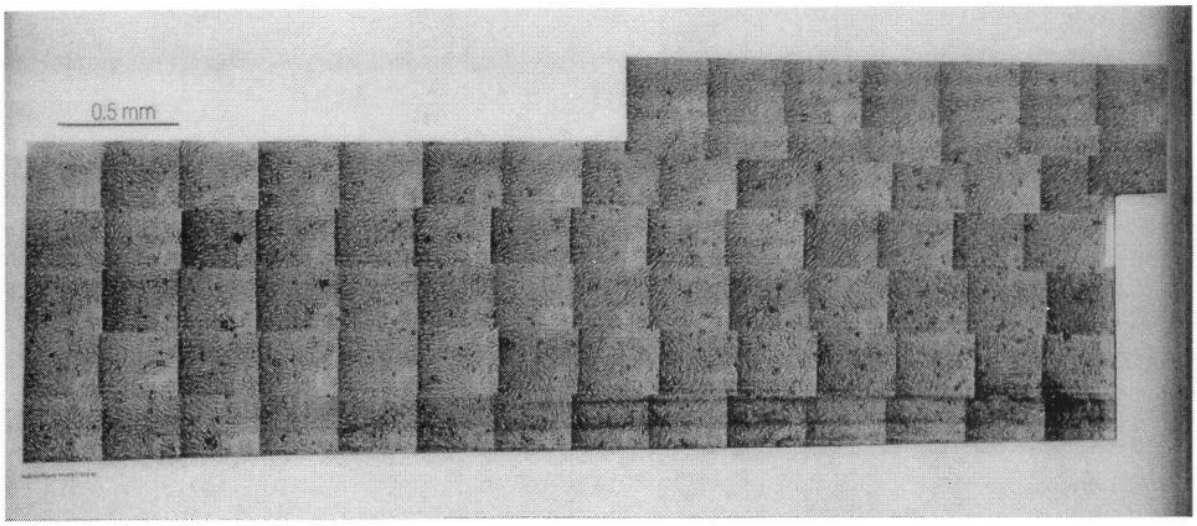

Fig. 3b. The photograph of subconfluent EC monolayers after shearing 24 hr. The right lower part of this figure is located on the separation area. Direction of the main flow is right to left

Table 1. Regional changes in shape index of the subconfluent EC monolayers subjected to shear in a horizontal flow chamber.

	Separation area	Fully developed area
Measured cell number at 0 hr	1376	1376
Measured cell number at 24 hr	1402	334
Average τ (dynes/cm^2)	0.51	26.00
Shape index at 0 hr	0.54 ± 0.15	0.54 ± 0.15
Shape index at 24 hr	0.63 ± 0.13	0.34 ± 0.14
t-test for shape index*	+	+

"+" represents significant difference ($p < 0.01$) between shape index at 0 and 24 hr

擾流對內皮細胞的影響

張振華*

摘 要

動脈硬化的損害區域常出現在動脈系統的彎曲處與分岔處，在這些地方的血流呈現擾動與不規則的狀態，本實驗之研究目的乃藉著水平層流槽的裝置進行擾流對人類臍靜脈內皮細胞之影響的體外研究，詳細的剪力分佈與流體結構可藉由處於曲線坐標系的有限體積法來加以計算。研究結果發現處於低剪力的分隔區之內皮細胞在形態上呈現較圓之趨勢，相對的，處於高剪力狀態下的內皮細胞之形態則有顯著的被拉長以及沿著流向分佈的趨勢。以上這些實驗結果可望助於了解活體中的內皮細胞受到擾流影響所造成之反應，進一步增加我們對動脈硬化成因的認識。

關鍵字：動脈硬化，擾流，內皮細胞，形態，剪力

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