Empirical Study on Grouping Behavior of Individual Endothelial Cells under Shear Stress*

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It has been recognized that the structure and function of the endothelial monolayer is affected by the applied mechanical stress. Microscopic observation of changes in cell morphology during the imposition of shear stress is necessary to identify the specific mechanism which mediates the cellular response to shear stress. In this study we observed the morphology and migration of individual cultured cells with the wall shear stress of 3 and 5 Pa under an optical microscope. We traced the particular group of cells under view, and examined the behavior of these particular cells during the imposition of shear stress. It was concluded that the initial orientation and shape of cells affect the process of receiving the external shear stress stimulus. The behavior of the individual cell depends on the geometry of surrounding cells. Thus, the external shear stress stimulus results in the "small grouping behavior", primarily due to the biological and mechanical interaction among cells.

Key Words: Endothelial Cell, Bio-Fluid Mechanics, Biomechanics, Biological Engineering, Cell Morphology

1. Introduction

An endothelial cell exposed to blood flow receives two kinds of mechanical stimuli; the wall shear stress due to the viscous blood flow and the circumferential normal stress due to the periodic stretching due to the pulsatile blood pressure. The structure and function of the endothelial monolayer are affected by these mechanical stresses which were determined by many previous studies (Nerem (1992)**, Davis and Tripathi (1993)**). In particular numerous in vivo and in vitro studies** have revealed that changes in the morphology and function of endothelial cells occurs solely due to the wall shear stress stimulus. Levesque and Nerem (1985)*** determined that the cell is elongated and aligned to the main flow direction by the exposure to shear stress for 24 hr at 1, 3 and 8.5 Pa. Subsequently many researchers from various fields have revealed disclosed new findings of cell structure and functions which are affected by the wall shear stress stimulus. Because the wall shear stress is derived from the hemodynamics in the vessels, the fluid dynamical studies from macroscopic viewpoints deal with the blood flow in the artery, to quantify the local wall shear stress distribution (Suzuki et al. (1992)***, Yamaguchi and Kohtoh**).

To identify the specific mechanism which mediates the cellular response to shear stress, the theoretical description of the flow near a wavy surface of endothelial cells has been discussed. Satcher et al. (1992)*** assumed the geometry of the cell surface to be of sinusoidal shape and obtained an analytical solution for the wall shear stress profile by the linear perturbation theory. Barbee et al. (1995)** calculated the wall shear stress and the shear stress gradient on the surface of endothelial cells based on their actual geometry, measured by atomic force microscopy.

These studies identified that the local geometry of cells determine the microscopic wall shear stress distribution along the cell surface. We should note that the shear stress on the cell surface depends on the
geometry of surrounding cells. Furthermore the mechanical and biochemical interactions between the cells possibly contributes to the variation of the cell structure and functions. In other words, the individual endothelial cell does not respond independently to the mechanical stimulus, but multiple cells forming a group cooperatively respond to the stimulus. This is referred to as the grouping behavior. The grouping behavior is associated with the interaction between cells, which is one of the fundamental cell functions. This grouping behavior hypothesis offers a more logical method of understanding cell functions.

We studied the change of cell morphology empirically under the shear stress stimulus, and observed the behavior of individual cultured cells in the flow chamber under an optical microscope. In particular, we traced the morphology and migration of cells under view, and examined the behavior of these particular cell groups under shear stress. This study is empirical and qualitative, and it demonstrates an important aspect of cell behavior under the shear stress stimulus, which few previous studies have examined.

2. Materials and Methods

2.1 Cell culture

Porcine aortic endothelial cells (PAECs) were harvested and cultured in an incubator under an atmosphere of 95% air and 5% CO₂, maintained at 37°C using a tissue culture medium (Dulbecco's Modified Eagle Medium, Gibco) containing 10% fetal bovine serum, 100 U/ml penicillin and 100 mg/ml streptomycin. Cells used in these experiments were in their fifth to seventh passage.

2.2 Flow chamber

PAECs were incubated on the inner surface of a flow chamber which was fabricated from polycarbonate. The inner surface of polycarbonate chamber was not coated by any chemical substances, because any cell separation was not observed without coating. The response of cells against the shear stress stimulus may depend on the nature of coated substances, we tried to observe the changes in cell morphology without the effect of coated substances. After the PAECs were confluent, the culture was circulated in the flow circuit. The structure of the flow chamber is shown in Fig. 1. It had a flow section which was 500 mm in height, 30 mm in width and 90 mm in length. Small reservoirs were installed at both the inlet and the outlet of the flow chamber to ensure an uniform velocity profile at the entrance of the flow section. Because the aspect ratio of the cross section of the chamber was 60, the flow structure was regarded as a planar flow between the parallel plates; a planar Poiseuille flow. The flow circuit consisted of two reservoirs, a flow chamber and a roller pump, and the circulation circuits were filled with the culture medium. The flow rate, measured using an electromagnetic flowmeter, was controlled by adjusting the height of the upstream reservoir. The wall shear stress imposed on the cells was 3 and 5 Pa for Reynolds numbers of 5.2 and 8.7, respectively. The micrographs of the cell morphology using phase contrast microscopy were taken using a still camera (Nikon F2) every one hr until the end of the experiment (24 hr). The view of the microscope was fixed during the observation period of 24 hr to trace the behavior of particular cells. Because the specific view arbitrarily selected was traced for 24 hr, the present results should be regarded as the specific case study rather than a general study of cell functions.

3. Results and Discussion

As shown in Fig. 2, we constructed the contours of individual cells in two small groups observed in the microscopic image under the wall shear stress of 5 Pa and traced the change of location and shape of the cells in the group until 24 hr had elapsed.

At the beginning of the experiment, the shape of the cells in the confluent condition was polygonal. As the cells were exposed to shear stress, the cell shape and orientation changed due to shear stress. The shear stress profile near the surface of the cell is influenced by the microscopic geometry of the cells. This mutual interaction between the shear stress and the cell geometry promotes further changes in cell shape and orientation. The individual cells responded to the external stimulus by influencing the surrounding cells and vice versa. This is how the cells became organized into a small group. The tracing of cell shapes observed from the obtained pictures shows that the polygonal cells gradually change into elongated ones. The cells in a group tend to orient toward a particular direction, which is not necessarily the main flow direction. We should note that some cells...
Fig. 2-1 The traces of cell boundaries in the two small groups at the beginning of the shear stress stimulus of 5 Pa. The bar represents 20 μm.

Fig. 2-2 The traces of cell boundaries in the two small groups at the shear stress stimulus of 5 Pa for 4 hr elapsed.

Fig. 2-3 The traces of cell boundaries in the two small groups at the shear stress stimulus of 5 Pa for 6 hr elapsed.

Fig. 2-4 The traces of cell boundaries in the two small groups at the shear stress stimulus of 5 Pa for 8 hr elapsed.

Fig. 2-5 The traces of cell boundaries in the two small groups at the shear stress stimulus of 5 Pa for 12 hr elapsed.

Fig. 2-6 The traces of cell boundaries in the two small groups at the shear stress stimulus of 5 Pa for 16 hr elapsed.

Fig. 2-7 The traces of cell boundaries in the two small groups at the shear stress stimulus of 5 Pa for 20 hr elapsed.

Fig. 2-8 The traces of cell boundaries in the two small groups at the shear stress stimulus of 5 Pa for 24 hr elapsed.
migrate rapidly downstream between cells. The direction of migration is not necessarily the same as that of the main flow, but is always influenced by the surrounding cells in the group.

Carefully observing the morphological changes in the cells, we determined an interesting response of the cells to shear stress stimulus. The cell designated as 1 in the left group under view (Fig. 2-1) did not exhibit any remarkable changes until 12 hr elapsed (Fig. 2-5). However, cell 1 elongates after 16 hr and penetrates into the space between cells 2 and 6 (Fig. 2-6). After 20 hr, the cell advances further between the cells. The migration velocity was determined by the moved distance of nucleus center in the individual cell during 1 hr. The migration velocity appeared to accelerate after the cell was elongated. The cell designated as 3 does not exhibit any remarkable change after 20 hr, but elongates after 24 hr have lapsed. The cell designated as 7 elongates after 6 hr and is accompanied by migration. The direction of migration and elongation is not in the flow direction, but that in the lower left direction. The cell shows a significant elongation between 6 and 12 hr. The neighboring cell, designated as 8, accompanies cell 7 during elongation and migration and reduces its size between 12 and 16 hr. At 24 hr, the cell 7 was divided into two cells.

The behaviour of cells in the small right group under view is different from that of the left group. The general feature of the cells in the right group is, that most of the cells do not change until 16 hr have elapsed. After that, they tend to elongate in a direction normal to the main flow. After 20 hr, they migrated toward the direction of the lower left. It is also interesting to note that the cell designated as 3 was always accompanied by cell 7. The cell designated as 10 oriented towards the upper left for a period of 12 hr, but turned its direction to the lower left after 16 hr. Generally speaking, the migration of cells in the group was remarkably accelerated after 16 hr had elapsed.

The summarized behavior of these cells is as follows. First, the direction of elongation and migration does not necessarily match the main flow direction. Second, the distance moved is not uniform for each duration. For instance, the velocity of a particular cell was 1 - 5 μm/h in the beginning, which accelerated slightly after 12 to 16 hr, then reached approximately 16 μm/h after 16 hr. Third, some cell shapes resemble the surrounding cells.

In Fig. 3, the major axis of each elongated cell is denoted by the bars under view. The elongated cell was distinguished by the enlarged ratio of major and minor axis of the polygonal cell shape. The length of each bar corresponds to the maximum length of the cells. After 24 hr had elapsed, the number of elongated cells increased significantly, but the distribution of the direction of the major axis of each elongated cell preserved the initial confluent state. We should note that all cells were not aligned in the direction of the main flow but 4 small groups with their major axes in the same direction were observed under view after 24 hr as shown in Fig. 3. This tendency illustrates that an individual cell responds to the shear stress stimulus by influencing the surrounding cells. This shows the previously mentioned grouping behavior of the cells.

Figure 4 shows some examples of cell migration trajectories in the small group. At the beginning of the shear stress stimulus, all the cells did not respond promptly and did not migrate downstream. On the contrary, cells B and C even migrated upstream. The cells A and D turned to the normal direction after the slight migration. Thereafter, these cells migrated downstream. Furthermore, we should note that the individual cell did not migrate independently after 6 hr, but were accompanied by the surrounding migrating cells.

Figure 5 shows the histogram of the cell directions from the initial state to after 24 hr had elapsed. We determined the cell orientations by measuring the
angle of the major axis from the main flow direction and counted the number of cells for every angle. Figure 5-1 is the histogram of cell angles at a shear stress of 3 Pa. In Fig. 5-1, the number of cells nearly aligned in the main flow direction is already large in the beginning and this tendency is gradually increased. Finally, the majority of the cells are aligned in the main flow direction after 24 hr had elapsed. This is a typical result recognized by many previous works. However, we did not always observe such an alignment toward the main flow direction. Figure 5-2 is the histogram of the cell angle at a shear stress of 5 Pa. In Fig. 5-2, the initial histogram appeared to have two peaks, roughly at angles of 50 and −70 degrees. Few cells were aligned in the flow direction. After 24 hr had elapsed, the number of cells with angles between 10 and −40 degrees increases, however, the number of cells aligned in the flow direction does not necessarily increase. These results indicate that the distribution of cell angles in the initial state influences the subsequent change of cell morphology and alignment. In other words, an individual cell will receive the external shear stress stimulus, which is dependent on the microscopic morphology of the cells.

Eskin et al. (1984) observed the morphological change in cultured endothelial cells, and determined that the duration required for the alignment depended on the initial state; the confluent state. The duration for alignment is 21 - 22 hr for a confluent culture, but 6.5 hr for a subconfluent culture. Their study indicated that the process of cell elongation and alignment was associated with the initial state of cells and that the initial state of the cells should be specified for the observation of the cell behavior. In this study we observed that the initial state was confluent and our observation demonstrated that the cells did not respond uniquely to the shear stress stimulus.

Masuda and Fujiwara (1989) dealt with the behavior of single cells under shear stress and they determined
that an overall alignment in the flow direction was not present. They observed that some cells aligned normal to the flow direction. Therefore, we should note that the response mechanism of a single cell is essentially different from that of multiple cells. Namely, the function of the multiple cell assembly is derived from interaction among cells. The interaction consists of two groups of factors, biological and mechanical.

The possible biological factors are as follows.
1. Cell-substratum interaction, nature of substrates
2. Cell adhesion characteristics
3. Endothelial cell growth factor
4. Intercellular interaction by surrounding cells
5. Reorganization of the cytoskeleton
6. Regulation of permeable functions

The biological interaction among cells contributes to the formation of tissues and plays an essential role in the biological system. However, many biological interactions among the cells have not been revealed yet, and require future studies in the area of cellular biomechanics.

The mechanical factors depend on the magnitude, direction of shear stress, and time varying characteristics. The cell membrane tension due to the imposed shear stress may be transmitted at the junction of two neighboring cells\(^{(1)}\). Furthermore, the microscopic flow field along the cell surface depends on the geometry of surrounding cells.

4. Concluding Remarks

The present study empirically argues that an individual endothelial cell does not respond to the shear stress stimulus independently but cooperatively, by interacting with the neighboring cells. This results in the cells demonstrating a grouping behavior during the shear stress stimulus.

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References