Cellular detachment characteristics of human umbilical vein endothelial cell by laminar shear stress

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INTRODUCTION

Cellular adhesion plays an important role in many biomedical applications including development of artificial organs, large-scale cell culture, and blood-tissue interactions of biomaterials. Analysis of the morphologic change and strength of cellular attachment force can be useful in the development of biomedical device where an intimate cell-biomaterial contact is needed or, alternatively, must be avoided. In the development of antithrombogenic vascular grafts, a firm attachment is needed when endothelial cells are seeded on the inner wall of artificial grafts in order to create a simple epithelium. The vascular endothelium bears the hemodynamic stress with changing the alignment of endothelial cells.

Previous studies of cellular adhesion have been focused on the molecular biology of adhered proteins in cell attachment and movement. In principle, the strength of attachment between the cell and substrate can be determined through breaking the contact by applying a known hydrodynamic force. Micro-pipette aspiration [1,2], centrifugation of cells [3], and exposure of cells to fluid shear stress [4-13] are used to measure the strength of cellular adhesion. Especially, morphologic change and the strength of attachment of endothelial cell were investigated through *in vitro* and *in vivo* considerations. [5-8, 10, 13-15] According to these studies, endothelial cells were oriented with the flow direction under influence of shear stress and become more elongated when exposed to higher shear stress. But, these experiments were performed under the low Reynolds number. Different from above cases, endothelial cells were aligned perpendicular to flow direction under the case of the higher Reynolds number.[7,8]

In this study, we have examined cellular detachment and the effect of flow to the morphologic change following exposure of human endothelial cell to steady laminar flow on a parallel-plate flow channel. To investigate the morphologic change and strength of attachment for a single cell, density of seeded endothelial cells was suppressed under $3 \sim 4 \times 10^4$ cells/cm^2 with preventing from forming the confluency. The purpose of this study is to obtain the correlation between the cellular characteristics of an endothelial cell and known hydrodynamic forces.

MATERIALS AND METHODS

CELL CULTURE Endothelial cells were obtained from human umbilical cord veins by the method of Jaffe.[16] The cell pellet were cultured in medium 199 containing 20 % fetal bovine serum (HyClone, UT), 2 mM/l L-glutamine, 20 mM/l HEPES, 100 units penicillin and 100 μg streptomycin (Sigma, MO). Cells were incubated in an atmosphere containing 5 % CO_2 at 37 °C.

Endothelial cells at passage 3 - 5 were seeded on a pre-treated polyurethane (PU) sheets. PU sheet, total surface area of 2.54 cm^2, was put in a plastic ring and fibroblasts isolated from fetal skin were cultured on PU surface. The cultured fibroblast on PU sheet were treated with hypotonic shock to remove the cytoskeleton and expose the extracellular matrix (ECM) on the surface. In the experiments endothelial cells were sparsely cultured ($3 - 4 \times 10^4$ cells/cm^2) on the natural ECM.

EXPERIMENTAL SETUP A parallel plate, channel flow device designed to provide steady, laminar flow was used to expose cultured endothelial cell with a known hydrodynamic forces. The flow path was formed by medical-grade silicon rubber gasket (300 μm thick). The channel is composed with a rectangular cross section whose height 2h, is much shorter than its length, L, and width 2B. The velocity profile for a Newtonian fluid has a simple

parabolic form which obtained by the Integral Weighted Residual Method.

Fig. 1 shows the geometry of the parallel-plate, channel flow chamber, used both for the experiments and in the computational fluid dynamics. Endothelial cells with polyurethane sheet immobilized plastic ring were assembled with channel-flow chamber. Channel flow chamber was mounted on the stage of an light microscope (Olympus, CK2, Japan). Cell shape changes were examined by a CCD camera (TOSHIBA, LK-636, Japan) attached to the microscope and connected to the image analysis system and a video recorder.

RESULTS AND DISCUSSION

Cellular detachment by laminar shear stress was investigated for the adhesion of human umbilical vein endothelial cell on ECM coated PU. In the experiments, HUVECs were sparsely cultured on the natural ECM which was secreted by human fibroblasts. A parallel plate, channel flow device designed to provide steady, laminar flow was used to expose cultured HUVEC with a known hydrodynamic forces up to 131 dyne/cm^2. Cells detached significantly for first 1 minute after flow starts and the number of detached cells were directly proportional to the magnitude of applied shear stress. (Fig.2 and Fig. 3) Maximum adhesion strength of HUVEC seeded on the natural ECM occurred on the 4th day after seeding. (data are not shown.) Endothelial cell adhesion is mediated by ECM proteins, especially fibronectin (FN), and their receptors, the integrin. To confirm the role of FN on the receptor-mediated adhesion, the same experiments were taken on the HUVEC cultured on FN-coated (5 μg/cm^2) PU. Maximum adhesion strength of HUVEC seeded on FN was occurred on the 2th day after seeding, and for these two days, adhesion strength of HUVEC on FN was larger than that on natural ECM. (Fig. 3) Higher adhesion strength implies that immobilized FN offers higher ligand density to integrin than natural ECM. Integrin ß chains physically interconnect FN with F-actin filaments via actin-associated proteins on the inner surface of plasma membrane, elevated ligand binding on integrins evokes the secure rearrangement of cytoskeleton on adhered cell. FN seems to play a dominant role in the initial cell shape control through the rearrangement of cytoskeleton during adhesion, which results different mechanotransduction to applied stresses. Proximal decrease of cell height can reduce up to 40 % of shear stress acting on the plasma membrane, and this stress-damping reaction

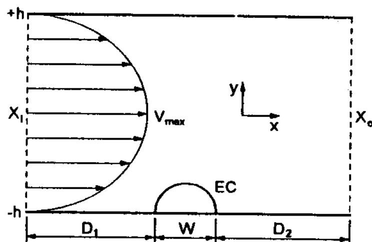

Fig. 1 Geometry of the parallel-plate flow chamber. Test fluid moves from left to right over a model endothelial cell of length L.

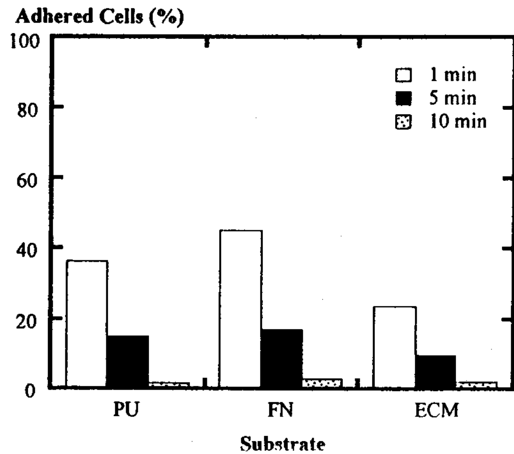

Fig. 2 Human umbilical vein endothelial cell adhesion on 2 days after seeding (131 dyne/cm^2, 380 ml/min, Re = 597.2)

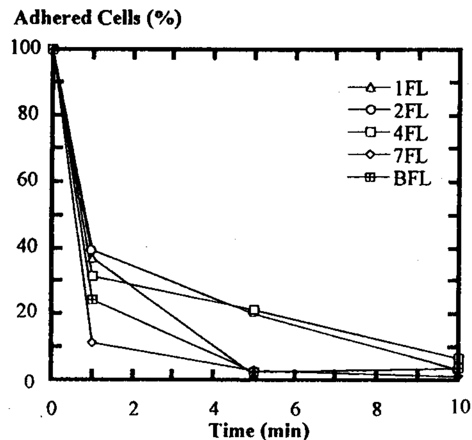

Fig. 3 Human umbilical vein endothelial cell adhesion on fibronectin coated surface (66 dyne/cm^2, 190 ml/min, Re = 298.6)

seems to preserve the right function of the stress activated ion channel on plasma membrane under large stress conditions. These results indicate that coupling of cytoskeletal rearrangement to auto-stress-damping reaction is involved in the mechanotransduction of adhered cell.

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