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# Design of Laminar Flow Chamber Apparatus for Endothelial Cell Physiology Study

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# 혈관내피세포의 생리적 반응 연구를 위한 평판형 층류발생장치의 설계

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**요 약**—혈관내피세포는 혈관의 내벽에 단일 층을 구성하고 있는 상피세포로 동맥경화나 혈관협착의 원인에 매우 중요한 역할을 하는 것으로 알려져 있다. 그리고, 모든 혈관 질환의 발생장소가 혈관이 나뉘는 분지부에 집중되고 있어, 혈류역학과 혈관질환 간에 상호연관성이 있음을 짐작할 수 있다. 특히, 최근에 와서 혈관내피세포가 혈액유동에 의해 발생하는 전단응력을 인지하여 혈관의 제반 생리적 반응을 조절한다는 연구결과가 속속 발표되고 있어, 혈관질환의 극복을 위한 연구 개발에 혈관내피세포에 대한 이해의 중요성이 증대되고 있다. 이에 본 연구에서는 혈관내피세포에 혈류와 같은 크기의 전단응력을 부가하여 세포의 생리적 반응을 고찰할 수 있는 평판형 층류발생장치를 설계, 제작하였다. 설계된 평판형 층류발생장치는 유동환경 하에서의 혈관내피세포의 동적반응을 고찰 할 수 있도록 유동액의 온도, 산도, 전단응력의 크기를 조절할 수 있도록 설계하였으며, 제작된 실험장치를 이용하여 전단응력에 의한 혈관내피세포의 형태변화를 고찰하였다. 개발된 층류발생장치는 혈관내피세포의 연구 뿐 아니라, 백혈구의 점착, 암세포의 전이등에도 다양하게 활용이 가능하다.

Key words —laminar flow chamber, shear stress, endothelial cell, physiology, atherosclerosis.

## 1. Introduction

The preferential location of the atherosclerotic plaques at bifurcation's and at the orifices of the branch vessels has promoted speculation that fluid shear stress within these regions may contribute to the pathogenesis of the circulatory disease like atherosclerosis and stenosis [1]. At the same time, experimental studies continue to implicate the endothelial cell injury as an initiating factor in the lesion formation [2].

Endothelial cells form a continuous layer only a single cell thick that lines the entire vascular system. In large and small arteries, these cells respond to acetylcholine and other blood-borne neurohormones by releasing the substances causing the relaxation of the smooth muscle of the vessel wall. Endothelial cells also respond to the mechanical forces generated by blood flowing under the pressure. The idea that the endothelium acts as a transducer of the hemodynamic forces to control the release of the vasoactive substances offers a simple explanation for how local changes in the vascular tone. Yet how endothelial cells sense the fluid forces and couple the initial mechanical deformation of the plasma membrane to the release of vasoactive substances is poorly understood [3].

When exposed to hemodynamic shear stress, endothelial cells undergo a sequence of the rapid responses which, if the stimulus is sustained, are followed by predictable biochemical [4], gene regulatory [5], and morphological changes [2]. The eventual outcome for these cells exposed to unidirectional flow is a streaming of the cell that leads to a lessening of the shear stress magnitude. Arterial shear

stress levels on the order of 25 dyne/cm$^2$ cause enhanced arachidonic acid metabolism and altered protein synthesis [4]. These observations suggest that fluid mechanical forces can directly influence the endothelial cell structure and function. Modulation of endothelial behavior by the shear stress may be relevant to normal vessel wall physiology, as well as the pathogenesis of vascular diseases, such as atherosclerosis [6].

In this paper, I describe a system that can be used to study the response of endothelial cells to shear stress generated by flow. In order to evaluate the effect of flow in a biological environment accurately, a parallel plate flow chamber with the temperature and pH control was designed. One application of the system to a study of the effect of shear stress on cultured endothelial cells was reported in this paper.

## 2. Description of Laminar Flow Chamber System

### 2-1. Laminar Flow Chamber

The laminar flow chamber for endothelial cell adhered on the micro slide glass was designed to expose cultured cells with a known hydrostatic shear stress. The laminar flow chamber, shown in Fig. 1, 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. On the top of the base, a flow path, 60 by 20 mm by 200 $\mu$m in height, was machined with the numeric-controlled milling machine and two silicon gaskets were fixed in two grooves around the flow path. The micro slide glass with cells held tightly on the base chamber block by applying a vacuum with vacuum pump (007CA13, Thomas Industries, Inc., Sheboygan, WI) through 6 holes to the space between the two gaskets to prevent the leakage of circulating medium, as shown in Fig. 1. Laminar flow chamber sterilized with ethylene oxide prior to the experiments. The flow chamber with flow and vacuum lines was turned upside down and installed on the microscope (IMT-2, Olympus, Japan).

### 2-2. Flow Circuit for Laminar Flow Chamber

The flow chamber was connected between the upper and lower reservoir through two 4 mm I.D. ports

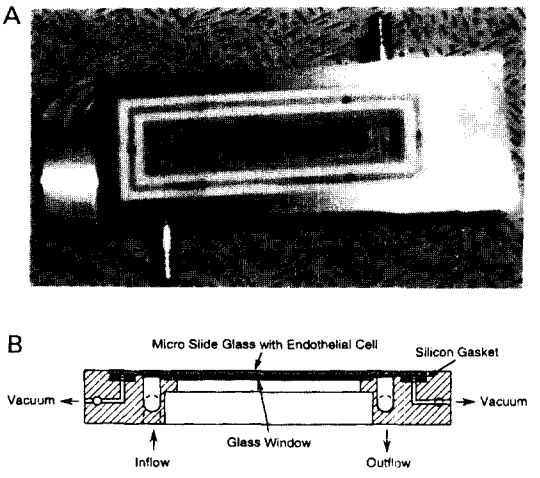


**Fig. 1. Laminar flow chamber driven by hydrostatic pressure head. (A) Stainless-steel base block was shown with silicon gaskets and a glass window. Six holes between silicon gaskets were connected to the vacuum pump to prevent medium leakage. I.D. 4 mm inlet and outlet ports were also shown in figure. (B) Schematic side view of laminar flow chamber. Flow path was generated between two glasses, a glass window and a micro slide glass with endothelial cells.**

and silicon tubing. A roller pump (MasterFlex L/S Drive with Easy LoadTM head, Cole-Pharmer Instrument Co., Chicago, IL) moves the perfusate from the lower reservoir to the upper reservoir. In the upper reservoir, medium cup was installed to maintain the same hydrostatic pressure head on the laminar flow chamber. The overflows from the roller pump were directly connected to the lower reservoir via overflow lines between the upper and lower reservoirs.

The temperature control of circulating fluid was accomplished with a heating mantle (S501, Misung Scientific Co., Seoul), a PID-temperature controller (Misung Scientific Co), a "surface mount" thermocouple (30 gauge, K-type, CO1, OMEGA Engineering, Inc., Stamford, CT), and a heat exchanging mini condenser. A heat exchanging mini condenser was connected between the upper reservoir and the laminar flow chamber. Hot-water heated with the heating mantle was supplied to condenser with the submergible water pump (UP-7W, HyupShin Water Design, Seoul) to maintain the perfusate temperature as 37°C. The supplied volume of the hot-water was adjusted with the flow regulator (300 cc/min, Dwyer,

Michigan City, IN). The PID-temperature controller, which get the temperature information from the "surface mount" thermocouples attached on the outer surface of condenser, controlled the gain of the heating mantle to adjust the temperature of the hot-water. The actual temperature of the perfusate was monitored invasively just after the laminar flow chamber with the needle type thermocouple (HYP2-21-1-1/2-T-G-48-OST-M, OMEGA Engineering, Inc.) and digital indicator (DP-11 Series, OMEGA Engineering, Inc.).

To regulate the pH of perfusate at 7.4, 5% $CO_2$ with 95% clean air was supplied to the upper and lower reservoirs. The supplied gas was humidified in the distilled water and filtered with 0.2 $\mu$m PTFE filter (ACRO 50, Gelman). The amount of filtered gas to both reservoirs was controlled with the air

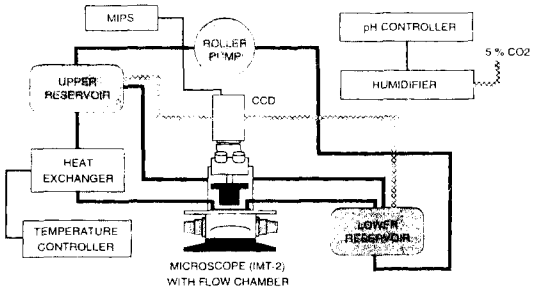


**Fig. 2. Schematic illustration of experimental setup with flow chamber apparatus.**

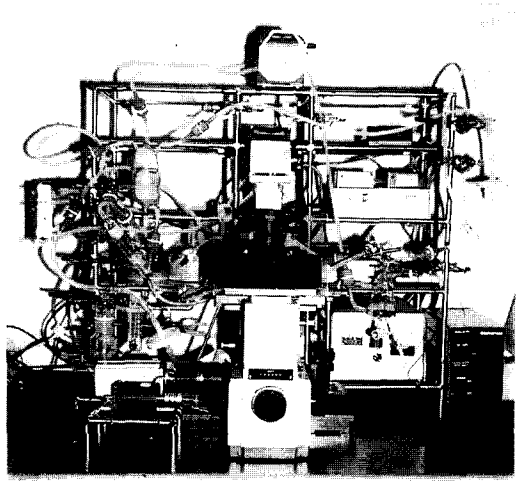

**Fig. 3. Photograph of experimental system. 4 mm I. D. flow lines were connected with autoclavable Keck quick disconnect adapters.**

flow regulator (25 cc/min air, Dwyer) and the excessive pressure of supplied gas from the gas tank was regulated with solenoid valve (SNS-C101, SAGInoMIYA, Japan) and check valve (AB-31-02-3, CKD/P, Japan). The gas outflow ports in the upper and lower reservoir were also connected to 0.2 $\mu$m PTFE filter to prevent the contamination of the gas environment of the flow system.

The flow lines were composed with 4 mm I.D. silicon tubing and autoclavable quick-disconnect adapters (L-06841-50 for tubing and L-06841-54 for coupling, Cole-Pharmer Instrument Co.). The cell culture medium was used as a circulating fluid. Fig. 2 shows the schematic illustration of experimental setup with flow chamber apparatus.

The morphology of cells was examined by a CCD camera (LK-636, TOSHIBA, Japan) attached to the microscope. Fig. 3 was taken the whole experimental system during the experiments.

### 2-3. Shear Stress developed in Flow Chamber

The magnitude of shear stress, $\tau_w$, developed in laminar flow chamber which driven by the hydrostatic pressure head, $H$, was calculated from the pressure difference, $\Delta P$, between the upper and lower reservoirs and the gab, $h$, between wide parallel plates (two glasses in this flow chamber). The pressure difference between two reservoirs was calculated simply from the hydrostatic relationship:

$$\Delta P = \rho g H \tag{1}$$

where $\rho$ is the density of the fluid (1 g/cm$^3$ for tissue culture medium) and g is the acceleration of gravity (980 cm/s$^2$ with CGS unit). For pressure drops in the reservoirs and flow lines were negligible, the pressure drop within the flow chamber was calculated with the pressure difference in Eq. (1) as follows:

$$\frac{\Delta P}{\Delta X} = \frac{dp}{dx} = \mu \frac{12Q}{wh^3} \tag{2}$$

with $\Delta X$ the laminar length of the flow chamber, $\mu$ the viscosity of fluid (0.007 g/cm/s for culture medium at 37°C), $Q$ the flow rate (mL/s), and $w$ the width of the flow path. Shear stress $\tau_w$ (dyne/cm$^2$) generated on the endothelial cell monolayer could be calculated as follows:

$$\tau_w = \mu \frac{6Q}{wh^2} \tag{3}$$

For 200 $\mu$m gab height flow chamber, shear stress

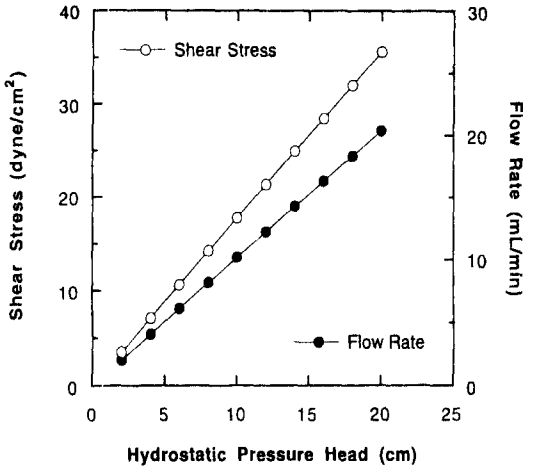


**Fig. 4. Shear stress and flow rate as a function of the hydrostatic pressure head. For 200 $\mu$m gab height flow chamber, shear stress (dyne/cm$^2$) could be expressed as $\tau_w$, and the flow rate (mL/min) as $Q$ =1.0182$H$, respectively.**

$\tau_w$ could be expressed with the first-order equation:

$$\tau_w = 1.7818H \quad (4)$$

with $H$ the length of hydrostatic pressure head, expressed in centimeter. Shear stress and flow rate (mL/min) were plotted as a function of the hydrostatic pressure head in Fig. 4.

## 3. Application to Cultured Endothelial Cell Studies

### 3-1. Isolation and Culture of Endothelial Cells

Human umbilical vein endothelial cells were isolated from human umbilical vein as previously described with modified collagenase digestion technique [7]. Umbilical cords obtained after normal vaginal deliveries are placed in cord buffer (phosphate buffered saline (PBS), 137 mM NaCl, 2.7 mM KCl, 8 mM $Na_2HPO_4$, 1.5 mM $KH_2PO_4$ with 11 mM glucose) and stored at 4°C until used. The cord was removed and then cannulated at one end with a stainless-steel cannula (syringe adapter, A.H. Thomas, PA). The vein was flushed with warm cord buffer to rinse out the blood and allowed to drain. The vein was finally filled with enough warm 0.2% collagenase (Clostridium histolyticum, type I, Worthington Diagnostic System Inc., Freehold, NJ) in cord buffer and incubated at 37°C for 10 minutes.

After incubation, the cord was gently kneaded twice between the fingers up and down its length. The collagenase solution was flushed out of the vein with cord buffer into a 50 ml conical plastic centrifuge tube containing 10 ml of culture medium (Medium 199 (Gibco BRL, Gaithersburg, MD) with HEPES (25 mM, Gibco BRL). The tube was centrifuged for 7 minutes at 800 g and the supernatant decanted. The endothelial cells, form a small pellet in the bottom of the tube, were gently resuspended in 4 ml of culture medium and plated in a T-25 tissue culture flask.

To optimize conditions for growing and adhesion of endothelial cells on the artificial surface, human fibronectin (Boehringer Mannheim, Mannheim, Germany) of 5 $\mu$g/cm$^2$ was coated on the slide glass [8].

### 3-2. Morphological Response of Endothelial Cells to Shear Stress

Cultured endothelial cells were seeded on the fibronectin-coated slide glass with $5\times10^4$ cells/cm$^2$ seeding density, and incubated for two days before experiments.

After removing the culture medium, endothelial cells were incubated with cytochalasin D (0.1 $\mu$g/ml, Sigma Chemical Co., St. Louis, MO) at 37°C for one hour before flow starts. After the incubation of cytochalasin D, drug was removed and endothelial cells were gently washed in PBS. Micro slide glass was assembled on the sterilized flow chamber and the 20 dyne/cm$^2$ shear stress was applied to cultured endothelial cell for 2 hours. Micrographs were saved automatically in every 5 minutes in the host PC after a shear exposure to record the morphological changes of endothelial cells with shear stress. Increase of adhesion area was analyzed with the serial micrographs with time.

Very rapid recovery of cellular area and adhesion was evoked within 2 hours fluid shear exposure, as shown in Fig. 5. After the treatment of cytochalasin D, endothelial cells shrink around the nucleus with abundant microspikes (shown in Fig. 5 at 0 min). The arrow in the upper left corner presents the flow directions. The numbers below the photograph indicate the lapsed time after 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 the cell periphery, which was served from the leading edge of microspikes.

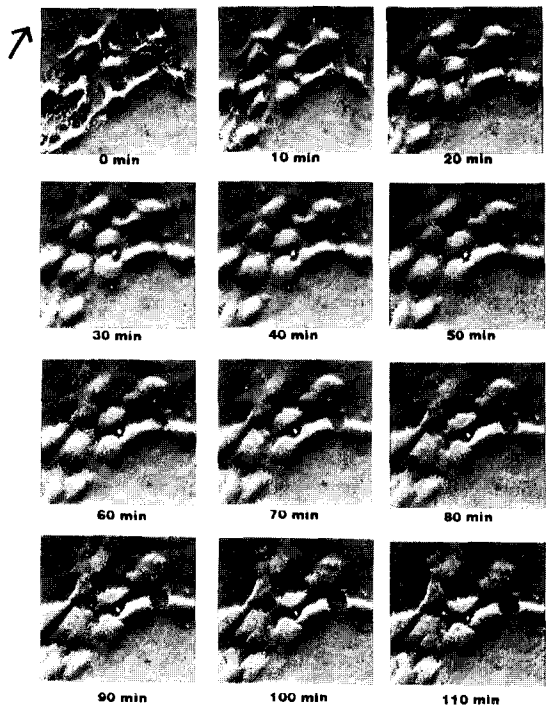


**Fig. 5. Shear stress-induced morphological changes of endothelial cells adhered on 5 $\mu$m/cm$^2$ fibronectin-coated glass with cytochalasin D (0.1 $\mu$g/ml) treatment for 1 hour. 20 dyne/cm$^2$ shear stress was applied for 2 hours. The arrow in the upper left corner presents the flow directions. The numbers below the photograph indicate the lapsed time after shear stress exposure.**

The cellular area of endothelial cells was recovered to the original level just after 40~50 minutes passed from the initiation of flow.

## 4. Conclusion

*In situ* laminar flow chamber experimental apparatus including real-time image analysis system was developed to investigate the mechanotransduction of endothelial cells. This system was suitable for the study of physiological response of anchorage-dependent endothelial cells with its capacity of controlling the temperature and pH of the circulating medium. Shear stress-induced morphological changes of endothelial cells were investigated with this apparatus. This experimental system is also of value for studies of other cells such as megakaryocytes, leukocytes, and tumor cell invasions.

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