# Effect of Pulsatile Shear Flow on Migration of Endothelial Cells Cultured on Tube

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## ABSTRACT

The effect of pulsatile shear flow on migration of endothelial cells was investigated *in vitro*. The inner surface of the silicone tube was sputtered in a vacuum to change the surface character from hydrophobic to hydrophilic, and coated with collagen gel. The endothelial cells of rat's descending aorta were cultured on the inner surface of the test tube. Two types of shear flow were applied to the endothelial cells: a continuous flow powered by a peristaltic roller pump, and a pulsatile flow powered by a diaphragm pump. The behavior of cells was observed through the tube wall by a phase contrast microscope. In pulsatile flow, the cells did not peel off at the mean shear rate of 15 1/s in 60 minutes. The change in orientation of cell occurs gradually with repeating peel off at the corner of the cell. The cultured endothelial cells on the collagen-coated wall of silicone rubber tube with sputtering did not peel off in pulsatile shear flow at 37 degrees C for 24 hours. The experimental results show that the longitudinal axes of the cells tilt toward the flow direction and that pulsatile flow may have an advantage for migration of cells.

**Keywords:** Endothelial Cells, Pulsatile Flow, Shear Rate, Silicone Tube, Hydrophilic, Collagen Coating, Sputter Etching

## 1. INTRODUCTION

Arterial endothelial-cells, which cover the inner surface of the blood vessel, are exposed to the pulsatile flow *in vivo*. The cyclic shear stress is induced in cells with the pulsatile flow. The effects of shear stress to the endothelial cells have been studied and several chemical responses were pointed out in the previous studies [1-5]. The longitudinal axes of endothelial cells are arranged along the streamline. The orientation may occur by migration of cells. In the present study, the effect of pulsatile shear flow on migration of endothelial cells was investigated *in vitro*.

## 2. METHODS

**Endothelial cells**

The descending aorta of an anesthetized rat was exposed and exfoliated from surrounding tissue under thoracotomy (Fig. 1). The descending aorta as long as 10 mm was excised from the rat. The excised aorta segment was sunk in a medium including an antibiotic substance (Fig. 2). The endothelial cells were gently peeled off from the inner layer of descending aorta by the flow of the culture medium with a syringe, and collected at the downstream into another syringe (Fig. 3). The surface of the inner wall were gently rubbed with a surgical knife to peel off endothelial cells (Fig. 4).

After cultivation for one week on a piece of glass plate in a dish preserved in an incubator to be cloned (Figs. 5 & 6), the endothelial cells were cultured on the inner surface of the test tube.

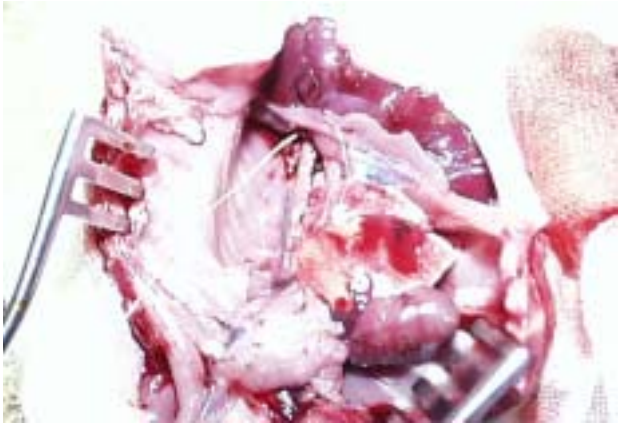

**Fig. 1:** The descending aorta of an anesthetized rat was exposed and exfoliated from surrounding tissue under thoracotomy.

**Fig. 2:** The excised aorta segment was sunk in a medium including an antibiotic substance.

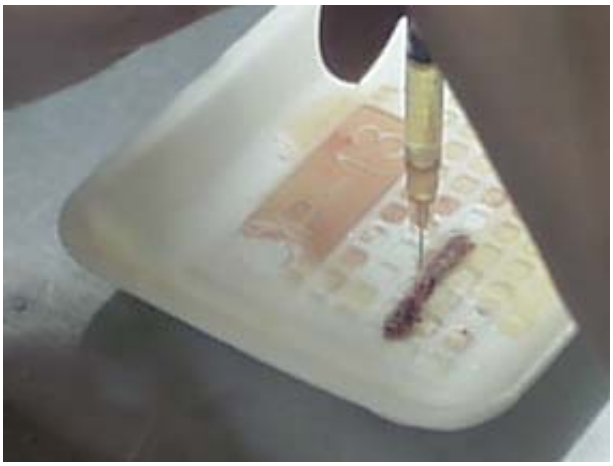

**Fig. 3:** The endothelial cells were gently peeled off from the inner layer of descending aorta by the flow of the culture medium with a syringe.

**Fig. 4:** The surface of the inner wall were gently rubbed with a surgical knife to peel off endothelial cells.

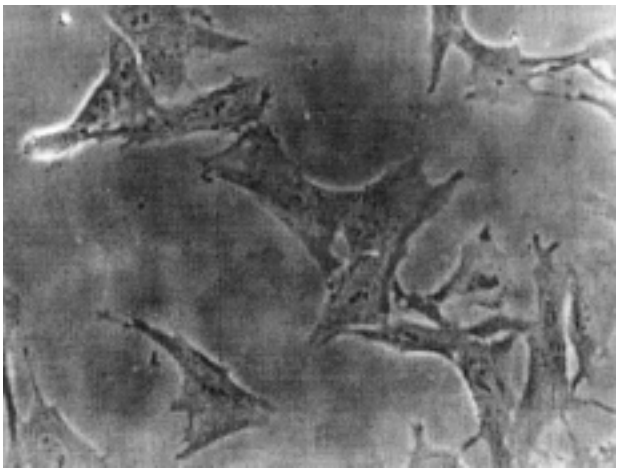

**Fig. 5:** Endothelial cells were cultured on a piece of glass plate in a dish preserved in an incubator to be cloned.

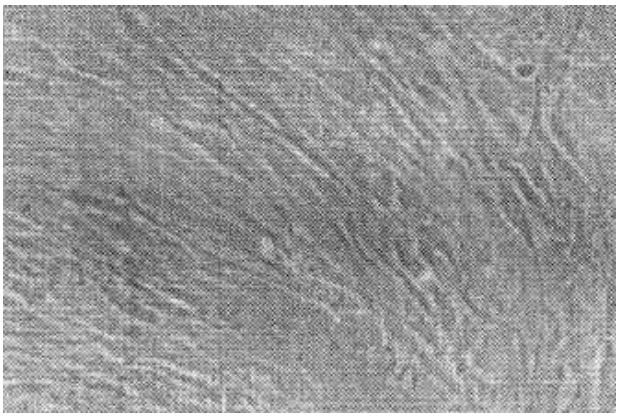

**Fig. 6:** Endothelial cells after cultivation for one week on a piece of glass plate in a dish preserved in an incubator.

**Test tubes**

Two kinds of test tubes were prepared; one is made of glass and the other of silicone-rubber. The internal and external diameters of the glass tube are 0.56 mm and 1.6 mm, respectively. The internal and external diameters of the silicone-rubber tube are 1.5 mm and 2.0 mm, respectively. A 20mm-length segment of the silicone rubber tube was turned inside out. The inner surface of the silicone tube was sputtered in a vacuum to change the surface character from hydrophobic to hydrophilic (Fig. 7). After the sputter etching, the tube was immediately sunk in a water solution including collagen molecules. Through the procedure, the inner surface of the silicone-tube wall was coated with collagen. The cells contained in the suspension were poured into the tube to be cultured on the inner surface of the tube.


**Fig. 7:** The inner surface of the silicone tube was sputtered in a vacuum to change the surface character from hydrophobic to hydrophilic.

**Mock circulation circuit**

A mock circulation circuit was constructed with the silicone tube and the pump. Before cells were cultured to be confluent on the surface, the tube with cells was contained in a chamber made of the transparent styrene plate (Fig. 8), and connected to the pump via silicone tubes. The culture medium was filled in the chamber, where concentration of carbon dioxide was kept at 5 % in the incubator. The chamber also plays a role of the buffer for the stroke volume in pulsatile flows. Some lines were marked on the bottom plate of the chamber to observe cells at the same point of tube intermittently during the test (Fig. 8). A liquid culture medium (0.002 Pa s) was circulated in the circuit at 25 degrees C. In a long-term test, the whole circulation system was set in the incubator, and cells were exposed to the flow for 24 hours at 37 degrees C (Fig. 9).

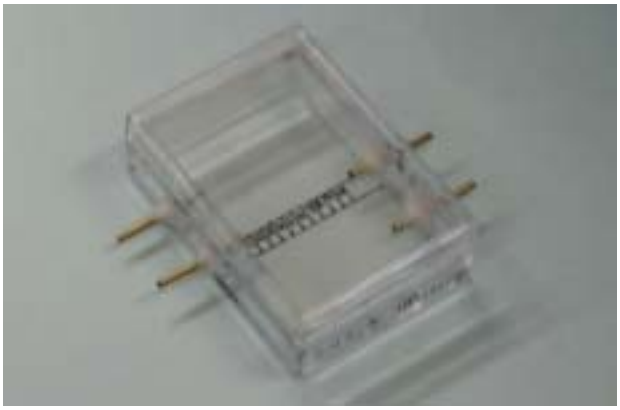

**Fig. 8:** Chamber made of the transparent styrene plate.

**Fig. 9:** Whole circulation system was set in the incubator.

**Pumps**

Two types of shear flow were applied to the endothelial cells: a continuous flow and a pulsatile flow. The continuous flow was powered with a peristaltic roller pump (Fig. 10). To apply the pulsatile flow, a micro diaphragm pump system was manufactured (Fig. 11). The system consists of a battery, a DC motor, a crank, a diaphragm chamber, and leaflet one-way valves. In the pulsatile flow, the flow condition was controlled to simulate that with the natural heart of rat: the beating rate was 300 per minute and the stroke volume was 0.06 ml.

**Fig. 10:** The continuous flow was powered with a peristaltic roller pump.

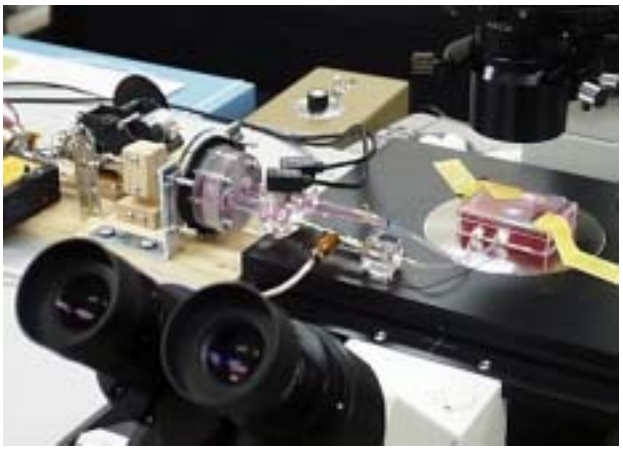

**Fig. 11:** The pulsatile flow was powered with a micro diaphragm pump.

**Shear rate**

The shear rate at the inner wall surface of the tube is estimated by Eq. (1).

$$G = 4\,Q / (P\,R^3) \qquad (1)$$

Where. $G$ is shear rate at the wall (1/s), $Q$ is flow rate ($cm^3/s$), $P$ is the ratio of the circumference of a circle to its diameter, and $R$ is radius of the tube (cm). The calculated value is an approximation under Poiseuille flow. The flow rate ($Q$) was measured with an electromagnetic flow meter installed in the downstream of the tube with cells.

**Microscope**

The behavior of cells was observed through the tube wall by a phase contrast microscope (Figs. 10 & 11). The wall of the tube was thin enough to keep transparency for observation at the inner surface with an optical microscope.

## 3. RESULTS

The most of cells were peeled off by continuous shear flow with shear rate of 15 1/s in 10 minutes. By pulsatile flow, however, the cells were not peeled off at the mean shear rate of 15 1/s in 60 minutes. The cells were repeatedly peeled off only at their corner, while keeping adhesion at another corner. The change in orientation of cell occurs gradually with repeating peel off at the corner of the cell. The longitudinal axes of endothelial cells tilted toward the direction of the streamline (Figs. 12 & 13).

The affinity between collagen-gel and silicone rubber increased with sputtering, which made strong adhesiveness between wall and cells. The cultured endothelial cells on the collagen-coated wall of silicone rubber tube with sputtering did not peel off in pulsatile shear flow at 37 centigrade for 24 hours, while the cells on that without sputtering peeled off in 5 minutes.

**Fig. 12:** The endothelial cells on the surface of the tube before exposure to flow.

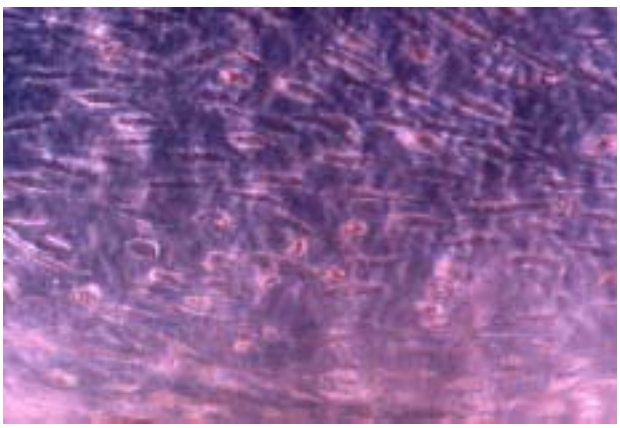

**Fig. 13:** The longitudinal axes of endothelial cells tilted toward the direction of the stream line (from left to right).

## 4. DISCUSSION

The glass tube is popular to be used for cell culture because of its hydrophile property. The arterial wall repeatedly deforms with pulsatile flow [6]. The deformation generates both tensile stress and shear stress in the endothelial cell. The compliant tube of silicone rubber is preferred to the rigid tube of glass to simulate mechanical condition of vessel wall. Both the sputter etching and the collagen coating were effective to change the property of silicone rubber to be used for cell culture.

Several studies have been performed about the difference between pulsatile and non-pulsatile flows [7, 8]. The manufactured micro diaphragm pump system was available for long-term test of pulsatile flow in the incubator.

## 5. CONCLUSIONS

The experimental results show that the longitudinal axes of the cells tilt toward the flow direction and that pulsatile flow may have an advantage for migration of cells.

## 6. ACKNOWLEDGEMENTS

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