COMBINATION OF HYDROSTATIC PRESSURE AND SHEAR STRESSES CONTRIBUTE TO ENDOTHELIAL CELL GROWTH IN A MICROFLUIDIC DEVICE

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ABSTRACT

This paper reports a microfluidic cell culture device capable of studying combination of hydrostatic pressure and shear stress contribute to endothelial cell growth. The device is constructed by a single layer microfluidic pattern made of polydimethylsiloxane (PDMS). Microfluidic channels are designed using equivalent fluidic circuit model in order to create combinations of various hydrostatic pressure and shear stress. In the experiments, human umbilical vein endothelial cells (HUVECs) are cultured in the device, and their orientation, geometries, and protein expression are analyzed. The developed device provides a powerful tool to systematically study the cell behaviors under combinations of different mechanical stimulations.

KEYWORDS

Endothelial Cell, Hydrostatic Pressure, Shear Stress, Microfluidics

INTRODUCTION

Endothelial cells (ECs) are exposed to three significant mechanical forces *in vivo*: fluid shear stress, pulsatile stretch and hydrostatic pressure [1]. Many microfluidic cell culture devices have been developed to mimic physiological mechanical microenvironments to study EC behaviors. Most studies focus on single force, and the results demonstrate that these forces can influence EC growth and function, which are critical in endothelial physiology and pathophysiology [2]. Recently, some studies started to focus on the combination of these mechanical forces, and the results show that EC morphology is changed by flow pattern [3]. However, most present experiments designed for creating combined mechanical force environments are complicated and hard to be scaled up. In this study, we develop a microfluidic device, which provides cell culture microenvironments with various combinations of hydrostatic pressure and shear stresses. In the experiments, we studied cell morphology and protein expression of human umbilical vein endothelial cells (HUVECs) exposed to combined forces.

EXPERIMENT

Figure 1(a) shows the device fabricated by the well-developed soft lithography. According to fluid mechanics, the same shear stress exhibits at four cell culture chambers because of the identical total flow resistances created by meander-shaped channels in four branched channels. In contrast, the hydrostatic pressure in the four chambers is different due to the chamber positions. As the numerical simulation result shown in figure 1(b), while flowing at 100 μ l/min, shear stress is 16 dyn/cm² and hydrostatic pressures are 50, 80, 110 and 140 mmHg in four chambers. HUVECs are first seeded and cultured in chambers until reaching confluent, and the medium is pumped with a flow rate 100 μ l/min for 6 hours.

RESULTS AND DISCUSSION

The HUVEC morphology under different conditions is observed as shown in figure 2. HUVECs are oriented to flow direction in flow chambers compared to the static control, in which HUVECs are seeded in the device without flowing (no shear stress and no hydrostatic pressure). To study the cell morphology and protein expression, vascular endothelial cadherin (VE-cadherin), filamentous actin (F-actin) and nuclei are fluorescently stained. The resulted photos (Fig. 3) show the strong VE-cadherin expression (green) in static control and weaker expression in flow chambers. The VE-cadherin expression is also down regulated by higher hydrostatic pressure. F-actin (red) filaments are clear and distributed in a rounded shape in static control, while they are indistinct and

distributed randomly in flow chambers. By analyzing the photos, the intensity of VE-cadherin expression (I_{VE}), cell orientation angle (Ang) and cell shape index (SI) can be calculated as shown in table 1. Compared to the static control, the VE-cadherin expression is reduced in flow chambers. As the hydrostatic pressure increases, the VE-cadherin expression further decreases. The flow direction is defined as 0 degree and the cell orientation is closer to flow direction in higher hydrostatic pressure. In addition, the shape index results show that HUVECs are slightly elongated in flow chambers.



Figure 1. (a) Schematic of the microfluidic device for EC studies. A, B, C and D are the cell culture chambers. (b) Simulation results when flow rate is 100 μ l/min: shear stress is 16 dyn/cm² and hydrostatic pressures are 50, 80, 110 and 140 mmHg in four chambers.



Figure 2. Bright field photos of (a) static control in device and flow chambers with a 16 dyn/cm² shear stress and a hydrostatic pressure of (b) 50 mmHg (c) 80 mmHg (d) 110 mmHg (e) 140 mmHg. Scale bar indicates 200 μ m.

Table 1. The table shows the mean values of VE-cadherin intensity (I_{VE}), orientation angle (Ang), shape index (SI) of HUVECs under different conditions. The flow direction is defined as 0 degree. Shape index = $4\pi^*$ (cell area)/(cell perimeter)².

| | Static Control | | With Flow (shear stress: $16 \text{ dyn/c}m^2$) | | | |
|-----------------------------|----------------|--------|--|---------|----------|----------|
| | Dish | Device | 50 mmHg | 80 mmHg | 110 mmHg | 140 mmHg |
| IVE $(\mu m^2/\text{cell})$ | 464 | 377 | 240 | 90 | 66 | 58 |
| Ang (degree) | 47.7 | 41.8 | 34.3 | 31.6 | 29.3 | 26.2 |
| SI | 0.61 | 0.61 | 0.52 | 0.49 | 0.53 | 0.50 |

CONCLUSION

In summary, with the device developed in this study, the endothelial cell behaviors under the various combinations of hydrostatic pressure and shear stress conditions can be easily studied.

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Figure 3. Immunofluorescence stain photos of (a) static control in dish, (b) static control in device and flow chambers with a 16 dyn/cm² shear stress and a hydrostatic pressure of (c) 50 mmHg (d) 80 mmHg (e) 110 mmHg (f) 140 mmHg. The photos are taken by a confocal laser scanning microscope. Scale bar indicates 100 μ m. Flow direction is from the right side to the left side.

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