Toward Coating-Based Tissue Engineering: Microstructure Control of Vascular Endothelial Cell Sheets Using Shear Flow

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1. Introduction

Tissue engineering is a technique to assemble cultured cells into 2D/3D structures for replacing damaged tissues/organs. Function of the assembled "tissue" is strongly affected by its microstructure. For example, in the case of vascular tissue engineering, unidirectionally-aligned structure, as is the case in native blood vessels, is preferred for endothelial cell sheets to achieve required functions. However, the control of this microstructure is still a great challenge.

It is known that native endothelial cells maintain the aligned structure by blood flow, though detailed mechanism is not fully understood. This cellular property inspired us to develop an alignment control process of endothelial cell sheets using shear flow, which is commonly used in coating technologies. When shear flow was applied to randomly-oriented endothelial cell sheet, cells elongated spontaneously and a well-aligned structure was formed in the cell sheet. The effect of process parameters, such as flow rate and cell density, on the aligned structure formation was examined in this study. Possible physicochemical mechanism underlying this process was also discussed.

2. Experimental

Human umbilical vein endothelial cells (HUVECs) were used in this study. HUVECs suspended in culture medium (EBM2, Lonza) were added to a collagen-coated culture dish. The dish was maintained in a humidified incubator at 37 °C and 5% CO_2 overnight to form a HUVEC monolayer. After that, the dish

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was inserted into a hand-made parallel-plate flow chamber, shown in Fig. 1a. The flow chamber has a flow section of 0.5 mm in height and 50 mm in width. The flow chamber was then connected to a medium perfusion loop and the HUVEC monolayer was exposed to shear flow (Fig. 1b). During perfusion, alignment of HUVECs induced by shear flow was observed via phase-contrast microscope in real time.

For quantitative analysis of the aligned structure formation of HUVECs, two parameters were employed in this study: shape index *S.I.* and order parameter S_θ. Shape index *S.I.* reflects the elongation of cells and given as:

$$S.I. = \frac{4\pi A}{P^2}$$

where A and P is the area and perimeter of cells, respectively. *S.I.* is defined as 1.0 for a circle and it approaches zero for highly elongated shapes. On the other hand, order parameter S_θ is often used to characterize the orientational ordering of anisotropic objects, such as liquid crystals[1] and rod-like particles.[2] In 2D case, it is given as:

$$S_\theta = \left(\sum_{i=1}^{n} 2\cos^2 \theta_i \right) / n$$

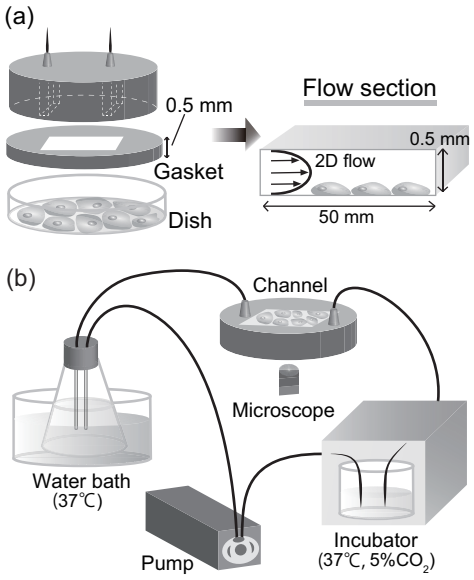

Fig. 1 Schematic illustration of (a) flow chamber and (b) medium perfusion loop used for the alignment control of endothelial cell sheets.

where n and θ is the number of cells and the angle between the flow direction and the axis of a cell. S_θ is unity when cells are unidirectionally aligned, and it approaches zero when cells are randomly oriented.

3. Results and Discussions

The aligned structure formation of HUVECs induced by shear flow is shown in Fig. 2. Phase-contrast microscopic images of HUVECs for selected times after shear flow exposure are shown. By the exposure to shear flow, HUVECs spontaneously elongated and aligned in the flow direction, which was the similar structure with native blood vessels. By 24 h shear flow exposure, *S.I.* decreased from 0.64 to 0.37, which

corresponds to the HUVEC elongation, while S_0 increased from 0.01 to 0.77, which corresponds to the HUVEC alignment. These results suggest that by using our flow circuit system, we can fabricate the unidirectionally-aligned endothelial cell sheet, which is necessary for the vascular tissue engineering.

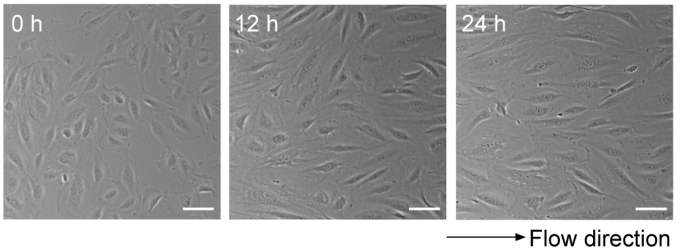

Fig. 2 Phase-contrast microscopic images of HUVECs after 0, 12, and 24 h shear flow exposure.

The final structure of HUVEC sheet after shear flow exposure depends on various process parameters, such as shear rate. Among these, we found that cell density in HUVEC sheet dominantly affected the final structure of HUVECs. The effect of cell density on alignment of HUVECs is shown in Fig. 3a. Variation of elongation (*S.I.*) and alignment (S_0) of HUVECs with cell density after shear exposure is also quantitatively analyzed in Fig. 3b. With increase in cell density, the degree of HUVEC elongation was decreased (Fig. 3a), which was also confirmed by the increase in *S.I.* (Fig. 3b). With increase in cell density, free space between HUVECs decreases. Therefore, when cell density was high, there would be insufficient free space for HUVECs to elongate, which resulted in the decrease in elongation. Orientational ordering of HUVECs was also revealed to be cell density-dependent. We found that HUVEC monolayer at low cell density (5.0×10^3 cells cm^{-2}) did not form aligned structure even after exposed to shear flow, while that at high cell density (2.0×10^4 and 8.0×10^4 cells cm^{-2}) showed aligned structure after exposed to shear flow (Fig. 3a). This trend is quantitatively shown in Fig. 3b. When cell density was low, S_0 of HUVEC monolayer after shear flow exposure was around zero, meaning that HUVECs were randomly oriented in the monolayer. On the other hand, when cell density exceeded a threshold value (around 1.0×10^4 cells cm^{-2}), S_0 jumped to ca. 0.8, meaning that HUVECs were almost unidirectionally aligned in the monolayer. These results indicate that existence of other cells (i.e., cell-cell interaction) is essential for the aligned structure formation of HUVECs. It is reminiscent of the orientational ordering of liquid crystals or rod-like particles, which is driven by excluded volume of

molecules or particles.[1] Alignment of HUVECs may also be attributed to the increase in excluded volume of cells, which is caused by the elongation of HUVECs induced by shear flow. From these results, it can be said that cell density is a dominant factor for determining the final structure of HUVECs after exposed to shear flow. The time course of the aligned structure formation was also revealed to be cell density-dependent. We have to care this factor to achieve coating-based vascular tissue engineering.

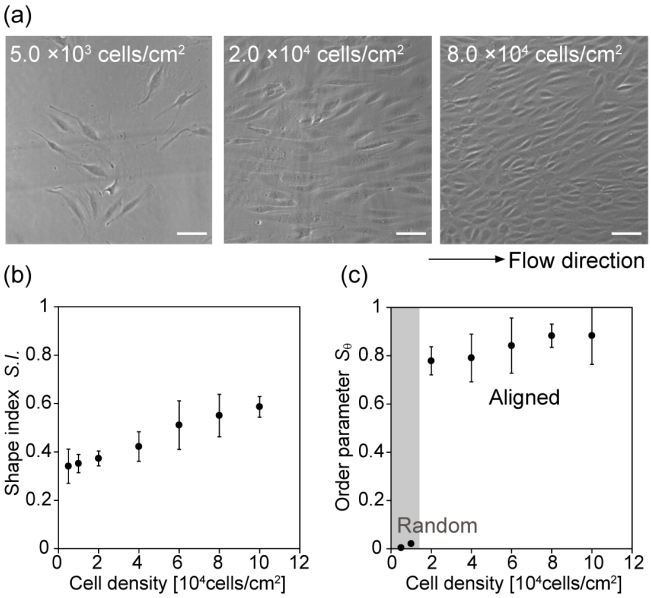

Fig. 3 (a) Phase-contrast microscopic images of HUVECs with different cell densities after shear flow exposure. (b-c) Effect of cell density on (b) *S. I.* and (c) S_θ after shear flow exposure.

In this study, we showed that alignment control of endothelial cell sheets could be achieved by shear flow, which was commonly used in coating process. To achieve vascular tissue engineering, further, we have to assemble this sheet with other cells and fabricate 3D tubular structure. For these processes, coating techniques, such as layer by layer coating and ink-jet printing, can also be used.[3] Combining coating techniques with biomedical engineering, we can open the way to the coating-based tissue engineering.

4. Conclusions

By using shear flow, alignment control of HUVEC sheets can be achieved. It was revealed that the aligned structure formation of HUVECs was cell density-dependent. We have to optimize cell density and other process parameters to achieve the future coating-based tissue engineering.

References

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