RECONSTRUCTION OF CAPILLARY NETWORKS IN HUVEC-MSC COCULTURE CULTURED IN STATIC/FLOW CONDITIONS IN A MICROFLUIDIC PLATFORM
Kohei Tanimura*, Kyoko Yamamoto*, Ryo Sudo*
* Department of System Design Engineering, Keio University, Yokohama, Japan

ABSTRACT
There is a demand for creating stabilized capillary structures covered by pericytes in the field of tissue engineering. In this study, we performed coculture of human umbilical vein endothelial cells (HUVEC) and mesenchymal stem cells (MSC) in a microfluidic platform to investigate the effect of static/flow conditions on constructing capillaries with aligning pericytes. The results indicated that capillary formation was attenuated in flow conditions in HUVEC-MSC coculture.

KEYWORDS: Angiogenesis, HUVEC, MSC, Microfluidic platform

INTRODUCTION
The field of tissue engineering has encountered a particular challenge in creating a large tissue. Since diffusive transport is limited in short distance, large tissues become necrotic without functional vasculature. Therefore, the mechanism for creating functional vasculature needs to be clarified.

Pericytes is an important cell type to create functional vasculature since it is known that microvasculature is stabilized and matured by pericytes in vivo. However, it is difficult to isolate and culture pericytes. Therefore, other cell types need to be considered as an alternative to pericytes. Mesenchymal stem cell (MSC) is a cell source for pericytes since MSC can differentiate into pericytes.

Recently, we developed a microfluidic platform to create an in vitro 3D angiogenesis model [1]. In this model, human umbilical vein endothelial cells (HUVEC) formed 3D microvascular networks without pericytes. Here, we extended this single culture model to coculture of HUVEC and MSC for investigating the mechanism to create functional vasculature. A specific purpose of this study was to investigate the effect of interstitial flow on capillary formation in the HUVEC-MSC coculture.

EXPERIMENTAL
Microfluidic platform
The microfluidic device used in this study was made of poly(dimethylsiloxane) (PDMS) and cover glass (Fig. 1A). The PDMS device was bonded with cover glass to form microfluidic channels between the PDMS and cover glass. Type I collagen gel (5 mg/ml, pH 7.4) was formed between two parallel microchannels (Fig. 1B).

HUVEC-MSC coculture
HUVEC were seeded by injecting 10 µl of cell suspension at 1×10⁶ cells/ml and cultured for 6 days to allow HUVEC to form vascular sprouts (Fig. 2A). Similarly, MSC were then added into another microchannel to start coculture (Fig. 2B). Cells were cultured in static conditions (Fig. 2C) or interstitial flow conditions (Fig. 2D). Interstitial flow was generated by 5-mm H₂O pressure difference across the gel region (Fig. 3). The cells were cultured in a 1:1 mixture of EGM-2 and DMEM supplemented with 10 ng/ml bFGF and 10 ng/ml VEGF.

Capillary morphogenesis was monitored by phase-contrast microscopy. Immunofluorescent staining was carried out to visualize fine structures of microvasculature, and the stained samples were examined by a confocal laser-scanning microscope.
RESULTS AND DISCUSSION

In static conditions, microvascular networks were covered by pericytes, formed lumens and extended toward another microchannel (Fig. 4A, B). On the other hand, in interstitial flow conditions, microvascular networks were covered by pericytes but failed to extend across the gel region resulting in increased the network density (Fig. 4A,B). In both static and flow conditions, capillary structures covered by pericytes were observed (Fig. 3C & 4C).

These results indicated that interstitial flow significantly attenuated capillary morphogenesis, which might be due to a paracrine effect of MSC. MSC may secrete cytokines which promote vessel maturation(Fig. 6).
**CONCLUSION**

We developed a microfluidic platform to create an in vitro 3D angiogenesis model in HUVEC-MSC coculture. Our findings revealed that interstitial flow plays an important role in capillary morphogenesis in the coculture model.

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**REFERENCES**


**CONTACT**

*Kohei Tanimura, E-mail: kouhei-tanimura@z6.keio.jp*