Capillary network formation by the co-culture of endothelial cells and mesenchymal stem cells in a microfluidic device

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Abstract

In the field of tissue engineering and regenerative medicine, there is a demand for construction of three-dimensional (3D) organs such as liver, pancreas, and kidney. Although recent advances in tissue engineering allowed us to construct two-dimensional tissues such as skin and cornea, it is still challenging to construct 3D organs. Since these vital organs are complex in structure and contain abundant capillary networks, it is difficult to construct these organs in vitro. In particular, construction of capillary networks and their integration to epithelial tissues are important for 3D tissue engineering. Therefore, we have been focusing on construction capillary networks. The aim of this study is to investigate the mechanism how we can construct functional microvascular networks by the co-culture of endothelial cells and mesenchymal stem cells while we utilize a microfluidic device to control culture microenvironments.

Microfluidic devices used in this study were made by soft lithography. Our microfluidic device is made of silicone rubber (polydimethylsiloxane) and a cover glass. The device has two parallel microfluidic channels and an intervening hydrogel region, which allowed us to control cellular distribution and interstitial flow conditions. Using this microfluidic device, we cultured human umbilical vein endothelial cells (HUVEC) and mesenchymal stem cells (MSC). Phase-contrast and confocal fluorescent microscopy methods were used to investigate the process of capillary formation.

HUVEC, which were seeded into a microfluidic channel, attached on the sidewall made of hydrogel and started to penetrate into the gel for constructing vascular sprouts. These vascular sprouts extended into the gel and finally developed into branching capillary networks. However, when HUVEC were cultured alone, capillary networks were not able to be maintained in long-term culture. On the other hand, when HUVEC were culture with MSC, MSC migrated into gel region, and some cells attached to HUVEC differentiated into pericytes. After MSC attached to HUVEC, HUVEC started to form vascular sprouts and finally developed into branching capillary networks, which were stable for more than 3 weeks. Confocal microscopy revealed that these capillary networks contained continuous lumens, which were partially wrapped by pericytes, suggesting that pericytes play important roles in stabilization of constructed capillary networks in vitro. In addition, the effect of interstitial flow on the construction of microvascular networks was investigated by applying a series of interstitial flow magnitude. The results revealed that capillary morphogenesis was enhanced with increasing interstitial flow magnitude.

In conclusion, HUVEC-MSC interactions and interstitial flow conditions play important roles in regulating capillary formation. Control of these culture conditions allows us to construct long-lasting functional microvascular networks.

Keywords: Angiogenesis, Tissue engineering, Microfluidics, bioMEMS, Microvascular network