QUANTITATIVE ANALYSIS OF THE INTERACTIONS BETWEEN 3D HEPATOCYTE TISSUE AND CAPILLARY NETWORKS IN A MICROFLUIDIC PLATFORM

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ABSTRACT

Tissue vascularization is one of the biggest challenges in tissue engineering. We constructed 3D hepatocyte tissue-like structures and endothelial capillary networks in a microfluidic platform. Here, we analyzed interactions between these structures quantitatively by recording the cell migration and morphogenesis. The analysis of capillary network extension rate under various combinations of growth factors (e.g., HGF, VEGF) revealed that the growth rate significantly increased by the growth factor addition. The capillary networks, however, stopped extending after contacting with hepatocytes, suggesting that additional factors are necessary for achieving hepatocyte tissue-like structures including microvascular networks.

KEYWORDS: Hepatocyte, Vascularization, Tissue Engineering

INTRODUCTION

Understanding the mechanism of tissue vascularization is one of the biggest challenges in tissue engineering, since vascularization is essential to overcome a limit for passive transport of nutrient, oxygen, and waste products by diffusion, which leads to construction of a large-scale tissue. We previously implemented a microfluidic platform to create a co-culture model of hepatocyte tissues-like structures and rat microvascular endothelial cell (rMVEC) capillary-like structures [1]. Although we found that rMVEC formed capillary structures only in the presence of hepatocytes, little is known about the mechanism of the interactions between hepatocyte tissue-like structures and rMVEC capillaries, especially under various combinations of growth factors such as hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF).

Here, we quantitatively analyzed the interactions between hepatocytes and rMVECs by recording the cell movements and morphogenesis.

EXPERIMENTAL

Microfluidic platform

The microfluidic device used in this study is made of poly-dimethylsiloxane (PDMS) cured on a silicon template. The PDMS device was bonded with coverslip to form microfluidic channels between the PDMS and coverslip. Collagen gel was injected into a gel region from gel injection ports (Fig. 1).

Hepatocyte –Endothelium coculture

To engineer vascularized hepatic tissues, we mimicked microenvironments of *in vivo* angiogenesis in the microfluidic platform (Fig. 2). First, hepatocytes were seeded on the sidewall of a collagen gel scaffold via one of the microfluidic channels. Next, rMVECs were seeded on the other sidewall of the collagen gel scaffold after hepatocytes formed 3D tissue-like structures. The morphogenesis of hepatocytes and rMVECs was monitored by phase-contrast microscopy, and the images were quantitatively analyzed.

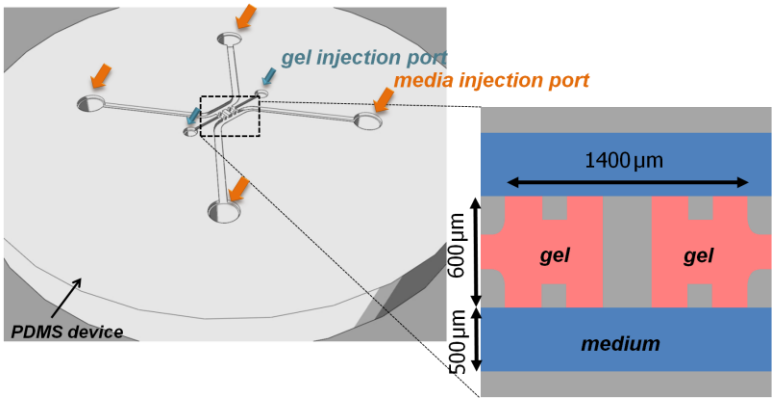

Figure 1: Microfluidic platform

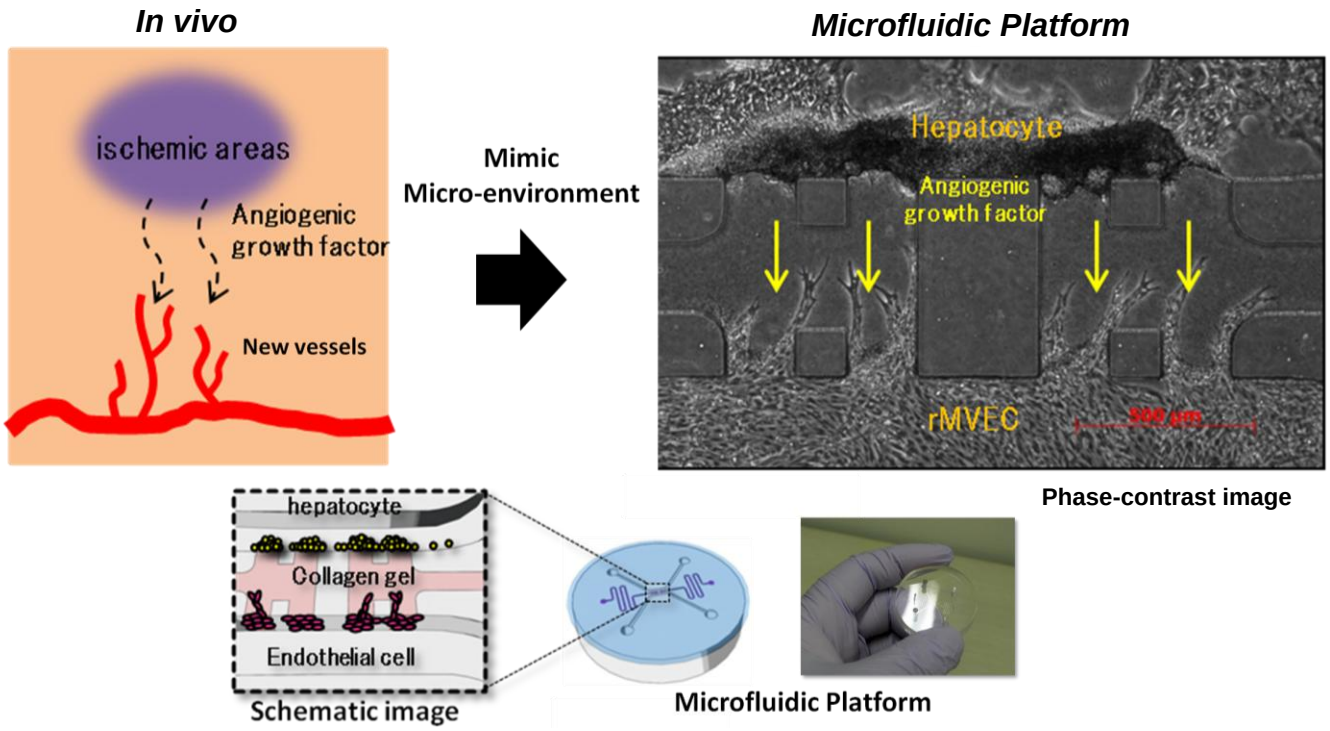

Figure 2: rMVEC - Hepatocytes coculture model

RESULTS AND DISCUSSION

Contact of capillary networks and hepatocytes

rMVEC capillary networks finally came in contact with a hepatocyte tissue. But, the capillary networks stopped extending at the contact point (Fig. 3A). After the contact, capillary networks increased their lumen size, and then, some networks crawled under the hepatocyte tissue or pass through the gel region on the top of the hepatocyte tissue (Fig. 3B).

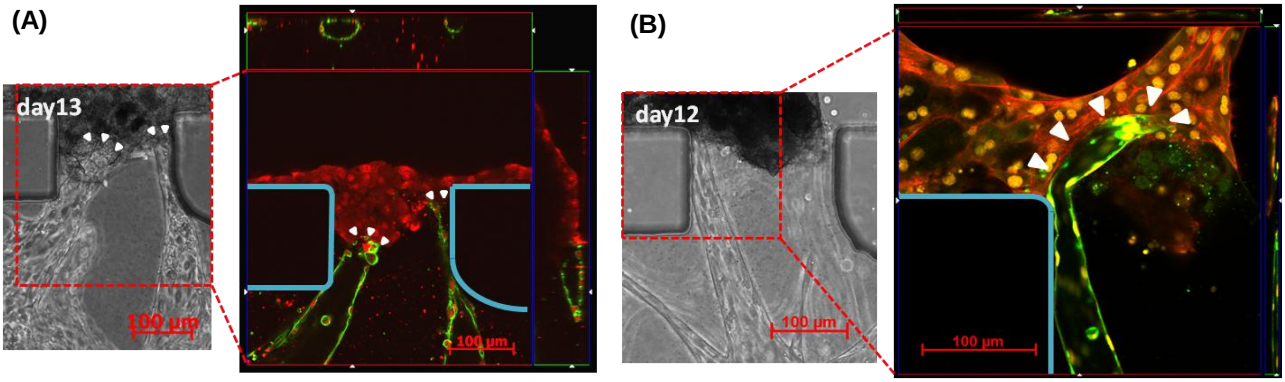

Figure 3: Immunofluorescent staining images. Cells were fixed and stained with anti-cytokeratins (red, hepatocytes) and RECA-1 (green, rMVEC) and PI (red, nuclei). (A) Capillary networks stopped extending, when they attached to hepatocyte tissues. (B) Some networks crawled under hepatocyte tissues. The edge of PDMS posts were outlined by blue lines. Arrow-heads indicated a contact point of hepatocyte tissue-like structures and rMVEC capillaries.

Vascularization of hepatocyte tissue-like structures

rMVEC capillary networks failed to penetrate hepatocyte tissues in this study. This result suggests that rMVEC don't have ability to dissolve cell-cell junctions of hepatocytes. *In vivo*, vascularization of avascular, matrix-poor hepatocyte islands following partial hepatectomy is thought to be initiated by hepatic stellate cells, which extend projections into cell-to-cell junctions between hepatocytes and secrete laminin, providing a beachhead for subsequent invasion by sinusoidal endothelial cells [2,3]. Therefore, hepatic stellate cells may be essential in promoting endothelial integration into hepatocyte tissues.

Capillary network extension rate

To construct hepatocytes-rMVECs-stellate cells tri-culture model, stellate cells were seeded on the same side of hepatocytes via one of the microfluidic channels. After a few days, we observed outgrowth of activated-stellate cells which were positive for both desmin and α-SMA from hepatocyte tissue-like structures. These stellate cells inhibited capillary network extension (Fig.4A). The direct contacts between stellate cells and tip cells of rMVEC capillaries seemed to be critical for this inhibitory mechanism.

Quantitative analysis of the capillary network extension rate revealed that VEGF and HGF significantly promoted the network extension. Especially, the inhibitory effect of stellate cells became suppressed by HGF addition (Fig. 4B). But, even in the tri-culture model, rMVECs failed to penetrate into the hepatocyte tissue-like structures. Further investigations will be needed to clarify the mechanism of the tissue vascularization.

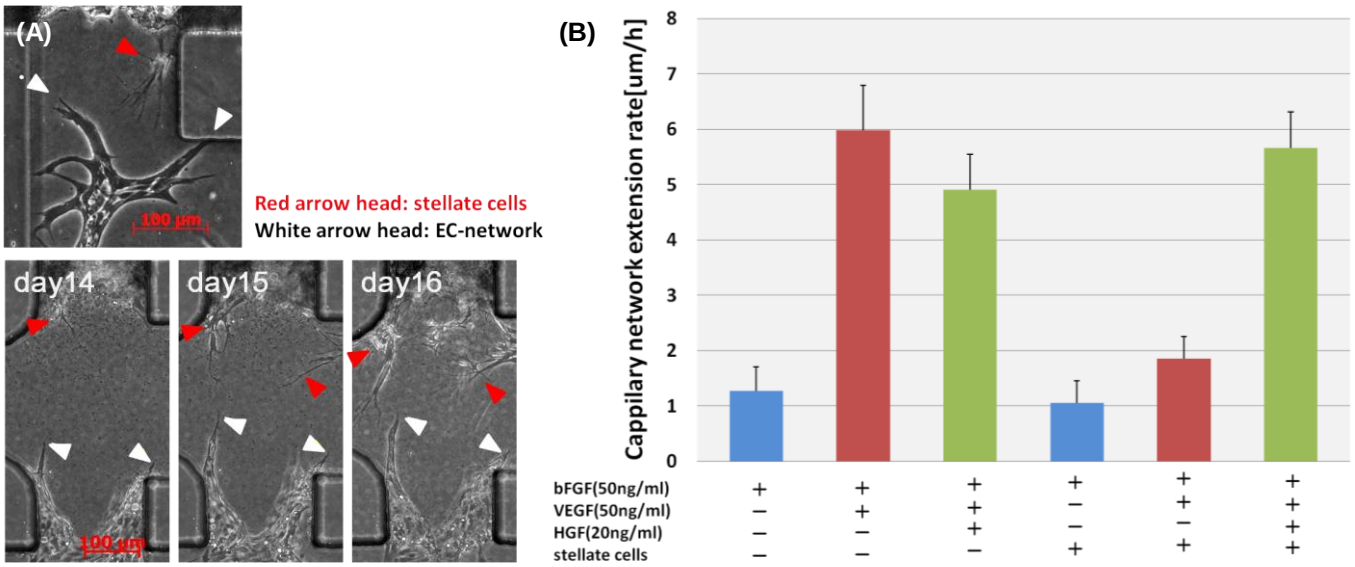

Figure 4: (a) Capillary network growth inhibited by stellate cells. (b) Quantitative analysis of capillary network extension rate under various combinations (n ≥13 for each condition)

CONCLUSION

We created a co-culture model of hepatocyte tissues-like structures and rMVEC capillary structures in a microfluidic platform. Quantitative analysis of the capillary network extension rate revealed that VEGF and HGF significantly promoted rMVEC capillary network extension. But, in a tri-culture model, stellate cells inhibited the network extension. Additional factors are necessary for achieving the tissue vascularization.

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