IN VITRO 3D COLECTIVE ANGIOGENIC RESPONSE UNDER ORCHESTRATED MULTIPLE CHEMICAL GRADIENTS

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ABSTRACT

Angiogenesis is a process requiring a coordinated guidance from multiple factors. We examined the effects of angiogenic endothelial cell sprouting under the gradient of soluble factors, VEGF and ANG-1. This resulted in a different cellular morphology than the condition with single factors; the presence of VEGF gradient induced a greater number of tip cells, and addition of ANG-1 to the VEGF gradient stabilized collectively migrating cells. Interestingly, as shown by the average filopodial angle measured, tip cell was affected by VEGF only when ANG-1 gradient was not present, possibly because the tip cells attached to stalk cells have reduced freedom.

KEYWORDS: Angiogenesis, Cell migration, Cell-on-a-chip

INTRODUCTION

Sprouting angiogenesis is the initiation of microvessel growth and requires a coordinated guidance from various angiogenic factors. This also plays an essential role in development and reproduction, and is also a prominent feature in tumor formation as well as in a variety of diseases[1]. There have been efforts to elucidate the complexity of the angiogenesis, but previous studies were limited in 2D culture models or tested a single contributing factor at a time[2]. Previously, we have shown that the microfluidic platform can mimic 3D physiological microenvironment with precise control of vascular endothelial growth factor (VEGF) or other single factor gradients generated[3]. Utilizing the developed platform, we examined the effects of angiogenic endothelial cell (EC) sprouting under the gradient of two soluble factors, VEGF and ANG-1.

The chemotatic gradient generated can be sensed by cells through signal molecule receptors, and process the received information to adjust and orient the cell polarity appropriately. This process is achieved through "inner workings" of eukaryotic chemical compass. The compass model proposes that the receptors transmit the received information and make the cells undergo spatial actin rearrangements to point towards the source of stimulant and thus leading towards the direction of cell's interest[4,5].

This paper presents experimental analysis of sprouting angiogenesis in response to imposed concentration gradient of VEGF and ANG-1 and shows how VEGF affects the turning angle of endothelial cells.

EXPERIMENTAL

The experimental setup was fabricated using microfluidic devices made of PDMS. The usage of microfluidic device is advantageous for its merit including but not limited to easier control and possible decoupling of biophysical and biochemical factors, creation of 3D micro-environment, and optical access. For these reasons, I have used microfluidic devices for human dermal microvascular endothelial cell (hMVEC) culture under VEGF gradient and VEGF & ANG-1 gradient as shown in figure 1.

The experiment procedure includes mainly following four steps: device fabrication, hydrogel injection, cell seeding, and media insertion. Microfluidic devices were made by PDMS using soft lithography methods. Collagen type I gel with density 2.0mg/ml filled the scaffolding T-section region, and gel was polymerized after 30 min. This gel represented the extracellular matrix (ECM) in three-dimensional space. The gel scaffold separated the independent flow channels as shown in figure 1, and this was also the region where the chemotactic gradient was induced later through diffusion. Then endothelial cell suspension ($2 \cdot 10^6$ cells/ml) was inserted in the bottom channel, which later provided a confluent monolayer within 2 days of seeding.

The chemotactic gradient was achieved by introducing VEGF added to endothelial cell medium. While the control devices received the regular endothelial cell medium in all three channels, the VEGF condition devices had regular medium with 50ng/ml VEGF introduced at the right channel, and VEGF & ANG-1 devices had 50ng/ml VEGF in the right channel and 50ng/ml ANG-1 in the center channel. All media were replenished on daily basis.

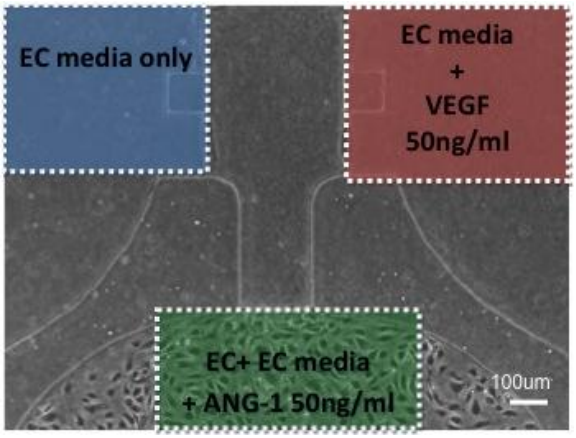

Figure 1: Experimental set-up uses microfluidic device with collagen scaffold filling the T-section, endothelial cell covering the bottom channel, and chemo-attractant factor VEGF introduced on the right channel.

The devices were monitored on a daily basis for movement using phase microscope over period of 8 days. Figure 2 below shows an image of VEGF conditioned device at day 4. The collective migration behavior of endothelial cell has been quantified using the optical image data. From the image, I could identify the forefront of migrating cell, known as tip cell, and measure the filopodial extrusion angles of all the tip cells in each device. Although I observed both chemotactic migration as well as proliferation behavior of the cell in the experiment, it is known that the response of VEGF regarding proliferation and migration behaviors are decoupled in a way that chemotactic migration due to VEGF is guided by the tip cells guide while the proliferative response of VEGF occurs in the sprout stalk cells[6]. It is therefore important to measure the turning angle of the tip cells.

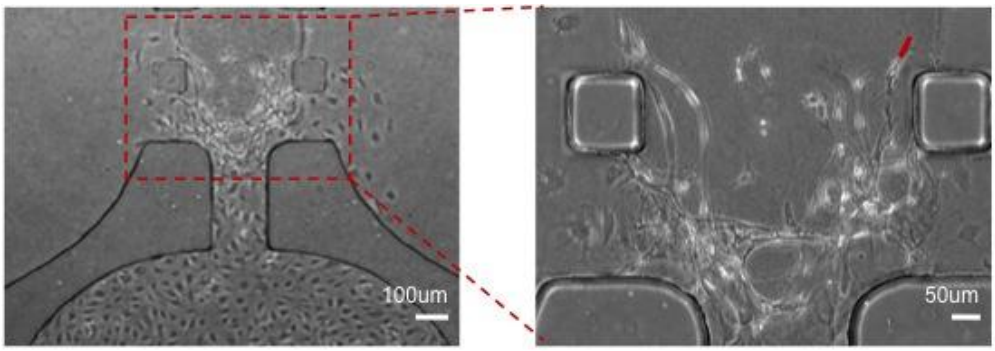

Figure 2: Image obtained from VEGF conditioned device after day 4, which shows both chemotactic and proliferative behavior of endothelial cells. The image on the right is magnified version, which shows how each image is analyzed by measuring the turning angle of each tip cells.

RESULTS AND DISCUSSION

Collective sprouting angiogenic behavior of endothelial cells under VEGF gradient has been analyzed. Endothelial cells migrated and formed a 3D sprout in the collagen scaffold. This behavior were quantified for the turning angle of the most forefronts cell, which is assumed to be the tip cell. The polar histogram graph in Figure 3 shows the result from the experiment as well as the mean value of tip cell direction.

The average angle of the tip-cell filopodia were measured to be 82.7°±5.0°, biased toward the condition channel under VEGF gradient. However, the VEGF+ANG-1 conditioned devices had the average filopodial angle of 93.7°±4.4°, which close to 90°. Thus, VEGF+ANG-1 conditioned devices can be claimed to be not directed by the horizontal VEGF gradient, unlike the result from only VEGF conditioned devices, which indeed seemed to reflect the VEGF gradient.

Another morphological difference was that under both VEGF and VEGF+ANG-1 conditions, the number of tip cells in the collagen scaffold increased significantly compared to the control. Interestingly, most of those tip cells were clearly separated from the stalk cells 3-4 days after the cell seeding without ANG-1 supplement. This result suggests a new role for ANG-1 in regulating the connection between tip cells and stalk cells during capillary morphogenesis. The reduced degree of freedom of tip cell filopodia from VEGF+ANG-1 conditioned sample may be due to connected status of tip cells to stalk cells, and also may help to explain tropism that is independent of the gradient.

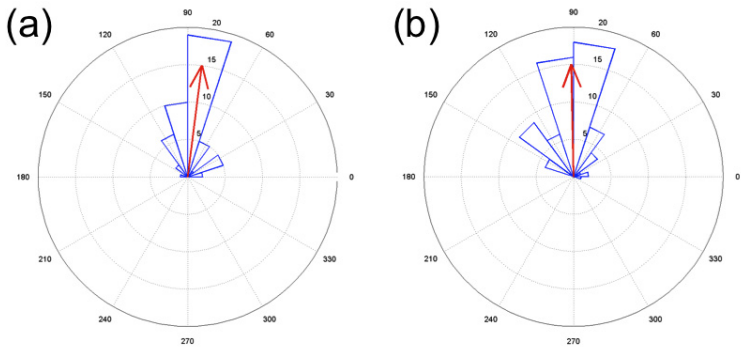

Figure 3: Tip-cell filopodia turning angle direction. Tip cells from VEGF gradient only samples were biased towards VEGF gradient (a), but VEGF+ANG-1 gradient sample remained close to 90° (b). Arrows in graphs indicate the average angle of the tip-cell filopodia, whereas blue triangles indicate the histogram.

CONCLUSION

The sprouting angiogenic behavior of endothelial under VEGF gradient has been analyzed experimentally using microfluidic devices. We incorporated the basic cell culturing methods while imposing the precise chemical gradients of two soluble factors, VEGF and ANG-1, highlighting microfluidics' biggest advantage. The cells exhibited a greater average tip cell filopodial turning angle toward the higher concentration of VEGF when VEGF only gradient was applied. On the other hand, the cells remained not affected when both VEGF and ANG-1 were applied, possibly because of firm connection of tip and stalk cells obtained with the addition of ANG-1. The difference in cellular morphology for these conditions confirms the coordinated action of VEGF and ANG-1, and also identifies the role of ANG-1 as a stabilization reagent.

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