Endothelial Cell Morphogenesis and Capillary-like Network Induced by Soluble and Bound VEGF in a Definite Biogel Composed of Collagen and Fibronectin

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Abstract: In vitro culture of endothelial cells to form capillary-like networks is essential in tissue engineering. Vascular endothelial growth factor (VEGF) is one of the primary signal proteins stimulating blood vessel formation. This growth factor can be soluble in the medium or protein-bound to the substrate. However, less attention has been paid to distinguishing the specific stimulations by soluble and bound VEGF. We conducted a series of experiments to explore the respective effects of these two VEGF forms. An in-house synthesized biogel comprising a definite concentration of collagen and fibronectin was designed to cultivate human umbilical vein endothelial cells to form the capillary-like network. Collagen served as the primary substrate for cell attachment. Fibronectin provided the surface to bind soluble VEGF in the culture medium to create the bound VEGF. The experiment of adding VEGF-blocking-peptide was conducted to prevent the formation of VEGF bound to the fibronectin domains, to distinguish the respective effects of the soluble and bound VEGF. With the in-house biogel of definite components, we were able to clarify the different roles of soluble and bound VEGF. The results indicated that the soluble VEGF promptly induced the cells to change from round to elongated shape, which contributed to forming network cords. Simultaneously, the bound VEGF provided long-term stimulation, causing the cells to migrate and differentiate into the final capillary-like network.

Keywords: capillary-like network; endothelial cells; morphology; vascularization; VEGF

1. Introduction

Regenerative medicine is an increasingly important issue in the biomedicine field, which emphasizes the usage of healthy cells to replace necrotic ones to repair functionless tissues or organs [1]. Tissue engineering, aiming to generate biological tissues from the cells, has become one of the most popular research topics in regenerative medicine [2]. The engineered tissues should connect with the patient blood circulation system through the network-like capillaries so that the organ transplant acquires oxygen and nutrient supplies and discharges metabolic wastes [3].

The capillary formation has two processes: vasculogenesis and angiogenesis. Vasculogenesis is one of the characteristics of early embryonic development, which refers to dispersed endothelial cell aggregation to form cords in an avascular environment and the network construction with the center-hollowed tubes [4]. Angiogenesis is for capillary-existing sites, where the endothelial cells that make up the capillaries develop new networks through sprouting, common in self-healing at the wound sites [5]. The way to achieve in vitro capillaries culturing is similar to the vasculogenesis process. A capillary-like
network will gradually develop from scattered endothelial cells via cell migration and morphological variation [6].

Many biocompatible materials have been tried as the substrate for culturing endothelial cells. A phenomenon of biogel remodeling occurs during the capillary network formation, in which the endothelial cells exert traction force and thereby change the base material distribution [7–9]. Scholars have used biogel composed of ingredients such as a mixture of type I collagen and gelatin to mimic the environment of burns and scalds denatured [10]. Researchers have also adopted poly (methyl acrylate) (PMA) or poly (ethyl acrylate) (PEA) 3D printing scaffolds with a fibronectin layer on the surface to study the attachment structure and the function of promoting cell attachment [11,12]. Fibronectin is a protein dimer consisting of two nearly identical polypeptide chains. Modules III9–10 in fibronectin correspond to the integrin-binding site, where the synergy sequence PHSRN of III9 has a role in modulating binding with α5β1 integrin, and the RGD sequence of III10 is the site of cell attachment via α5β1 and αVβ3 integrins [13]. Modules III12–14 correspond to the heparin-binding areas. They can bind several growth factors, including platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF) families [14,15]. Because growth factors can be attached in advance and released gradually, fibronectin is designed as a chemical signal carrier and regarded as a potential material for in vivo experiments [16,17].

Vascular endothelial growth factor has been widely used in cultivation of capillaries. VEGF can be categorized into types A to D. Type A activates the phosphorylation pathway of VEGF-receptor 2 (VEGFR2), which is essential to the process of blood vessel formation [18–21]. There are four most notable VEGF-A isoforms found in humans, composed of 121, 145, 165, and 189 amino acid sequences. Except for 121, the other three isoforms have their protein structures located in exons 6a and 7, providing the binding ability to fibronectin with different binding degrees [22]. VEGF-A145 and A189 can strongly bind both the cell surface and extracellular matrix [23–25], and VEGF-A165 is the most abundantly expressed one and the most potent initiator of vasculogenesis [26].

The effect of VEGF on vasculogenesis is the key to cultivate a capillary network in the engineered tissue. In vivo, VEGF could be soluble in the medium or protein-bound [22,27,28]; however, how the soluble and bound forms of VEGF regulate the network forming is still not completely clear. The biogel-bound VEGF (abbreviated as the bound VEGF afterward) dominates angiogenesis and vasculogenesis by providing the prolonged VEGFR2 activation and subsequent enhancement of the phosphorylated pathway [29–31]. Additionally, the synergy of integrin and VEGFR2 on endothelial cell membranes has been discovered [32]. With the integrin-binding and growth factor-binding domains located closely enough on fibronectin, the chemical stimulation for endothelial cell migration is believed to be further enhanced. However, to date, a correct understanding of soluble or bound VEGF binding phenomena and their corresponding function is still unclear.

In this study, we conducted an experimental study focusing on the reactions of human umbilical vein endothelial cells (HUVECs) stimulated by VEGF signals in vitro. The aim is to develop a biogel of definite components, with which we could explore the developing process of capillary-like networks and discuss the network features under different culturing conditions. The in-house biogel was composed of a definite concentration of collagen and fibronectin rather than adopting a commercial gel to remove other growth factors that may have been contained in the commercial biogel. The in-house biogel was tested for its bio-compatibility and used to investigate the effects of soluble and bound VEGF on the endothelial cells. The fibronectin in the biogel provided the sites for VEGF binding and HUVECs attachment, respectively. We further used a synthetic VEGF-blocking-peptide (VBP) to block VEGF from binding to fibronectin to clarify whether the effect was derived from VEGF binding or cell attachment. This study distinguishes the different effects of soluble and bound VEGF on stimulating endothelial cells to form the capillary network, which has been overlooked before. A deep insight into the relationship between the net-
work features and the two VEGF forms will advance the research progress of culturing artificial capillaries in vitro.

2. Materials and Methods

2.1. Cells and Materials

Human umbilical vein endothelial cells (HUVECs) were obtained from the Bioresource Collection and Research Center (BCRC, Hsinchu, Taiwan, #H-UV001). Cells were cultured to the 7th generation for each experiment. The medium base used was Medium 199 (#31100035, Gibco/Thermo Fischer, Waltham, MA, USA), and with additional heparin sodium (#375095, EMD Millipore, Temecula, CA, USA), endothelial cell growth supplement (#02-102, ECGS; EMD Millipore, Temecula, CA, USA), fetal bovine serum (FBS; Gibco/Thermo Fischer, Waltham, MA, USA), and penicillin-streptomycin (P/S; Gibco/Thermo Fischer, Waltham, MA, USA). The substrate used for the subculture was gelatin (#G1890, Sigma-Aldrich, Burlington, MA, USA). An in-house biogel comprising type I collagen (#C9791, Sigma-Aldrich, Burlington, MA, USA) and fibronectin (#F2006, Sigma-Aldrich, Burlington, MA, USA) was used for in vitro vasculogenesis experiments. We adopted VEGF-A165 (#V5765, Sigma-Aldrich, Burlington, MA, USA) because, among the isoforms, VEGF-A165 has moderate diffusible and binding abilities, which facilitated the examination of soluble and bound VEGFs.

2.2. HUVECs Subculture

In the subculture stage, we used 1 wt% gelatin aqueous solution as biogel. The medium comprised 45 mL FBS, 5 mL P/S, and 450 mL Medium 199, with 60 mg heparin sodium, 15 mg ECGS, and 1.5 g sodium bicarbonate, recommended for HUVECs culturing provided by BCRC. Before subculture, 5 mL gelatin solution was added to a 10 cm petri-dish. The liquid was removed after 30 min so that a layer of gelatin adheres on the petri-dish surface to enhance cell attachment. Then, the 1 mL frozen HUVECs suspension was warmed up in a 37 °C water tank, mixed with a 9 mL medium, and added to the petri-dish. After 3 h of incubation at 37 °C, 95% relative humidity, and 5% carbon dioxide, the medium was refreshed to remove the dimethyl-sulfoxide (DMSO) from the suspension and the not yet attached less-active cells. After that, the medium was refreshed every other day until the cells covered more than 90% of the petri-dish. The medium was then removed, the cells were rinsed twice with Dulbecco phosphate-buffered saline (DPBS), treated with 2 mL Trypsin-EDTA, and neutralized with 8 mL fresh medium. Finally, the cells were collected by centrifugation and distributed to new dishes, and experiments started when the cells reached their 7th generation.

2.3. Biogel Preparation

For the in vitro experiments, the components selected for the in-house biogel were type I collagen and fibronectin, which were pre-prepared into 3 mg/mL collagen/acetic acid and 0.05 mg/mL fibronectin aqueous solution, respectively. The medium formulated in capillaries culturing was 485 mL Medium 199, 10 mL FBS, 5 mL P/S, and together with 60 mg heparin sodium and 1.5 g sodium bicarbonate. With the low content of FBS, HUVECs could obtain limited nutrients to survive while could not divide and proliferate. The ECGS extracted from the bovine hypothalamus was eliminated to avoid its complex components from affecting the results. Before the experiment, 400 µL collagen/acetic acid solution was added and air-dried per well in 12-well plates, and DPBS was added to neutralize the acid and then removed. After that, 400 µL fibronectin aqueous solution was added and removed 1 h later.

2.4. VEGF-Blocking-Peptide (VBP) Design

To clarify the effect of soluble and bound VEGF on endothelial cells, we used the VEGF-blocking peptide (VBP) synthesized by Abcam to conduct a second set of experiments. This peptide was derived from the exon-7 sequence of VEGF-A165. The amino acid sequence of
exon-7 conveys heparan sulfate proteoglycan (HSPG)- and Neuropilin 1 (NRP1)-binding motifs, which control the binding ability [22]. As shown in Figure 1, the VBP could only bind fibronectin but not interact with the receptors on the endothelial cell membrane. Before culturing, the VBP was added to the fibronectin-coated wells to block the growth factor-binding domain on fibronectin. Thus, the VEGF remained soluble in the medium. By introducing VBP to ensure soluble VEGF as the single influential variable, we could confirm the VEGF influence exerted by the soluble and bound form, respectively. A dosage of 0.125 ng/µL VBP dissolved in DPBS was added (400 µL per well) and removed an hour later. Finally, 800 µL of HUVEC suspension that contained 0.02 ng/µL VEGF-A165 was added into each well, with the cell concentration controlled to be 80,000 cells per well. The results were recorded using inverted microscopic instruments every 6 h in a 24-h incubation period.

Figure 1. Exons of VEGF-A165, in which exon-7 provides the ability to bind fibronectin. A VEGF-blocking peptide (VBP) was made with the amino acid sequence derived from exon-7. Amino acid numbers in the VEGF-A165 sequence are also labeled at two VBP ends.

2.5. Immunofluorescence

We collated the bound VEGF on the biogel using fluorescence immunooassay to assess whether VBP could prevent the forming of bound VEGF. This assay was also applied to evaluate whether HUVECs secreted VEGF to identify the possible autocrine signaling. In brief, the culture medium was removed, and the cells were fixed by adding 4% formaldehyde. The cells were incubated with Alexa Fluor® 488 conjugated antibodies specifically against VEGF (#ABS82-AF488, Millipore) overnight at 4 °C. After three washes with PBS, we observed the cells using the Nikon Eclipse 80i fluorescence microscope (Nikon Instruments, Tokyo, Japan) for image acquisition.

2.6. Images and Statistical Analysis

The cell network integrity and complexity were quantified using the number of network junctions, network segments, and the total segment length [12,33]. These indicating values were evaluated from the cell culture images using the plugin tool Angiogenesis Analyzer in Image J 1.52a (NIH, Bethesda, MD, USA). We set 10 pixels as the minimum cell size and 50 pixels as the minimum segment length, and applied these settings in our 2500 × 1600-pixel images. Different junction numbers, segment numbers, and total segment lengths were compared using the Student’s t-test. Statistical analyses were performed using Microsoft Excel 2016 (Microsoft, Redmond, WA, USA) and OriginPro 2021b (OriginLab, Northampton, MA, USA). The data shown were representative of 5 experiments with similar results. A p-value < 0.05 was considered significant.

3. Results

3.1. HUVECs Cultivation on Type I Collagen and Fibronectin

The primary purpose of this study is to explore the respective effect of soluble and bound VEGF on endothelial cells. Therefore, instead of using the commercial matrix (such as Matrigel®), which already contains growth factors, pure collagen type I and fibronectin were used to make the in-house biogel with a definite combination. Type I collagen as the primary substrate provided the endothelial cells with attachment sites when the cells
moved. Fibronectin attached to the collagen surface provided cell attachment and binding sites for soluble VEGF initially contained in the culture medium to form bound VEGF. The cell suspension was added to a 12-well plate coated with collagen with or without fibronectin. The initial number of endothelial cells was 80,000 cells per well, and the cell distribution images were recorded every 6 h.

The endothelial cells were cultivated under four conditions, considering whether fibronectin was coated on the collagen and whether VEGF was added to the culture medium. The endothelial cells in the four cases formed the network to different extents. The results are as shown in Figure 2. In the two cases with no VEGF but with or without fibronectin addition (−F−V and +F−V), HUVECs started the morphological change from round to elongated shape at about 6 h, and the change became evident at 12 h. The cells in these two cases were found to undergo a rapid cell death process that the number of adherent endothelial cells continually decreased. At 24 h of incubation, only a small number of cells remained in the sight field. It is worth mentioning that the cells could express the morphological change even without VEGF added.

Figure 2. HUVECs morphological forms and cellular networks in different cell culture conditions: without fibronectin (−F), with fibronectin (+F), without VEGF (−V), and with VEGF (+V). Black arrows in the enlarged photos indicate the cells with morphological change into the elongated shape, while white arrows indicate the cells still in the original cobblestone shape.

Under the culture condition without fibronectin but with VEGF (−F+V), many cells with morphological changes appeared at 6 h. However, the cell network could not ultimately form afterward. In the +F+V case, the beginning of morphological change occurred a bit later than in the −F+V case. The originally round-shaped cells became elongated, and the dividing boundary for adjacent cells became blurred at 12 h. These characteristics were more evident subsequently, which shows that VEGF stimulated the endothelial cells continuously. The cell-free area in the visual field became apparent as a result of cell aggregation. The cell colony evolved in the direction of forming the capillary-like network.
3.2. Effects of Soluble and Bound VEGF

Comparison between the –F+V and +F+V cases, as shown in Figure 2, reveals that the endothelial cells begin morphological changes earlier; however, a cell network could not form afterward if the biogel had no fibronectin. In contrast, although the cell commenced morphological change somewhat later in +F+V, the cells continued to migrate and aggregate into a cellular network. This result indicated that soluble and bound VEGF might have different effects on cell behavior. However, in addition to binding VEGF, fibronectin could bind cells through its integrin-binding domain. To clarify whether it was the cell-binding or VEGF-binding to the fibronectin responsible for the different cell behaviors, we conducted a series of experiments using the synthetic VEGF-blocking-peptide (VBP). The sequences of VBP were designed according to the fibronectin-binding domain of VEGF (exon-7). This peptide was added to the fibronectin-coating wells before adding the HUVEC suspension to shield the growth factor-binding domain of fibronectin. Because the bound VEGF was prevented from forming, the added VEGF would remain in the soluble form.

The VBP shielding effect was assessed using fluorescence immunoassay, where a FITC-conjugated anti-VEGF antibody was used to monitor the VEGF. Results are as shown in Figure 3 for four different cell culture conditions: without VBP (−P), with VBP (+P), without VEGF (−V), and with VEGF (+V). The darker fluorescence response in the case of +P+V than in −P+V indicated that VBP could avoid VEGF from binding fibronectin. The two subsets of −P−V and +P−V showed that even in the cases with no adding VEGF in the culture medium, the fluorescence response could still be detected, albeit the responses were weaker than the counterpart cases of −P+V and +P+V, respectively. The results indicated that HUVECs could secrete VEGF. This autocrine signaling explained why HUVECs presented a morphological change in the cases without adding VEGF from outside. However, because the cells experienced rapid cell death in the two cases without VEGF added, adding VEGF was essential to cell viability to prevent the cell un-attachment and cell death during the network developing process.

![Figure 3](image-url) Bound VEGF location after 6 h of incubation in different cell culture conditions: without VBP (−P), with VBP (+P), without VEGF (−V), and with VEGF (+V). A fluorescent signal from the primary antibody against VEGF was detected on the cell membrane and biogel surface. The VBP shielding effect avoided VEGF from binding biogel resulting in the darker VEGF field in both the +P−V and +P+V cases.

We further tested the VBP effect on cells by changing dose usage. The number of junctions quantitatively indicated the integrity and complexity of the capillary-like network. The results are displayed in Figure 4, which shows HUVECs could differentiate into a network and established an average of 140 junctions per view field for the cases with no
or adding a small amount of VBP. The junction number dramatically decreased with the peptide concentration larger than 0.0125 ng/µL, but the cells kept attaching to the biogel. The results showed that the usage of VBP interfered with the regulating effect of VEGF and affected the integrity of network structure but not the cell adhesion.

![Peptide 0 ng/µl](image1.png) ![Peptide 0.125 ng/µl](image2.png)

| Peptide Usage (ng/µl) | No. of junctions (＃) |
|-----------------------|----------------------|
| 0                     | 132                  |
| 0.000125              | 142                  |
| 0.00125               | 140                  |
| 0.0125                | 92                   |
| 0.125                 | 88                   |

**Figure 4.** The effect of VBP dosage on the cell network integrity. The average number of network junctions per view field served as a quantitative indicator of network integrity. Cell cords recognized by ImageJ software are shown, where the pink lines present the segments (connections between two junctions), the green lines the branches (connections between junction and node), and the blue lines the isolated elements (connections between two nodes). Data were collected after 24 h of cell culture and averaged with three repeated experiments.

Based on the optimum culturing environment (+F+V), as shown in Figure 2, we tested on HUVECs the stimulating effect of soluble and bound VEGF, respectively, by adding 400 µL DPBS per well with 0.125 ng/µL dissolved VBP. The cell culture images are shown in Figure 5. In the -P situation, i.e., without VBP addition, HUVECs showed the change in morphology and subsequently formed the capillary-like network. In the +P situation, i.e., with VBP addition, HUVECs could not ultimately create the cell network, albeit the cells presenting morphological change earlier than the -P case.

More details about the network integrity and complexity are summarized in Figure 6. The number of junctions, the number of segments, and the total segment length were measured as representative indicators. If no VBP was added (−P+V), these three values remain high for all the observation times, indicating that a cell network was developed to a high degree. In contrast, with VBP added (+P+V), these three values decreased significantly after 18 h, indicating no cell network could ultimately form with VBP added. Because adding VBP (+P) prevented VEGF binding onto fibronectin, the added VEGF would remain soluble. The soluble VEGF was shown incapable of sustaining the network formation.
**Figure 5.** HUVECs distribution with and without VBP addition. In the -P case, which means without the VBP addition, HUVECs showed the change in morphology at 12 h with some cells remaining in the cobblestone shape and subsequently formed the capillary-like networks at 18 h. In the +P case, which means with the VBP addition, most HUVECs showed the morphological change at 12 h; however, the network could not form afterward.

**Figure 6.** Junction number, segment number, and total segment length of the capillary-like network. The higher value of these three indicators, the more integrity of the network. All experiments were repeated five times. Data are presented as mean SD. The Student’s t-test was performed for statistical evaluation: * = $p < 0.05$, ** = $p < 0.01$, and ns = non-significant. The asterisk above the -P+V charts presents the $p$-value between the −P+V and +P+V cases at the same observation time.

### 4. Discussion

Previous experiments primarily focused on the VEGF amount added to the culture environment, lacking the discussion on the two states of VEGF [7,34]. Less attention was paid to telling the different effects of soluble and bound VEGF. The hypothesis that the formation of capillary-like networks is only regulated by the bound VEGF cannot fit all experimental data [35]. In theoretical modeling, on the other hand, capillary-like networks can be formed by using both soluble-VEGF-based or bound-VEGF-based mathematical models, and a consensus is still absent [36–38]. Moreover, although many in vitro capillaries culturing experiments have been successful, problems remained in the in vivo trials. Once the artificial scaffold and tissue are transplanted into the human body, the VEGF binding...
to the scaffold will gradually be consumed or decomposed over time. How to regularly provide an appropriate amount of VEGF becomes a difficult challenge [39,40]. A high initial dosage strategy is not adopted because the excessively high VEGF concentration will cause excessive proliferation of capillaries and potential carcinogenic risks [41]. Transplanting biomaterials, such as fibronectin, that can initially capture and gradually release VEGF, seems feasible, while it is essential to precisely predict the dynamic balance between soluble and bound VEGF [12,33].

Previous experiments showed that parts of the cells with morphological change to elongated shape are connected end-to-end to form the cords in the capillary-like network, while those without morphological change aggregate and create a cobblestone arrangement [34,42]. The same phenomena were found in our results (+F+V in Figure 2 and -P in Figure 5). Because the animal-extracted materials such as Matrigel and ECGS used in literature are originally rich in VEGF, previous studies lacked the discussion on the respective effects of soluble and bound form. We filled this gap by building a VEGF-free environment, which allowed us to explore the VEGF effects. We chose the type I collagen as substrate ingredient, which had been proven beneficial for HUVECs survival and biogel remodeling [7,10], and treated with the thin fibronectin layer coated on collagen surface, which provided VEGF-binding areas, and further enhanced HUVECs attachment and morphology [12,32]. By comparing the cases with and without adding VEGF, we discussed the different roles soluble and bound VEGF played in forming capillary-like networks.

The exogenous VEGF tests on HUVECs showed that more cells underwent a morphological change in the −F+V condition within 12 h of incubation than the standard +F+V case. Since in the −F+V case there was virtually no VEGF binding to fibronectin, the added VEGF remains in the soluble form; the results indicated a higher concentration of soluble VEGF induced the cell morphological change earlier. The primary response of endothelial cells to the stimulation of soluble VEGF was the shape change from round to elongated shape.

The experiment of adding VEGF-blocking-peptide (VBP) to prevent the formation of bound VEGF further confirmed the respective effects of soluble and bound VEGFs. Adding VBP resulted in a higher concentration of soluble VEGF in the culture medium than not adding VBP. Therefore, we could confirm that soluble VEGF mainly stimulated the endothelial cells to change from a round to elongated form, which might benefit the center-hollowed tube structure development [43]. On the other hand, the bound VEGF mainly stimulated the endothelial cells to migrate and ultimately formed a high integrity network.

In summary, the soluble and bound VEGF had different vital functions in inducing endothelial cells to differentiate into a capillary-like network. The soluble VEGF dissolved in the culture medium activated the cell morphogenesis quickly. The cells became elongated, which was conducive to the formation of network cords. Simultaneously, the VEGF binding to the biogel provided a slow and continuous chemical stimulation, guiding the cells to move and aggregate, forming a capillary-like structure with complete integrity.

According to previous literature, VEGF has multiple functions to regulate the growth, division, migration, and shape changes of endothelial cells [44]. Our results confirmed that the cell survival ratio is significantly lower in the cases with no VEGF added. We also showed that endothelial cells could secrete VEGF themselves if no exogenous VEGF was provided. These two phenomena might have some correlation in our cultural condition. Furthermore, our results explained why some theoretical studies could obtain cell networks under specific culture conditions conforming to the autocrine experimental setups [37,45,46]. However, to develop a stable capillary-like network in vivo, the VEGF source needed should be paracrine, secreted by other kinds of cells [34,47].

5. Conclusions

This study established a cell culture biogel of definite collagen and fibronectin, providing a pure scaffold for capillary network formation. As the biogel and the added VEGF had
fixed-content components, we investigated the respective effect of the solute and bound VEGF on the in vitro cultivation of HUVECs. The experiments with adding VEGF showed that the soluble VEGF dissolved in the culture medium, and through the quick interaction with the endothelial cells, activated the cell morphological change. The cells became elongated in shape, conducive to the formation of capillary cords. On the other hand, the bound VEGF induced cell migration and aggregation into a network and kept the network integrity. The experiments without adding exogenous VEGF showed that HUVECs could secrete VEGF and prompted them to commence morphogenesis, changing from round to elongated shape. However, as the cells would quickly die without adding VEGF, a sufficient VEGF supply was shown essential to the capillary-like network formation.

The limitation of this study is that we constructed a simplified model. The essential biomolecules participating in the vasculogenesis are enormous. Our study may not fully reflect all the phenomena involved in the capillary-developing process. However, the current experimental design provides a definite environment to study the effect of each biomolecule that participates in vasculogenesis. After exploring the effect of each biomolecule, we may decode the entire vasculogenesis process in the future.

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