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A novel *in vitro* angiogenesis model based on a microfluidic device

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Angiogenesis is very important for many physiological and pathological processes. However, the molecular mechanisms of angiogenesis are unclear. To elucidate the molecular mechanisms of angiogenesis and to develop treatments for "angiogenesis-dependent" diseases, it is essential to establish a suitable *in vitro* angiogenesis model. In this study, we created a novel *in vitro* angiogenesis model based on a microfluidic device. Our model provides an *in vivo*-like microenvironment for endothelial cells (ECs) cultures and monitors the response of ECs to changes in their microenvironment in real time. To evaluate the potential of this microfluidic device for researching angiogenesis, the effects of pro-angiogenic factors on ECs proliferation, migration and tube-like structure formation were investigated. Our results showed the proliferation rate of ECs in 3D matrix was significantly promoted by the pro-angiogenic factors (with an increase of 59.12%). With the stimulation of pro-angiogenic factors gradients, ECs directionally migrated into the Matrigel from low concentrations to high concentrations and consequently formed multi-cell chords and tube-like structures. These results suggest that the device can provide a suitable platform for elucidating the mechanisms of angiogenesis and for screening pro-angiogenic or anti-angiogenic drugs for "angiogenesis-dependent" diseases.

angiogenesis, microfluidic device, microenvironment, pro-angiogenic factors

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Angiogenesis is very important for many physiological and pathological processes. Physiological angiogenesis often occurs in development, reproduction and tissue repair. Pathological angiogenesis occurs not only in tumor formation, but also in a range of non-neoplastic diseases that could be classified together as "angiogenesis-dependent diseases" [1]. However, the molecular mechanisms and the treatments of angiogenesis are far from clear. To elucidate the molecular mechanisms of angiogenesis and to develop treatments for cancer and other "angiogenesis-dependent diseases", we established an *in vitro* angiogenesis model.

Angiogenesis is a multi-step dynamic process involving complex interactions between vascular cells and the corresponding extracellular environment [2–4]. During this process, numerous pro-angiogenic factors, such as fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), angiopoietin-1, and stromal-derived factor 1 (SDF-1), are secreted from new blood vessel formation sites. With the induction of pro-angiogenic factors, endothelial cells (ECs) are activated from a quiescent state into tip cells. Then matrix metalloproteinase family members are activated and released from the "activated" ECs to degrade the basement membrane surrounding the existing vessel. The "activated" ECs are induced to migrate into the interstitial

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space, to proliferate and to form a network of tubes and loops. Finally, the new basement is generated with the recruitment of pericytes to stabilize and maintain tube formation [5,6]. Many *in vitro* models have been designed to mimic the basic steps of the *in vivo* process [7,8]. An ideal angiogenesis model should have a known spatial and temporal concentration distribution of angiogenic factors and inhibitors being studied for forming dose-response curves, and it should be able to quantify the structure and function of the new vasculature (including the ECs migration rate, proliferation rate, canalization rate, blood flow rate, and vascular permeability) [8,9]. However, many traditional models are carried out in two dimensions (2D) and may not take into account the more complicated three dimensional (3D) arrangements involved in cell and extracellular environment interactions.

Microfluidic technologies have paved the way for new approaches to manipulate and monitor cells in an environment that closely mimics *in vivo* conditions. The major advantages of microfluidic systems are their abilities to use small quantities of cells and reagents, to have precise control of spatial and temporal environments and to visualize the cellular events in real time [10,11]. Some microfluidic devices have been constructed as *in vitro* angiogenesis models to study the angiogenic mechanisms [10–14]. However, many are limited as they are only in 2D. Vickerman et al. [10] developed a controlled multi-parameter microfluidic platform to study capillary morphogenesis and to demonstrate the role of gradients of pro-angiogenic factors, surface shear and interstitial flow in angiogenesis in a defined 3D environment. However, patterning matrix gel in this device with microinjection is challenging and requires a very complex system including a manual micromanipulator, microliter syringe, digital microscope and a monitor for visual guidance. These experimental setups and equipments are not readily available in most biomedical labs. Thus, their applications were limited.

In this study, we developed a microfluidic device which allows for precisely patterning 3D gels into a microfluidic channel using only a pipette. This device is composed of three parallel main channels and several smaller horizontal microchannels which connect to the main channel. The middle channel contains the gel patterning channel. This device provides an *in vivo*-like microenvironment for cultures of ECs, for controlled time-dependent delivery of biochemical agents, uniformly or with a spatial gradient, and for real-time monitoring of ECs in response to changes in their microenvironment. To demonstrate the potentiality of this microfluidic device for angiogenesis model research, we investigated the effects of pro-angiogenic factors on ECs proliferation, migration and tube-like structure formation. The results suggest this microfluidic device can provide a suitable platform to elucidate mechanisms of angiogenesis and to screen pro-angiogenic or anti-angiogenic drugs for "angiogenesis-dependent" diseases.

1 Materials and methods

1.1 Design and fabrication of the microfluidic device

The microfluidic device is composed of three main parallel channels connected by several smaller horizontal microchannels (Figure 1(a)). Each main channel is 400 μm in width, 150 μm in height and 20 mm in length, while each bridge channel is 100 μm in width, 150 μm in height and 300 μm in length. The device was prepared by standard soft lithography. Briefly, it was fabricated by replicate molding on the master using polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning Corp. Midland, MI, USA). The master was prepared by spin coating a 150 μm layer SU8 negative photoresist (Microchem Corp, Newton, MA, USA) onto a glass wafer and patterned by photolithography. The PDMS precursor and curing agent (10∶1, w/w) were mixed thoroughly, degassed under vacuum, poured onto the master and incubated in an oven at 80°C for 2 h. Subsequently, the PDMS device was peeled off from the master wafer and then punched to form inlets and outlets with a sharpened flat-ended 16 gauge needle. To form a closed microfluidic channel, the PDMS device was irreversibly bonded to a microscope glass slide after oxygen plasma treatment for 60 s. The device was sterilized by autoclaving at 121°C for 30 min before using.

1.2 Gel preparation and injection

Matrigel (BD Biosciences, San Jose, CA, USA) was thawed overnight at 4°C on ice before use, and the pipettes, tips and microfluidic device were precooled. The Matrigel was mixed to homogeneity with cooled pipettes. While the precooled microfluidic device was kept on ice, 10 μL Matrigel solution was carefully injected into the middle channel of the microfluidic device with a pipette. The microfluidic device was then placed in a Petri dish and transferred to a 37°C incubator for 30 min to polymerize the gel. After the gel polymerized, media was added to the inlet reservoirs of the side channels and gently suctioned at the outlet holes using a pipette.

1.3 Demonstration of the concentration gradient across the gel channel

To confirm the diffusion and the distribution of biochemical factors across the gel channel, FITC-dextran (40 kD; Invitrogen, Carlsbad, CA, USA), which is similar in size to pro-angiogenic factors such as VEGF, was used to visualize the gradient of biochemical factors. After gel polymerization, PBS was loaded into both side channels to equilibrate for 2 h. The diffusion of FITC-dextran solution across the gel channel was characterized by using fluorescence microscopy. Tips were used as reservoirs for the side channels. A total volume of 60 μL PBS was loaded into the inlet reservoir

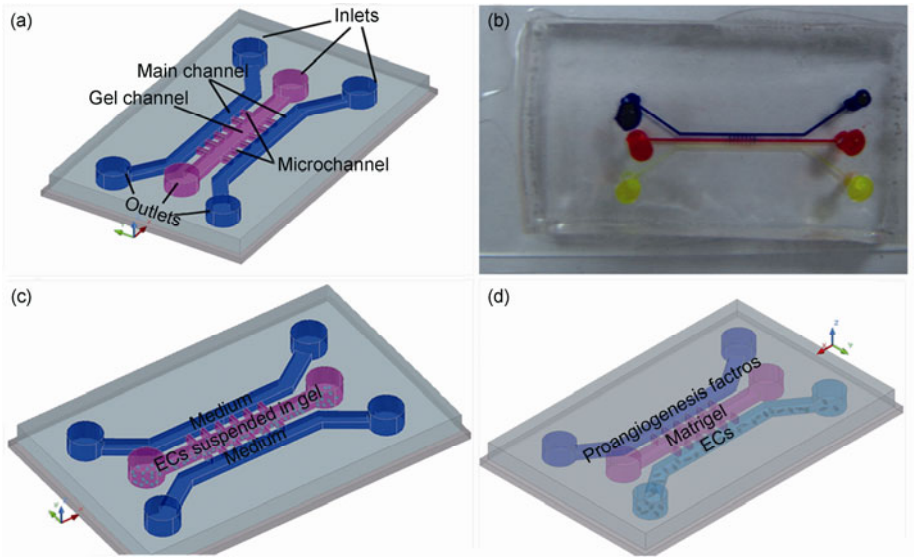

Figure 1 Design and structure of a microfluidic device for establishing an *in vitro* angiogenesis model. (a) Configuration of the device. The microfluidic device is composed of three main parallel channels connected by a series of smaller horizontal microchannels. (b) The fabricated device. The middle channel is injected with Matrigel and red dye, while the side channels are filled with medium containing either blue or yellow dye. (c) The schematic diagram of the microfluidic device for 3D culture. ECs suspended in Matrigel were injected into the middle channel, and both side channels were filled with medium to supply nutrition for the ECs in the 3D gel. (d) The schematic diagram of the microfluidic device for the migration assay of ECs. After Matrigel was filled into the middle channel and polymerized, ECs were seeded into one of the side channels. The other side channel was filled with medium with or without pro-angiogenic factors to induce ECs to migrate into the gel.

of the sink channel and 60 μL of 1 μmol/L FITC-dextran in PBS was loaded into the inlet reservoir of the source channel. After the solution in the inlet reservoir flowed through the channel to the outlet, the same volume of solution was loaded into the outlet reservoir to maintain a static condition in the channel. The time when FITC-dextran solution/PBS was loaded was set as t=0. The images for the concentration distribution were taken using a fluorescent inverted microscope (Olympus TH4-200, Tokyo, Japan) with a CCD camera (Olympus DP25) at every 5 min. The fluorescence intensity profile was analyzed by MATLAB (Math Works, Natick, MA, USA). For each frame, the intensity of the gel channel was measured and normalized to the maximum intensity in that frame. The distance from the source channel in the gel region was normalized by the length of the gel region.

1.4 Cell-gel formation and 3D culture

High density HUVECs (human umbilical vein endothelial cell) suspensions (10^7 cells/mL) were harvested and cooled on ice. Then cell suspensions were mixed (1∶1, v/v) with the thawed Matrigel solution. The cell-gel mixture was injected into the middle channel of the microfluidic device. After the Matrigel was polymerized, a gel network with embedded cells was created. DMEM complete medium (control) or medium supplemented with pro-angiogenic factors (VEGF, bFGF (basic fibroblast growth factor) and EGF (epidermal growth factor), 10 ng/mL for each) was injected into the side channel to supply nutrition for the cells in the 3D gel. Then, the viability, proliferation and tube formation behaviors of HUVECs were investigated in the 3D culture.

1.5 Cell viability assay

After 3-d culture, the viability of HUVECs in the 3D matrix was assessed by labeling live and necrotic cells with 2 μmol/L Calcein AM (Molecular Probes, Carlsbad, CA, USA) and 10 μg/mL propidium iodie (PI; Molecular Probes) respectively and viewed immediately by an inverted fluorescence microscope (Olympus TH4-200).

1.6 Proliferation assay

The Cell-Light™ EdU DNA Cell Proliferation Kit (Invitrogen, Carlsbad, CA, USA), which is a novel alternative to the bromodeoxyuridine (BrdU) assay, was used to evaluate the proliferation of HUVECs in 3D Matrigel culture. Briefly, HUVECs suspended in Matrigel were first seeded into the middle channel of microfluidic chamber. On the following day, medium enriched with pro-angiogenic factors was used to induce cell proliferation for 8 h. Then 50 μmol/L EdU was perfused into the side channels to label cells for 2 h. After EdU labeling, cells were fixed with 4% paraformaldehyde (Sigma, St Louis, MO, USA) for 30 min at room temperature, incubated with 2 mg/mL glycine for 10 min, and then permeabilized with 0.5% Triton X-100 (Sigma) for 20 min. Finally, EdU was detected by its reaction with the

Apollo reaction buffer and nuclei were stained with Hoechst 33342. Fluorescent images were obtained using an inverted fluorescent microscope (Olympus TH4-200).

1.7 Gradient of pro-angiogenic factors induces HUVECs to migrate into Matrigel

Matrigel solution was injected into the middle channel. After injection, the device was then kept in the incubator at 37°C for 30 min to form the gel. Prior to cell seeding, DMEM medium equilibrated in the side channels for 4 h. HUVEC suspension (5×10^6 cells/mL) was prepared and injected into one side channel. The cells were permitted to adhere to the substrate for 4 h. Then medium with pro-angiogenic factors was injected into the side channel without cells, and medium without pro-angiogenic factors was injected into the cell-containing channel. This resulted in a pro-angiogenic factor gradient across the gel region to induce HUVECs to migrate into the gel. To maintain the gradient, medium was changed every 12 h. Cell migration was monitored by phase-contrast microscopy (Leica DM750, Wetzlar, Germany) every day. The effect of pro-angiogenic factors on ECs migration was quantified by measuring the greatest distance from the contact line to the location of "lead cells" and the projected area of regions containing migrated cells using Image J (http://rsbweb.nih.gov/ij/) [12]. At the end of the experiments, actin filament staining was performed to visualize the final cell distribution. Briefly, samples were fixed with 4% paraformaldehyde for 30 min at room temperature, permeabilized with 0.5% Triton X-100 for 30 min and blocked with 5 mg/mL BSA for 60 min. Actin filaments were then stained with FITC-phalloidin (Alexis, San Diego, CA, USA). Fluorescent images were obtained using an inverted fluorescent microscope.

1.8 Tube-like structure formation assay

HUVECs in Matrigel were cultured in complete medium or medium supplemented with pro-angiogenic factors for 5 d. Media were replenished every day. The tube-like structure was characterized by an inverted microscope and an inverted fluorescent microscope. At the end of the experiment, actin filaments and nuclei were stained with FITC-phalloidin (Alexis) and DAPI (KeyGEN Biotech, Nanjing, China). Fluorescent images were obtained using inverted fluorescent microscope.

1.9 Statistical analysis

Statistical analysis was carried out using Origin 7.5 (OriginLab Data Analysis and Graphing Software). Data are represented as mean±SD of $\geqslant 3$ independent experiments. All statistical comparisons were made by performing Student's *t*-test analysis. A P value less than 0.05 was considered statistically significant.

2 Results

2.1 Design and characteristics of the microfluidic device

As shown in Figure 1, the microfluidic device was composed of three parallel main channels connected by several horizontal microchannels. The middle channel is the gel channel, which permits patterning of gels to create a 3D microenvironment. The horizontal microchannels connect the main channels and allow for precise patterning of the gels. The ratio of length to width (L∶W) of the microchannels is very important for patterning gels. Small L∶W ratios (short and wide channels) resulted in the gel leaking into neighboring channels. Excessively high ratios (long and narrow channels) prevented leakage but the microchannels were filled with bubbles. We found that an optimal L∶W ratio was 3∶1. One prominent advantage of our microfluidic device is that it allows one to precisely pattern a 3D Matrigel only with pipettes.

2.2 Concentration gradient profile across the gel channel

The concentration gradient of biomolecules across the gel channel was quantified by measuring the fluorescence intensity. As shown in Figure 2(a), FITC-dextran solution diffused from the source channel (up) to the sink channel (down) across the gel channel. The fluorescence intensity in the gel channel slowly increased and shaped a gradient across the gel with time. The fluorescence intensity of dashed lines indicated the region being measured and was normalized to the maximum intensity. The intensity profile across the gel channel was plotted during the gradient development (Figure 2(b)). For a 1000-μm long gel region, a nearly linear gradient was established within about 1.5 h, and it was maintained for about 10 h. Without replenishing either the source or sink channels, there was a depletion and an accumulation of fluorescence in the source and sink channels, respectively, which resulted in a gradual disappearance of the gradient.

2.3 Viability of HUVECs in the microfluidic device

HUVECs were cultured in the microfluidic device either in 3D culture or in 2D culture. In 3D culture, HUVECs suspended in 3D Matrigels were seeded in the middle channel. In 2D culture, HUVECs were seeded in one side channel after patterning Matrigel in the middle channel. Two dimensional cultures investigated the migration of HUVECs into Matrigel under the induction of pro-angiogenic factors gradient. To reveal the suitability of this device for further cellular research, the viability of ECs cultured in the microfluidic device was examined by Calcein-AM and PI staining. Viable cells were labeled by Calcein-AM and dead cells were labeled by PI. As shown in Figure 3(a) and (b), HUVECs in the 3D matrix formed viable multi-cellular

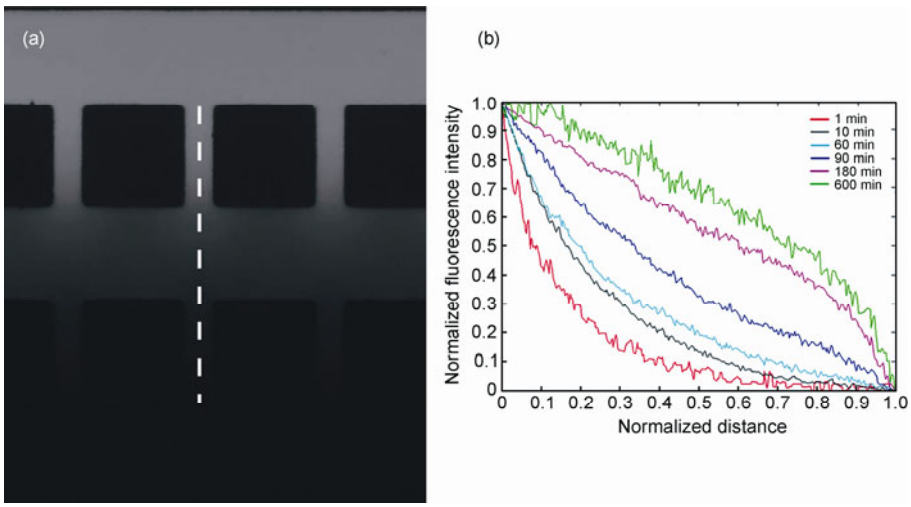

Figure 2 The gradient of fluorescence across the gel channel was quantified by measuring the fluorescence intensity. (a) After gel polymerization, the source channel (up) and the sink channel (down) were loaded with a FITC-dextran solution and PBS, respectively. The length of the Matrigel region was 1000 μm. The dashed line indicates the location of the fluorescence measurements. (b) The plot of the fluorescence intensity profile across the Matrigel region. A linear steady-state gradient was established after 90 min which was maintained for about 10 h.

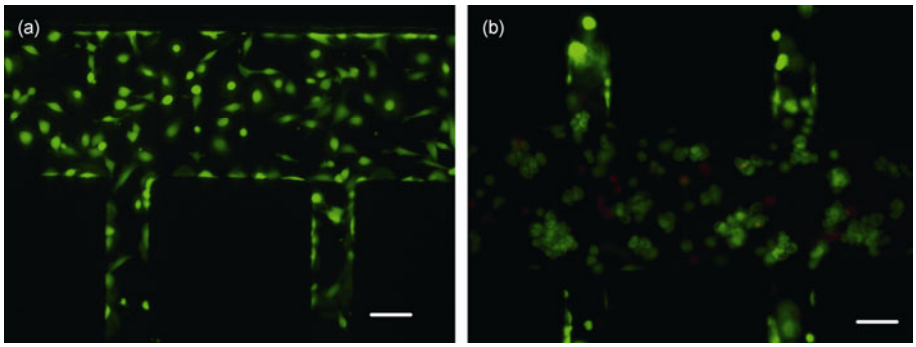

Figure 3 The viability of ECs in the microfluidic device was investigated by Calcein-AM and PI staining. (a) HUVECs were seeded into one side channel for 3 d; most cells were viable (green). (b) HUVECs in the 3D matrix formed viable multi-cellular aggregates after 72 h in culture and exhibited a rounded morphology distinct from that seen in 2D culture. The viability of HUVECs in 3D culture was up to 95% (the green label is viable cells, while the red label depicts dead cells). The scale bar is 100 μm.

aggregates after 72 h in culture and exhibited a rounded morphology distinct from that in 2D culture. HUVECs, either in 2D culture or 3D culture, exhibited a high viability (up to 95%) after being cultured in the device for 72 h, which suggests this microfluidic device is suitable for cell culture.

2.4 Proliferation of HUVECs in 3D culture under the induction of pro-angiogenic factors

During the process of angiogenesis, ECs are activated from a quiescent state because of the induction of angiogenic factors. The proliferation of ECs is essential for angiogenesis. An ideal angiogenesis model should be able to also allow proliferation assays of ECs. In this study, we investigated the proliferation of HUVECs under the induction of pro-angiogenesis factors using the Cell-Light EdU DNA Cell Proliferation Kit, which measures the proliferation of HUVECs by labeling cells during the S phase. HUVECs suspended in 3D Matrigel were injected into the middle channel to culture for 8 h with or without pro-angiogenic factors and then incubated with EdU for 2 h. After incubation, the nucleoli in S phase incorporated EdU (red) and were counterstained with Hoechst 33324 (blue) (Figure 4(a)). As shown in Figure 4(b), up to 71.63% ± 8.65% cells of the pro-angiogenic group were in the S phase, while only about 12.51% ± 3.1% cells were in the S phase in the control group. These results suggest that pro-angiogenic factors activated the ECs and promoted proliferation.

2.5 HUVECs directionally migrate into a 3D matrix because of the stimulation of the gradient of pro-angiogenesis factors

The migratory response of HUVECs to a gradient of pro-angiogenic factors was investigated over a 4 d period. As shown in Figure 5B (a–d), under the induction of the pro-angiogenic factors gradient, many HUVECs directionally

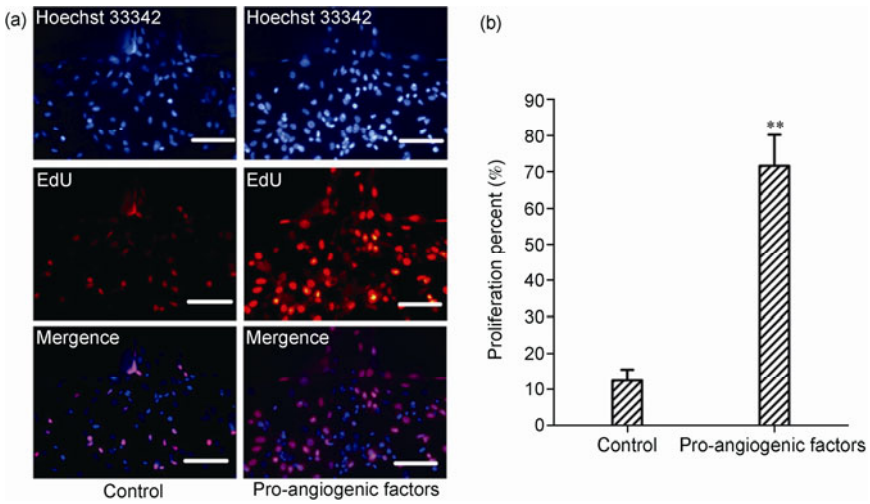

Figure 4 The proliferation of HUVECs in 3D culture induced by pro-angiogenic factors. (a) The proliferation of HUVECs was investigated using the Cell-Light EdU DNA Cell Proliferation Kit. HUVECs suspended in 3D matrigel were injected into the middle channel to culture for 8 h with or without pro-angiogenic factors and then incubated with EdU for 2 h. After incubation with EdU, dividing cells incorporated EdU (red). Cells were counterstained with Hoechst 33324 (blue). Only a few cells were labeled with EdU in the control group, while most cells in the experimental group were labeled with EdU after induction with pro-angiogenic factors. The scale bar is 100 μm. (b) This graph depicts the percentage of dividing cells incorporated with EdU. The results are given as mean ± SD (n=6, **, $P < 0.01$).

migrated into the region with high concentrations of pro-angiogenic factors. In the control group, only a few of cells migrated into the Matrigel (Figure 5A). During the invasion of HUVECs into the 3D Matrigel, the "lead-cells" extended filopodial projections into the gel while the neighboring cells remained non-invasive (Figure 5B(e)). The invading cells formed tube-like structures in the Matrigel (Figure 5C). The migration distance and area were used to quantify the migration behavior of HUVECs in the 3D Matrigel. Both the migration distance (Figure 5D(a)) and the area (Figure 5D(b)) increased significantly when HUVECs were simulated with a gradient of pro-angiogenic factors.

2.6 Tube-like structure formation

Previous studies on the tube formation of ECs were performed by culturing ECs on plates coated with matrix proteins such as Matrigel, collagen, or fibrins [15]. In such models, ECs were still in 2D culture which does not really mimic *in vivo* conditions. In this microfluidic device, HUVECs suspended in the 3D Matrigel were seeded into the middle channel of the device to culture for 7 d. As shown in Figure 6, 3D encapsulated HUVECs formed many multicellular chords and complex tube-like structures in the presence of pro-angiogenic factors, while most cells were isolated and exhibited a round shape in the control sample. The F-actin staining confirmed that these tube-like structures were composed of ECs with tight junctions.

3 Discussion

In this study, we established a novel *in vitro* angiogenesis model based on a microfluidic device. This angiogenesis model has many advantages including: (1) the device is simple to operate and has enormous flexibility in controlling the critical biochemical and biomechanical factors that influence the angiogenesis process; (2) the device provides an *in vivo*-like 3D microenvironment for the growth of ECs and can establish a controllable gradient of soluble factors in the 3D matrix; (3) it can quantitatively analyze the function of ECs; (4) the device allows for monitoring the behaviors of ECs in real time.

Angiogenesis occurs not only in many physiological processes, such as development, reproduction and endometrial cycle remodeling, but also in many pathological processes, including tumor formation and a range of "angiogenesis-dependent diseases". Therefore, the study of angiogenesis has clinical applications in therapies for numerous diseases. As such, more clinically relevant models of angiogenesis *in vitro* are crucial to the understanding of angiogenic processes. In 1980, Folkman et al. [16] found that bovine capillary ECs spontaneously formed tubes when cultured in gelatin *in vitro*. Since then, many *in vitro* models have been designed to mimic many of the basic steps of the *in vivo* process. However, angiogenesis is a multi-step dynamic process involving complex interactions between vascular cells and the corresponding extracellular environment. Thus, numerous challenges exist in properly modeling each of the steps involved in angiogenesis both *in vitro* and *in vivo.* Microfluidic technology provides an *in vivo*-like micro-environment for cell growth. Also, microfluidic technology can control many chemical and physical factors and monitor the effects of these factors on angiogenesis in real time [14].

During the angiogenic process, one of the essential steps

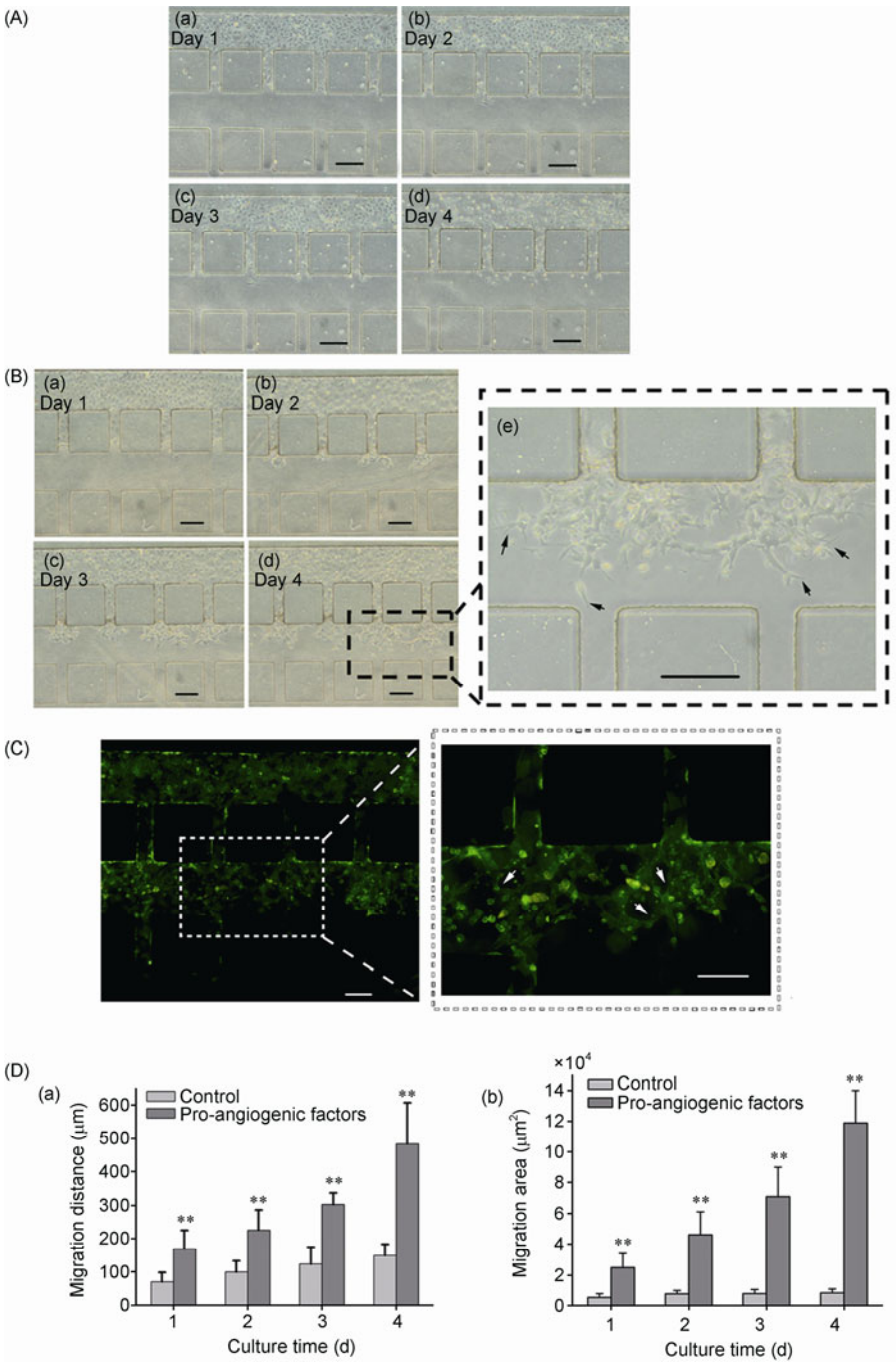

Figure 5 HUVECs migrated into the 3D matrigel to form tube-like structures under the stimulation of the gradient of pro-angiogenic factors (A,B). A sequence of micrographs recorded the migration of HUVECs into the 3D Matrigel over a 4 d period: (A) The control group without pro-angiogenic factors; (B) the migration of HUVECs because of the gradient of pro-angiogenic factors. (B(e)) During the invasion of HUVECs into the 3D Matrigel, the "lead-cells" extended filopodial projections (indicated by arrows) into the gel. The scale bar is 200 μm. (C) At the end of the experiments, cells were fixed and stained for actin. The results showed that tube-like structures were formed after cells invaded into the 3D Matrigel (indicated by arrows). The scale bar is 200 μm. (D) Quantitative migration assays of HUVECs into the 3D Matrigel. The migration distance (a) and area (b) of HUVECs into the 3D Matrigel significantly increased with pro-angiogenic factors, compared with those of the control without pro-angiogenic factors. Data are given as mean ± SD (n=6, **, P < 0.01).

is when ECs directionally migrate into the angiogenic site through the basement membrane to form new blood vessels [17]. Many pro-angiogenic factors (e.g. VEGF, bFGF) are being secreted at new blood vessel sites and establish a gradient across the extracellular matrix, which activates ECs to migrate into these new blood vessel sites. Therefore, an important feature of any *in vitro* angiogenesis model is to provide a gradient distribution for pro-angiogenic factors.

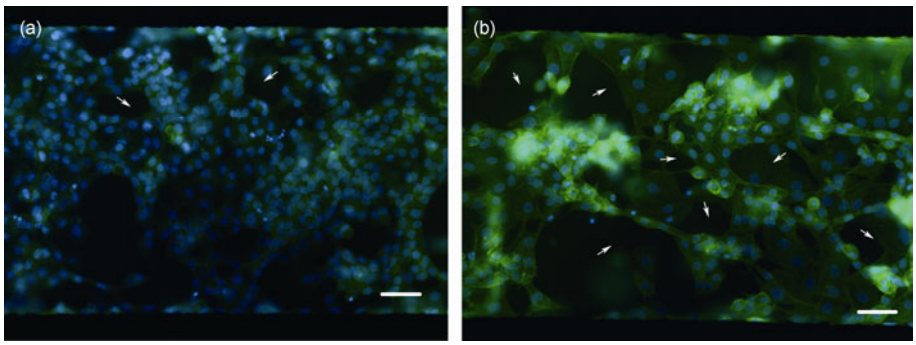

Figure 6 The tube formation of HUVECs suspended in the 3D matrigel. Fixed samples of HUVECs encapsulated in Matrigel were cultured for 5 d with or without pro-angiogenic factors. At the end of the experiments, samples were fixed and stained for the actin cytoskeleton (green) and nuclei (blue). (a) Cells cultured in DMEM medium (control); (b) cells cultured in DMEM supplemented with pro-angiogenic factors (VEGF, bFGF, EGF, each at 10 ng/mL final concentration). The results showed that 3D encapsulated HUVECs formed many multi-cellular chords and complex tube-like structures in the presence of pro-angiogenic factors, while most cells are isolated and present a round shape in the control sample. White arrows indicate the tube like structures. The scale bar is 50 μm.

Traditional *in vitro* gradient-generating methods have been instrumental in shaping our current understanding of gradient signaling, but they are not ideal to examine the quantitative or combinatorial nature of gradient signaling because of their inability to produce precise, user-defined gradients with tailored spatial and temporal profiles [18]. Our device has the ability to generate gradients of soluble factors across a 3D matrix. This provides the potential to precisely control the temporal and spatial distribution of pro-angiogenic factors, which direct ECs migration. Additionally, the response of ECs to the pro-angiogenic factor gradient across the 3D matrix can be monitored in real time, which cannot be carried out by more traditional methods such as Boyden Chamber or Transwell Assay.

After integrating into neo-angiogenesis sites, ECs form multi-cellular tube-like structures in the 3D matrix to form the initial vascular network. Previous studies on the tube formation of ECs were performed by culturing ECs on plates coated with matrix proteins such as Matrigel, collagen, or fibrins [15]. In such models, ECs were still in 2D culture which did not truly mimic *in vivo* conditions. In this study, HUVECs suspended in Matrigel solutions were seeded into the middle channel of the microfluidic device to form an ECs/matrix compound network. Pro-angiogenic factors significantly promoted the proliferation of ECs in the 3D matrix. Since ECs self-organize to form complex tube-like structures, pro-angiogenic factors could facilitate ECs to form tube-like structures.

In this study, we preliminarily assessed the feasibility of this novel angiogenic model by investigating the effects of pro-angiogenic factors on the proliferation, migration and tube formation ECs. We would like to further assess the functions of new vessels, such as the integrity of new vessels, vascular permeability, and blood flow rate. We would also like to investigate the synergistic angiogenic effects of biochemical and biomechanical factors, and perivascular cells, e.g. smooth muscle cells and fibroblasts.

4 Conclusions

In this study, a novel *in vitro* angiogenesis model was developed based on a microfluidic device. The device provides a well-controlled microenvironment mimicking physiological conditions of ECs culture and monitors the response of ECs to changes in their microenvironment in real time. To evaluate the potential usefulness of this microfluidic device for angiogenic research, we investigated the effects of pro-angiogenic factors on the proliferation, migration and tube-like structure formation for ECs in this microfluidic device. The results show that the device provided a suitable platform to elucidate mechanisms of angiogenesis and to test pro-angiogenic or anti-angiogenic drugs for "angiogenesis-dependent" diseases.

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