ENGINEERING STABLE MICRO-CAPILLARY STRUCTURES BY CONTROLLED 3D-COLLAGEN MICROCHANNELS

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ABSTRACT

This paper describes a simple and a rapid method to form stable microvasculatures in the collagen microchannels by geometrically controlling endothelial cells. We developed a semi-opened collagen gel microchannels that allow fluid-dynamically accumulating cells by loading with a simple pipetting method. Accumulated endothelial cells at a high density in the micro-space of collagen channel interacted each other, and formed continous microvasculature-like lumen structures within 24 hours. The developed platform can be used for angiogenesis study and regenerative medicine as the engineered grafts.

KEYWORDS

Tissue engineering, Microvasculatures, Microfluidic chip, Angiogeneis

INTRODUCTION

Microvascularization has a crucial role in all animal tissues. In this respect, *in vitro* microvascularization engineering has long been one of the biggest challenges for tissue engineering [1]. Over the past two decades, researchers showed culturing endothelial cells on/within three-dimensional (3D) extracellular matrices (ECMs) and two-dimensional (2D) micropatterning allow tubular formation of endothelial cells. These methods rely on endothelial cells to self-organize into capillary-like structures. More recently, researchers have been suggesting that controlled geometry of the cultured cells is important for microvasculature formation. For example, Chen's group [2] have demonstrated that geometrically controlling endothelial cells within the 3D environment (i.e. embedding cells within micro-structured collagen gel at higher cell density) efficiently accelerate tubular formation of endothelial cells. Even though this work opened new perspectives on *in vitro* microvasculature formation, perfusion properties into the constructed microvasculatures for the next application (i.e. fabrication of engineered tissues with vasculatures) remain to be studied. Thus, in this study, we developed a method allowing the rapid formation of stable lumen structures based on the vasculogenesis process as a tool for angiogenesis studies (Fig. 1).

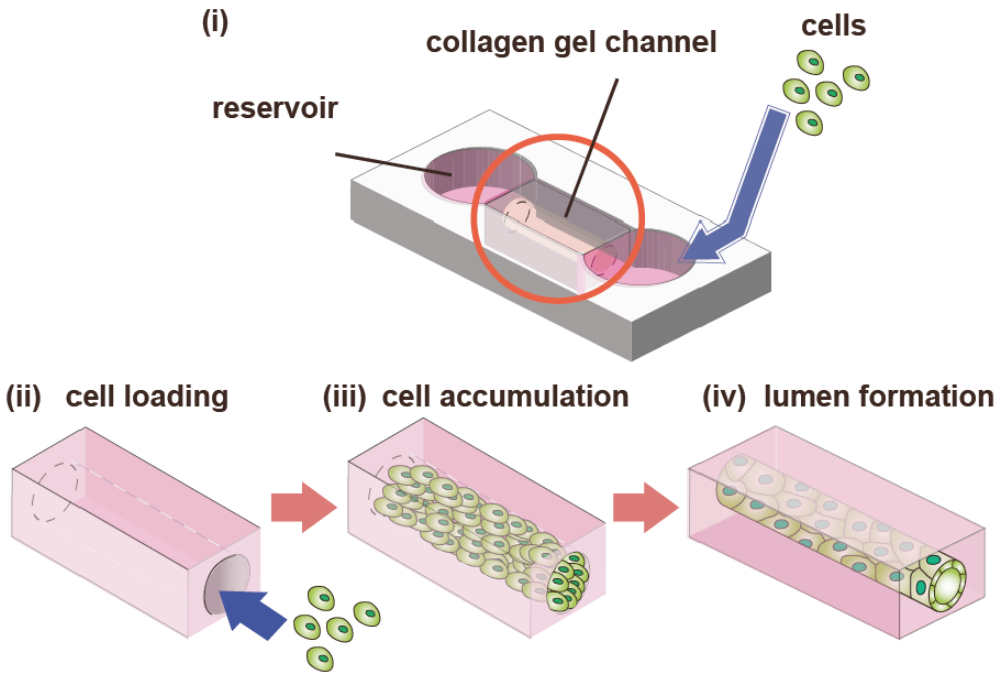

Fig. 1: Concept of this study. Engineering microvasculatures by geometrical control of endothelial cells using microfluidic end-closed collagen microchannels.

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EXPERIMENT

Fabrication of end-closed collagen gel microchannels

To prepare the end-closed collagen microchannels, we gelated collagen type I (2.4 mg/mL) for 20 min in a PDMS hosting chamber including a needle (120 μm in diameter) as a channel mold. Inlet and outlet reservoirs are surrounding the collagen chamber as shown in Fig. 1. The needle was then removed, and collagen gels were immersed with cell culture media for 10 min.

Cell culture in the collagen gel microchannels

HUVECs at passage 3 to 6 were used in this study. HUVECs were obtained from Lonza, and they were maintained at 37°C in 5% CO2 in EGM-2 on the tissue culture polystyrene dishes. Harvested cells were suspended into the EGM-2, then loaded in the inlet reservoir by pipetting to accumulate within the collagen gel microchannels. The cells within the channels were incubated at 37°C in 5% CO2. Cell culture media was changed every 12 hours.

Tube formation analyses

The samples were carefully washed with phosphate buffered saline (PBS), and then fixed with 4% paraformaldehyde in PBS for 30 min. After permeabilization with 0.5% Triton X-100 in PBS at 25°C for 10 min, cells were blocked with 1% bovine serum albumin (BSA) in PBS at 25°C for 60 min, and reacted with 1:200 Alexa Fluor 488-conjugated phalloidin (initial conc. 200 U/mL; Invitrogen) at 25°C for 2h. Hoechst 33342 (DAKO) was added at 1:1000 dilution, and reacted at 25°C for 5 min. After wishing with PBS, the stained cells were observed under a fluorescence confocal microscope (LSM710; Carl Zeiss, Hallbergmoss, Germany) using the objective lens (Plan-fluor, 20X, NA=0.5, Carl Zeiss).

Cell viability assay

After 6, 18, 24, and 48-hour culture period, the cells within the collagen gel microchannels were washed three times with cell culture media without FBS, and Live/Dead assay reagent (Invitrogen) constituted according to the supplier's protocol was added. The samples were incubated at 37ºC in 5% CO_2 for 15 minutes, and observed under a fluorescence confocal microscope.

RESULTS and DISCUSSION

We successfully accumulated HUVECs within the end-closed collagen channels (channel diameter: 120 μm). After 6 hours, we observed that the cells interacted with each other, then formed tube-like structures at 24 hours (Fig. 2). After 48 hours, the cells appeared to undergo internal rearrangements to finally invade into the collagen significantly, showing sprouting behavior known as angiogenesis process of vasculatures.

We also performed analyzing more in details whether these tubular structures had continuous lumen. To visualize the structure of cultured cells within the collagen microchannels, we performed immunostaining against F-actin and nuclei using Alexa488-conjugate phalloidin and Hoechst 33342, subsequently. We observed that cells form continuous lumen structures at 24 hours and 48 hours (Fig. 3).

To further investigate cell behavior within this 3D configuration, we analyzed that the cell viability using Live/Dead assay at the same time points. We found that the cells were significantly viable over the 48 hours. This result indicates that our cell loading process and the cell culture environment within the collagen channels are mild enough for cells.

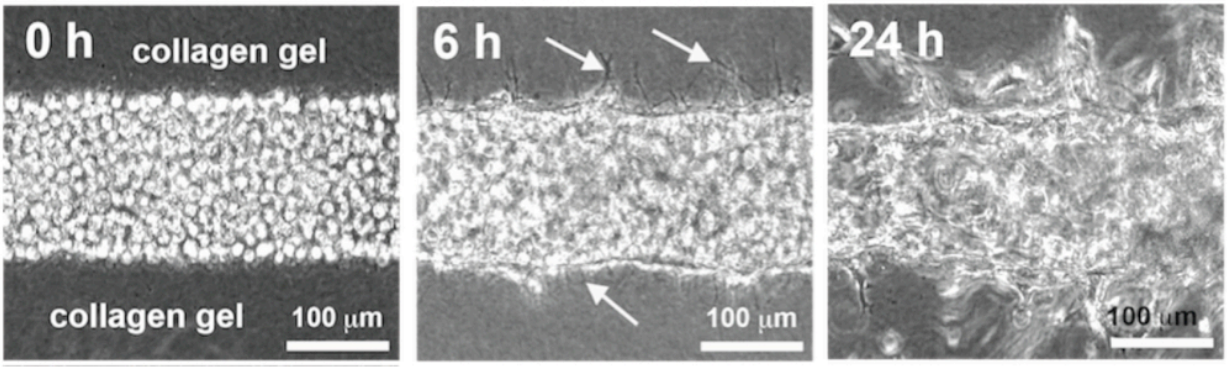

Fig. 2: Microscopic views of HUVECs accumulated within the end-closed collagen microchannels. We observed that the collagen microchannels are fully occupied with cells. After 6 hours, cells appeared to form tubular structures. White arrowheads show that the cells also started to sense their environment at 6 hours. Finally, the arrowheads at 24 and 48 hours show the presence of structures similar to endothelial sprouts. Scale bars: 100 μm.

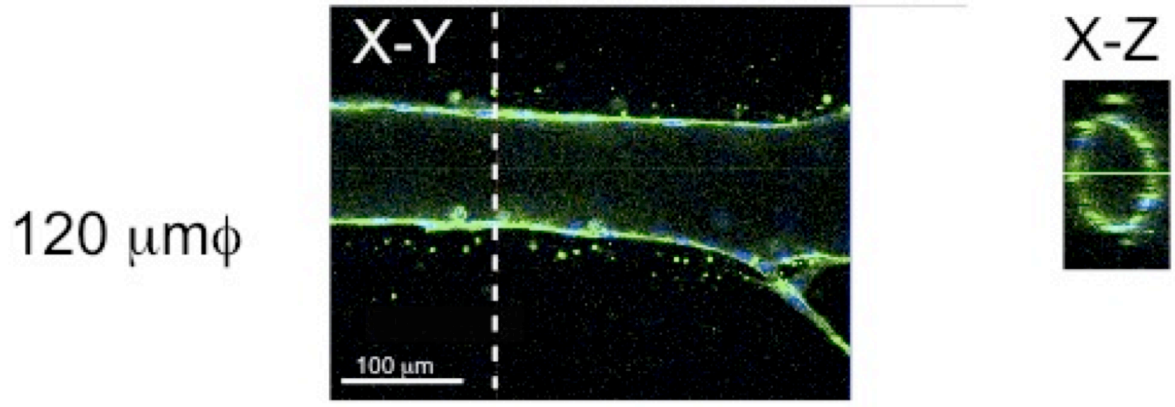

Fig. 3: Fluorescence confocal microscopies of HUVECs accumulated within the collagen microchannels after the 24-hour culture period. F-actin and cell nucleus were visualized using Alexa488-conjugated phalloidin (green) and Hoechst 33342 (blue), subsequently.

CONCLUSIONS

We demonstrated a reproducible method of stable tubular formation of endothelial cells by geometrical control of cells using a end-closed collagen microchannels. We successfully accumulated cells within the collagen channels, and cultured them as viable. At 6-hour culture period, cells started interacting each other, then formed tubular structure within 24 hours. At 48 hours, we observed that the formed microvasculatures showed sprouting behaviors, indicating that the formed microvasculatures behave as *in vivo*-like microvascultures. Since our system can be embedded different cell types of cells within outer collagen gels, engineered tissue grafts having microvasculature will be fabricated.

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REFERENCES

[1] Z. Lokmic and G.M. Mitchell, “Engineering the microcirculation.” Tissue Eng Part B Rev, 2008. 14(1): p. 87-103.
[2] S. Raghavan, et al., “Geometrically controlled endothelial tubulogenesis in micropatterned gels.” Tissue Eng Part A, 2010. 16(7): p. 2255-63.

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