Rapid Formation of Engineered Microvasculatures Using Microfluidic Techniques

Yukiko T. Matsunaga[1,2], Nathalie Brandenberg[1], Isamu Matsuda[1,3], Mitsuo Umezu[3], Yuuki Okubo[1]
1. The University of Tokyo, Japan, 2. PRESTO, JST, Japan, 3. Waseda University, Japan

INTRODUCTION

In vitro microvascularization 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, geometrically controlling endothelial cells within the 3D environment (i.e. embedding cells within micro-structured collagen gel at higher cell density) efficiently accelerates tubular formation of endothelial cells[2]. Even though these 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. 1a).

METHODS

Fabrication of end-closed collagen gel microchannels: To prepare the end-closed collagen microchannels, we gelated collagen type I 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. The needle was then removed, and collagen gels were immersed with cell culture media for 10 min.

Cell culture in the end-closed collagen gel microchannels: Human umbilical vein endothelial cells (HUVECs) 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. Formed tissues in the collagen microchannels were analyzed by staining with Alexa Fluor 488-conjugated phalloidin and Hoechst 33342.

RESULTS & 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, and then they formed continuous lumen structures at 24 hours (Fig. 1b). After 24 hours, the cells appeared to undergo internal rearrangements to finally invade into the collagen gel area that was determined by immunostaining of the tissue section. This result indicates that the formed microvasculatures show sprouting behavior known as angiogenesis process of vasculatures. Also our microchannel allowed perfusion of the medium by hydrostatic pressures.

CONCLUSIONS

We demonstrated a rapid and a simple method of stable tubular formation of endothelial cells by geometrical control of cells using an 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. After 24 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.

ACKNOWLEDGEMENTS

This work was supported PRESTO, JST, Japan.

REFERENCES

[1] Z. Lokmic and G.M. Mitchell, Tissue Eng Part B Rev, 2008. 14(1): p. 87-103.
[2] S. Raghavan, et al., Tissue Eng Part A, 2010. 16(7): p. 2255-63.

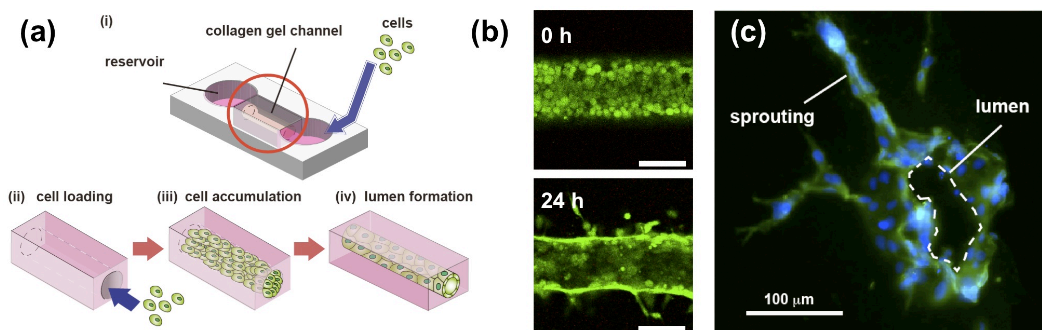

Figure. 1: Microvascular formation by cell accumulating method using an end-closed collagen gel microchannels. (a) Concept of this study. (b) Accumulated cells within the collagen gel microchanels. Cells were visualized by Calcein-AM. Scale bars: 100 µm. (c) Tissue section image of the formed microvasculatures after the 24-h cell accumulation. F-actin and cell nucleus were visualized using Alexa488-conjugated phalloidin (green) and Hoechst 33342 (blue), subsequently.

©2013 Society For Biomaterials