TUBLOGENESIS OF ENDOTHELIAL CELLS IN CORE-SHELL HYDROGEL MICROFIBERS

Hiroaki Onoe[1,2], Shoji Takeuchi[1,2]

[1]*Institute of Industrial Science, The University of Tokyo, Japan,*

[2]*Takeuchi Biohybrid Innovation Project, Exploratory Research for Advanced Technology (ERATO), Japan Science and Technology (JST), Japan*

ABSTRACT

This paper describes tube formation of endothelial cells in collagen/alginate core-shell hydrogel microfiber. We found that encapsulated MS1 (mouse pancreatic endothelial cell line) and HUVEC (human umbilical vein endothelial cell) spontaneously create tube-like monolayer cell structures along the axial direction of the fiber at meter-length scale. Because the produced endothelial tubes are free-standing and handleable in a culture medium with the support of the alginate shell, our endothelial cell fibers would be a fundamental tissue unit for constructing three-dimensional (3D) macroscopic tissues with vascular network and for analyzing cellular behaviors (ex. vasculogenesis or angiogenesis) under intercellular/chemical/mechanical/fluidic interactions.

KEYWORDS

Hydrogel, Microfiber, Endothelial cell, Vascular, Cell encapsulation.

INTRODUCTION

Creating vascular network in a macroscopic artificial tissue has been stated as an essential element for constructing long-life artificial tissues having nutrient/metabolite circulation [1]. Various attempts have been made to create blood vessels in an artificial tissue [2,3], the arrangement of vascular network in a designed 3D configuration have still been a challenging issue. Recently, we have proposed a cell-encapsulating core-shell hydrogel fiber, named "cell fiber" [4,5,6]. One important feature of our "cell fiber" is that we can freely manipulate the fibers with keeping cellular activities and assemble them into designed 3D configurations. In this paper, we report the successful tube formation of endothelial cells in our cell fiber for the first time, indicating that 3D arranged vascular network could be build with the fiber-based tissue assembly.

FORMATION OF ENDOTHELIAL TUBE-LIKE STRUCTURE

Formation of endothelial cell-encapsulating core-shell hydrogel fiber was conducted with double coaxial microfluidic device that we previously reported [4,5,6]. The formed microfiber provides a fiber-shaped 3D microenvironment (core material: collagen, core diameter: ~100 μm, length: >several meters) covered with sodium alginate shell (shell diameter: ~200 μm). We cultured two different endothelial cells, MS1 and HUVEC, and observed tube-like structures formed after day 2. Figure 2 (A) shows the growth and the formation of tube-like structures of MS1 endothelial cells in the core. The tube-like structure was uniformly formed along the fiber and no blanch was observed (Figure 2 (B)). As well as MS1, we succeeded in fabricating tube-like structures by encapsulating HUVEC in the microfiber.

OBSERVATION OF ENDOTHELIAL TUBE-LIKE STRUCTURE

We analyzed the formed tube-like structures. Observation with conforcal reflectance microscopy revealed that the tube-like structure of endothelial cells was embedded in collagen fibrils, not located at the boundary between the collagen core and the alginate shell. This fact suggests that the remodeling of endothelial cells in 3D

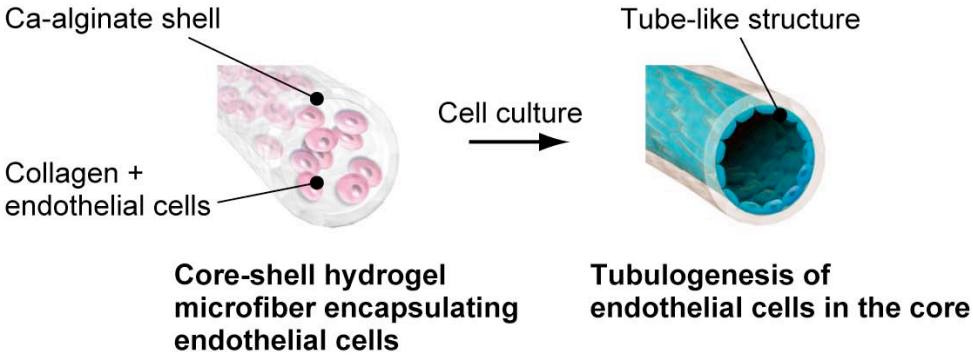

Figure 1. Concept of tubulogenesis of endothelial cells in core-shell hydrogel microfibers. Endothelial cells were encapsulated in a core-shell hydrogel microfiber generated with a double coaxial microfluidic device. The encapsulated endothelial cells remodel their configurations and form a tube-like microtissue (tubulogenesis) in the core collagen space.

978-0-9798064-5-2/μTAS 2012/$20©12CBMS-0001

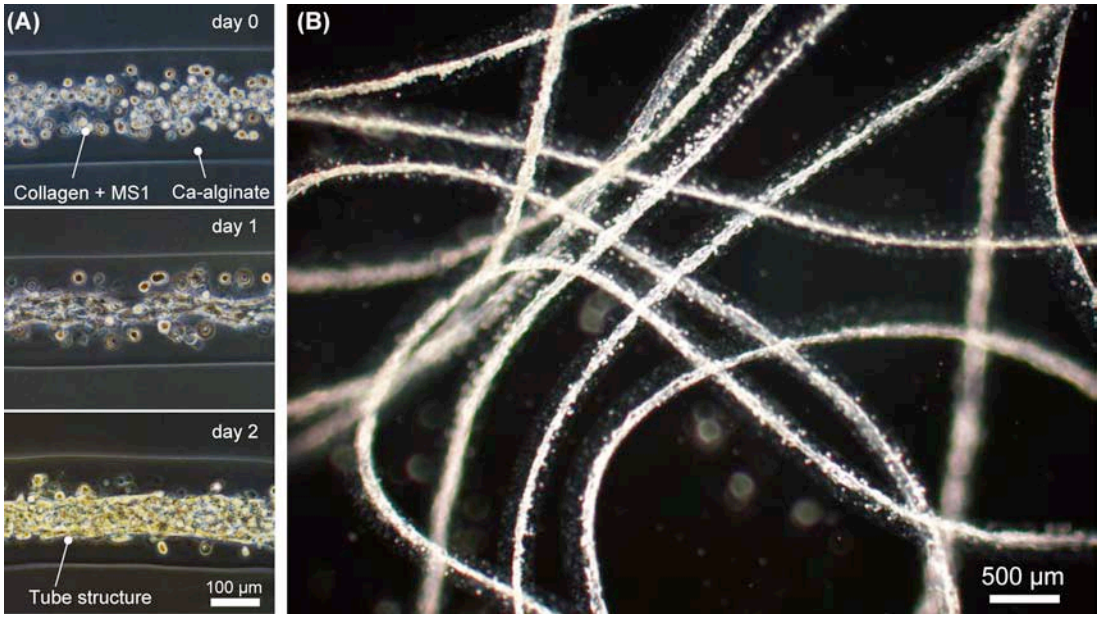

Figure 2. Tube formation of endothelial cells, MS1 in the core-shell hydrogel microfiber. ***(A)*** *Tube formation of MS1 in the core-shell fiber.* ***(B)*** *MS1 tubular cell fiber at day 2. The tube structure was formed along the longitudinal direction of the fiber at meter scale length.*

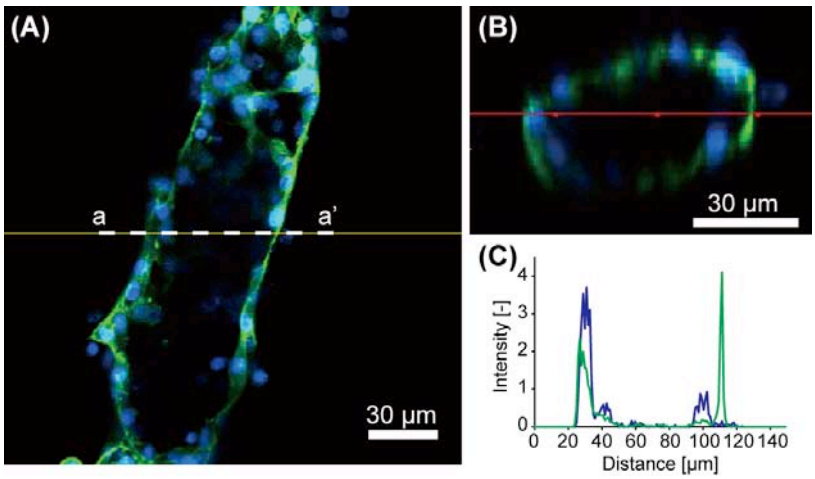

Figure 3. Conforcal fluorescent images of the MS1 microfiber. Alexafluor-conjugated phalloidin and hoechst were used for the immunostaining of actin filaments (green) and nuculeus (blue), respectively. ***(A)*** *Cross-sectional image of the tube structure of MS1 cells in the fiber. Monolayer cell wall was observed clearly.* ***(B)*** *Reconstructed cross-sectional image of the a-a' in (A). Monolayer cell tube was observed.* ***(C)*** *Intensity distribution of the image of (B).*

microenvironment [7] drives the formation of the endothelial tubes. Confocal images of immunostained MS1 cell fibers clearly indicated that monolayer cell tubes were continuously formed as shown in Figure 3 (A) and (B). The diameter of the tube was 63.8 ± 6.8 μm for MS1 (Figure 3 (C)). In the case of HUVEC cell fiber, the diameter of the remodeled tube was 22.2 ± 7.0 μm. We consider that the initial cell density or nature depending on cell property may cause the difference of the tube diameters in MS1 and HUVEC cell fibers.

One question arises after the observation: Is the inner space of the tube structure void for transferring liquid flow? We did not confirm whether the inner space was void though, we think that a void could be created in the tube-like structure by the digestion of collagen with the endothelial cells, because void formations of 3D encapsulated endothelial cells were reported elsewhere [7]. Thus, we believe that our endothelial fiber would have the ability to transfer liquid inside and would provide an useful tissue unit for constructing vascular 3D network and for analyzing behaviors of endothelial cells in 3D tubular microenvironment.

CONCLUSION

We succeeded in observing the tubulogenesis of endothelial cells, MS1 and HUVEC, in the core-shell hydrogel microfibers. The tube-like endothelial cell constructs was monolayer and formed along the longitudinal axis of the fiber. Our endothelial cell fibers would be a useful cellular unit for the bottom-up construction of 3D macroscopic tissues with vascular network and for analyzing cellular behaviors including vasculogenesis and angiogenesis of endothelial cells.

ACKNOWLEDGEMENT

The authors thank to A. Itou for her technical assistance. This work was partly supported by Grant-in-aid for Young Scientists (A) (Project No. 24686031) from the Japan Society of the Promotion of Science (JSPS), Japan.

REFERENCES

[1] Ruben Y. Kannana, Henryk J. Salacinskia, Kevin Salesa, Peter Butlerb, Alexander M. Seifalian, *The roles of tissue engineering and vascularisation in the development of micro-vascular networks: a review*, Biomaterials. 26, 1857, (2005).

[2] O. E. Teebken, A. Bader, G. Steinhoff and A. Haverich, *Tissue Engineering of Vascular Grafts: Human Cell Seeding of Decellularised Porcine Matrix,* Eur. J. Vasc. Endovasc. Surg. 19, 381-386, (2000).

[3] Simon P. Hoerstrupa, Gregor Zünda, Ralf Sodianb, Andrea M. Schnellc, Jürg Grünenfeldera and Marko I. Turinaa, *Tissue engineering of small caliber vascular grafts*, European Journal of Cardio-Thoracic Surgery 20, 164-169, (2001).

[4] Hiroaki Onoe, Riho Gojo, Yukiko Tsuda, Daisuke Kiriya, Shoji Takeuchi, *Core-shell gel wires for the construction of large area heterogeneous structures with biomaterials*, Proceedings of 22nd IEEE International Conference on Micro Electro Mechanical Systems (MEMS), pp. 248-251, (2010).

[5] Hiroaki Onoe, Riho Gojo, Yukiko Tsuda, Daisuke Kiriya, Midori Kato-Negishi, Kaori Kuribayashi-Shigetomi, Yuto Shimoyama, Shoji Takeuchi, *Cell fibers: construction of centimeter-scale 3D tissues by weaving*, Proceedings of Fourteenth International Conference on Miniturized Systems for Chemistry and Life Science (microTAS), pp. 629-631, (2010).

[6] Hiroaki Onoe, Midori Kato-Negishi, Shoji Takeuchi, *Differentiation Induction of Neuronal Stem Cells in 3D Fiber-Shaped Microenvironment*, Proceedings of Fifteenth International Conference on Miniturized Systems for Chemistry and Life Science (microTAS), Seattle, USA, pp. 1490-1492, (2011).

[7] Valerie L. Crossa, Ying Zhengb, Nak Won Choib, Scott S. Verbridgea, Bryan A. Sutermasterb, Lawrence J. Bonassara, Claudia Fischbacha, Abraham D. Stroockb, *Dense type I collagen matrices that support cellular remodeling and microfabrication for studies of tumor angiogenesis and vasculogenesis in vitro*, Biomaterials 31, 8596, (2010).

CONTACT

Hiroaki Onoe, IIS, The University of Tokyo, 4-6-1, komaba Meguro-ku, Tokyo, JAPAN, tel: +81-3-5452-6650, email: onoe@iis.u-tokyo.ac.jp