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3D image reconstruction techniques using STEM for arteriole and venule morphological analyses

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Abstract

Veins are thin-walled structures inside of which a set of valves keeps blood in the body flowing in one direction. The functions of veins have not yet been characterized in detail because they are regarded as simple pipes that carry blood to the heart. However, the functional properties of veins need to be clarified. A three-dimensional (3D) analysis of individual vascular smooth muscle cells, which are essential for understanding the morphological function of blood vessels, has not yet been conducted. We attempted to elucidate how the form of smooth muscle cells in the venous system differs from that of the arterial system by 3D reconstruction techniques using scanning transmission electron microscopy (STEM). Venules and arterioles (less than 100 μm in diameter) were selected. The smooth muscle cells of arterioles were spindle-shaped and densely surrounded in a circular manner. In contrast, a small number of smooth muscle cells were scattered around venules and were not arranged in the circular manner observed in arterioles. 3D reconstruction techniques revealed that venule smooth muscle cells had multiangular short spindle shapes. This 3D reconstruction technique stereoscopically shows all cell and tissue structures irrespective of blood vessels only, and may significantly contribute to functional and morphological studies on cells and tissues in the body.

Keywords: scanning transmission electron microscopy (STEM), three-dimensional reconstruction, arteriole, venule, smooth muscle cells

Introduction

Arteries and veins both have the same three distinct layers: the tunica intima/interna, tunica media, and tunica adventitia. The media is the middle layer of a blood vessel and is the thickest of the three tunics in most arteries and veins. The intima and adventitia are similar in structure and function to arteries, whereas the

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media is markedly thinner due to significantly less smooth muscle and elastic tissue. Therefore, veins do not have the same capacity for elastic recoil and vasoconstriction as arteries. The walls of arteries are thicker than those of veins in order to withstand pulsatile flow and higher blood pressure. As arteries become smaller, wall thickness gradually decreases; however, the ratio of wall thickness to lumen diameter increases. Arterioles are surrounded in a circular manner by spindle-shaped smooth muscle cells (Ham 1987, Saino 2002a, 2002b, 2008, Tamagawa 2009, 2013, Okubo 2016). The activation of the biochemical cascade of vascular smooth muscle contraction occurs through the binding of contractile agents, such as norepinephrine, serotonin (5-HT), adenosine triphosphate (ATP), and endothelin, to specific membrane receptors present in smooth muscle cells (Lands 1967, Starling 1975, Mark 1988, Raymond 1990, Rosendorff 1997, Raat 1998, Saino 2002a, 2002b, Lamont 2003, Masu 2008, Okubo 2016). The relaxation of vascular smooth muscle is triggered by different agents produced by endothelial cells, including prostacyclin and endothelium-derived hyperpolarizing factor (EDHF) (Balligand 1999, Vanhoutte 2001). A large number of morphological studies have investigated arterioles *in vivo*. For example, in terminal cardiac arterioles with an outer diameter of less than 30 μm, muscle fibers became branched and commonly encircle the endothelial tube. The presence of regional differences in the arrangement of vascular smooth muscle fibers indicates the heterogeneity of blood flow at the level of cardiac microcirculation (Higuchi 2001).

On the other hand, few studies have examined veins. In clinical settings, the vein is used to collect blood samples and supply water or liquid in order to maintain a healthy balance. Damaged vein walls hinder the circulatory system, allowing blood to collect and flow in a backward manner when the muscles relax. Veins have not been the focus of research because they are regarded as a simple tube system that carries blood from capillaries towards the heart. However, mouse portal veins have a spiral fold that is produced by the inner layer, i.e. the endothelium and smooth muscles of the wall protruding into the lumen. A transient and periodic increase in Ca^{2+} concentrations occurs in longitudinal smooth muscle cells and is transmitted spirally from the intestinal to the hepatic side. During peristaltic movement, the contraction of smooth muscle cells is transmitted along the longitudinal smooth muscles of the portal vein wall toward the liver (Takahashi 2002). This does not appear to be a general property of veins, and it currently remains unclear whether venule smooth muscle cells react to vascular stimulants. When some reactions in cells with the ability to contract are examined *in vivo*, their own shapes need to be closely compared. However, the morphological characteristics of venule smooth muscles have not yet been elucidated in detail.

In the present study, we examined the form of the smooth muscle cells of venules and how they are distinct from arterioles, which contribute to the maintenance of blood pressure. Electron microscopy has the capacity to identify the intracellular microstructures used; however, transmission electron microscopy (TEM) has the disadvantage of the observation field of view being limited. To overcome this drawback in our bioimaging center, the "TEM observation method (scanning transmission electron microscopy, STEM) by a scanning electron microscope" was improved (Hoffpauir 2007, Ponce 2012, Sawai 2013). The 3D reconstruction of serial sections using TEM is generally considered to require extensive labor and time. On the other hand, even if the specimen is thicker in STEM than in TEM, it is possible to confirm slice images, which is advantageous for 3D reconstructions by scanning the electron beam probe. In the present study, if it is possible to establish a method that evaluates reconstructions of serial sections of cells in elongated tissues, such as the vasculature, the same type of research may be efficiently performed in the future.

Materials and methods

Preparation of arterioles

Experiments were conducted in a manner consistent with the guidelines of the Ethics Committee for animal treatment of Iwate Medical University. Adult male rats (Wistar, 8–12 weeks old, body weight 250–400 g, n=10) were used. Rats were killed by carbon dioxide gas followed by exsanguination. The testes were removed and soaked in 0.2 M phosphate buffer saline (PBS). Small arteries and venules were then isolated and digested with collagenase (100 U/ml; Elastin Products, Owensville, MO, USA) in the HR at 37°C for 40 min. Connective tissues were then carefully removed under the stereoscopic microscope.

Fixation

The isolated arterioles and venules were observed by scanning transmission electron microscopy (STEM). Non-treated fresh arterioles and venules obtained from testes were fixed in 25% glutaraldehyde (Nacalai Tesque) and 4% paraformaldehyde (Merck, Germany) in PBS (0.1 M) at room temperature for approximately 6 hrs. After washing 3 times using distilled water, the cells were postfixed with 1% osmium tetroxide for an overnight incubation at room temperature, and then dehydrated in a series of ethanol and embedded in Epon 812 (TAAB, Berkshire, UK) for an overnight incubation at room temperature. Longitudinal sections were consecutively cut through the arterioles and venules using an ultramicrotome (ULTRACUT-UCD, Leica, Germany). Semithin sections (thickness of approximately 1 μm) were cut on an ultramicrotome using a Jumbo knife (Histo Jumbo, Nisshin EM, Tokyo), stained with toluidine blue, and observed by light microscopy. Ribbons of serial thin sections (thickness of between 70 and 100 nm) were retrieved on Indium-Tin-Oxide (ITO)-coated slides (Fig. 1) and doubly stained with uranyl acetate and lead citrate.

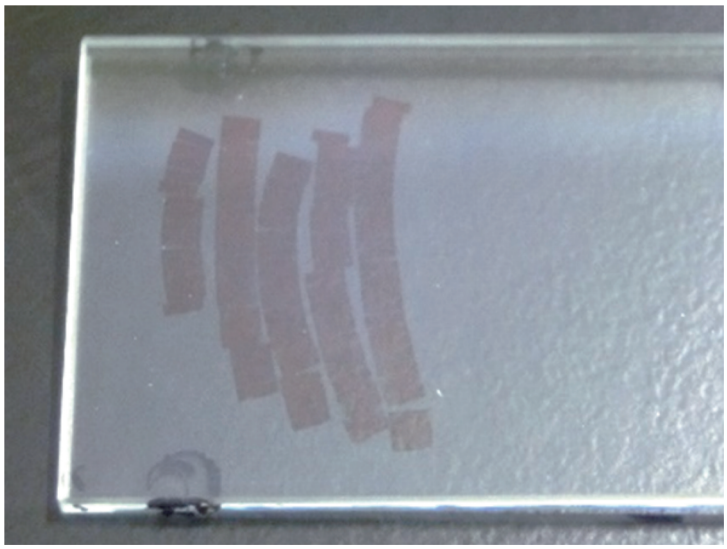

Fig. 1. Ribbon-like serial sections (approximately 100 nm) of testicular blood vessel specimens collected on a slide glass

Recording image data

The fine structures of the vessels were examined using a Field-Emission Scanning Electron Microscope (SU8010; Hitachi, Tokyo) at an accelerating voltage of 1.5 kV. Images were collected with dimensions of 2560 × 1920 pixels with averages of quadruplicates, and were viewed and analyzed by Image Fiji software.

Image processing

Image Fiji is a well-known and long-lived open source software for biomedical image analyses (Schindelin 2012). Multiple images taken using the tiling function for the scanning electron microscope of Image Fiji were automatically arranged to create a montage image (Ogura 2010). The image obtained by the scanning transmission electron microscope was reversed in contrast to the transmission electron microscope. In order to ensure that image quality was the same as that of a normal TEM image, image colors were inverted using Photoshop® (Adobe INC, CA, USA).

3D images

Before displaying 3D images, parameter settings in the Z axis direction of Image Fiji were performed. In the Image Fiji menu bar, [File] > [Import] > [Image Sequence] was selected. The sequence of images was loaded. [Analyze] > [Set Scale] was then opened. The Set Scale dialog was brought up, and the distance in pixels: 1, the known distance: 24.8, the unit of length: nm, and the scale: 0.0403 were entered (Fig. 2).

[Image] > [Properties] was opened and the numerical value of Voxel depth was entered. Since the input numerical value of the analysis this time was a section thickness of 100 nm, it was 100 (nm)/24.8 = 4.03 nm/pixels. The thinning processing method was performed; therefore, twice the thickness was entered to 24.8 × 4.03 × 2 = 199.888 in the voxel depth (Fig.3). By using this method, the setting was made taking into consideration the thin area of the blood vessels. By coloring smooth muscle cells using Photoshop beforehand, 3D imaging was performed on the colored parts of cells using the TrakEM2 function of Image Fiji. Therefore, it was possible to clarify the whole picture of smooth muscle cells.

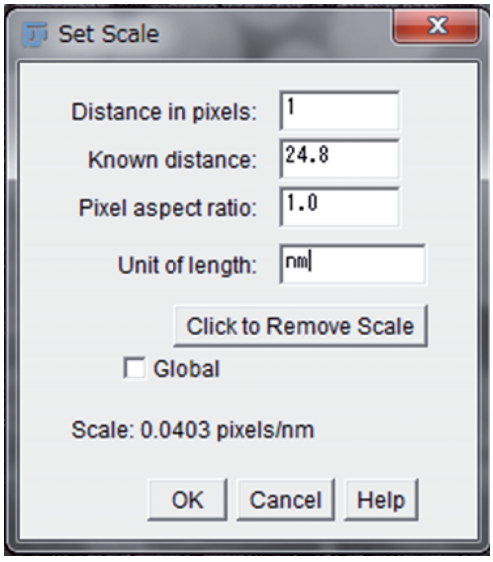

Fig. 2. The Set Scale dialog on Image Fiji (https://fiji.sc) software

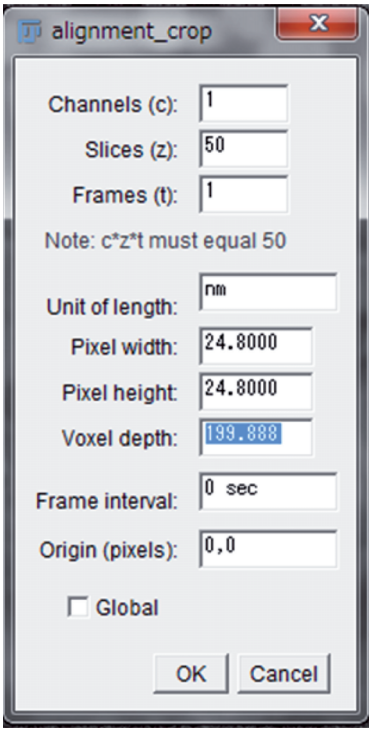

Fig. 3. The Properties dialog on Image Fiji (https://fiji.sc) software

Result

Using STEM, cross-sections of arterioles and venules with a diameter of approximately 50 μm were observed. Electron microscopy revealed that specimens maintained the typical structural integrity of testicular arterioles. In the case of arterioles, endothelial cells with clear nuclei were observed, and the cross-sectional shape of cells was cuboidal. The lumen of this arteriole was lined by 10 endothelial cells, which were closely attached to one another by junctions. Cytoplasmic protrusions of endothelial cells were observed on the luminal side. Since this arteriole was partly contracted, endothelial cell nuclei bulged into the lumen. A few lamellar layers of the basal lamina were interposed between the endothelium and smooth muscle layer. Smooth muscle cells in arterioles overlapped in 2 to 3 layers (Fig. 4). Smooth muscle cells formed a continuous layer, and the cytoplasm of the cells contained rich myofilaments. No significant ultrastructural damage (e.g. swollen mitochondria or the vacuolation of the sarco/endoplasmic reticulum) was detected. In longitudinal sections, smooth muscle cells continuously surrounded arterioles. Smooth muscle cells were uniformly spindle shaped, and most nuclei were elliptical in shape. Cell nuclei were oriented perpendicular to the longitudinal axis of a vessel; endothelial cell nuclei were parallel to the axis. Depending on the location of arterioles, the length of smooth muscle cells was similar to the transverse diameter of the blood vessel (Fig. 5).

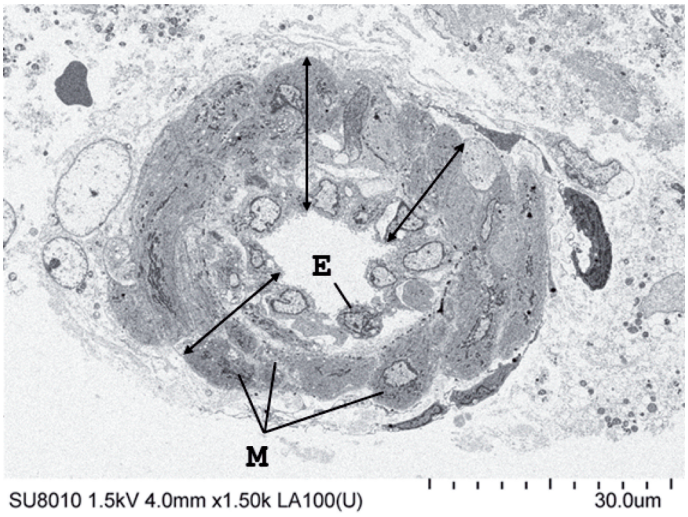

Fig. 4. Electron micrograph of a cross-section of testicular arterioles (E: endothelial cells, M: smooth muscle cells). The vessel is bounded by a single layer of endothelial cells. Arrows indicate the thickness of smooth muscle cells.

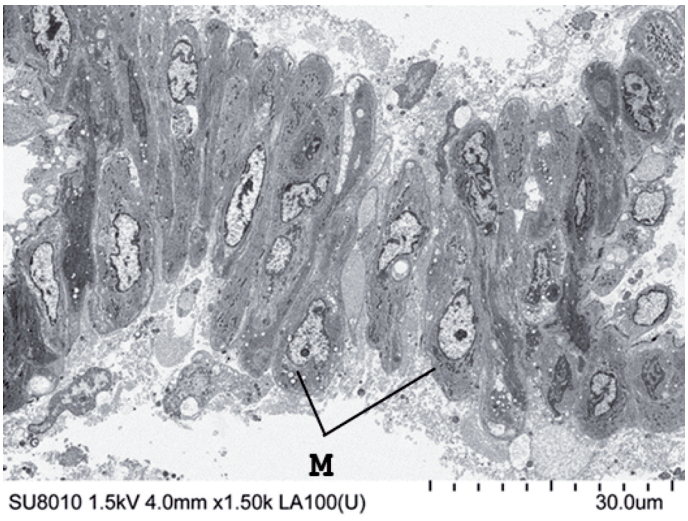

Fig. 5. Electron micrograph of a longitudinal section of testicular arterioles (M: smooth muscle cells)

On the other hand, the structure of the vascular layer, including endothelial cells and smooth muscle cells, was not clearly seen in the cross-section of the venules. Furthermore, the lumen of the vessels was irregularly shaped instead of being circular as seen in arterioles. Only a few endothelial cells were observed. The number of smooth muscle cells containing microfilaments of actin and myosin was smaller and sparser than in arterioles (Fig. 6). In longitudinal sections, few smooth muscle cells were observed in venules, and intercellular spaces were detected between smooth muscle cells. The nuclei of smooth muscle cells were parallel to the axis of the venule (Fig. 7). Comparisons of cross-sections and longitudinal sections revealed that arteriole smooth muscle cells had an elongated spindle shape, we were unable to determine the three-dimensional structure of the venular smooth muscle from our current findings. By focusing on luminal volumes, the thickness of blood vessel walls was measured in cross-sections of arterioles and venules with the same diameters. Wall thickness was two-fold greater in arterioles than in venules (arterioles, 17.45 ± 1.6 μm, and venules, 8.1 ± 1.82 μm (Mean ± SD)). Therefore, venules had markedly larger lumens and thinner walls than the corresponding arterioles.

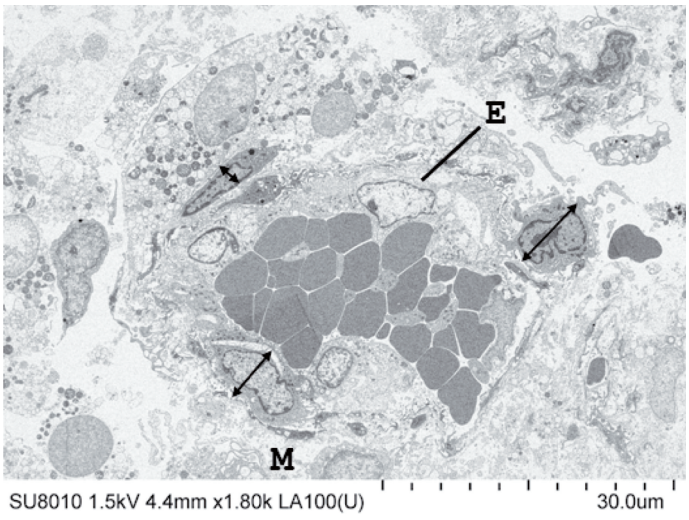

Fig. 6. Electron micrograph of a cross-section of testicular venules (E: endothelial cells, M: smooth muscle cells, R: red blood cell). Arrows indicate the thickness of smooth muscle cells.

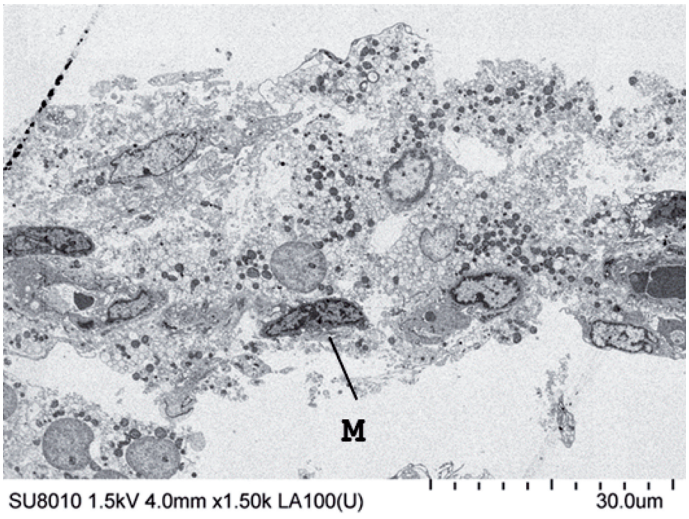

Fig. 7. Electron micrograph of a longitudinal section of testicular venules (M: smooth muscle cells)

To date, few studies have examined the morphology of venules in detail. Therefore, we observed the 3D structures of venules using Image Fiji software. A morphology-based approach to venular smooth muscle cells was performed using Image Fiji software (orthogonal view), with a 3D analysis based on XYZ. The results obtained showed that the smooth muscle cell had a spindle shape that was close to a circle based on information obtained from the Z axis direction image from the side view and the XY plane image (Fig. 8). In another example of a smooth muscle cell in the same venule, the cell had a squat spindle shape in the lower XY plane image, which was slightly long laterally (Fig. 9).

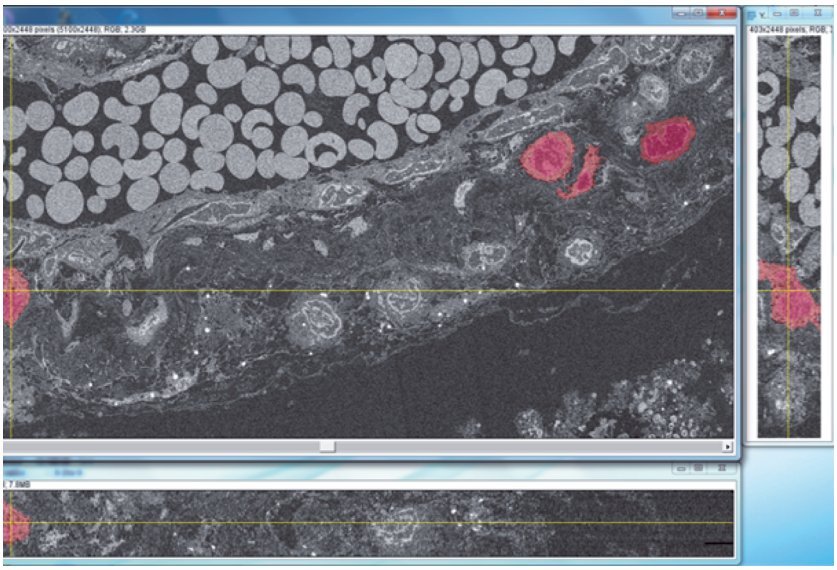

Fig. 8. An orthogonal view screen image of testicular venules on Image Fiji 1
Red: smooth muscle cell, the images on the right: Z-axis direction surface from the side, lower image: XY plane images

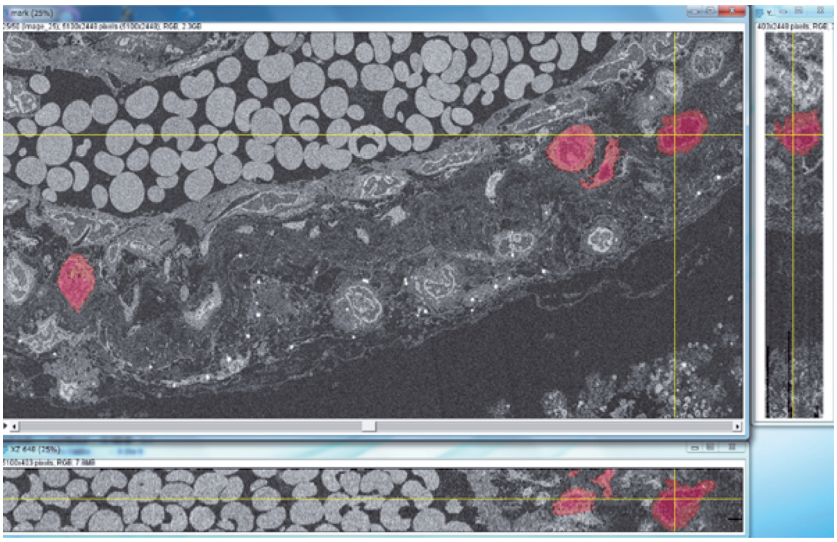

Fig. 9. An orthogonal view screen image of testicular venules on Image Fiji 2
Red: smooth muscle cell, the images on the right: Z-axis direction surface from the side, lower image: XY plane images

A 3D construction of smooth muscle cells using the StackEM 2 function of Image Fiji was then performed. The smooth muscle cell shown in Fig. 8 had a short spindle shape with short protrusions. The nucleus was nearly in the center of the cytoplasm, but was atypical (Fig. 10). Furthermore, the results obtained confirmed that triangular-like cells and spindle-shaped cells were present in close proximity (Fig. 11).

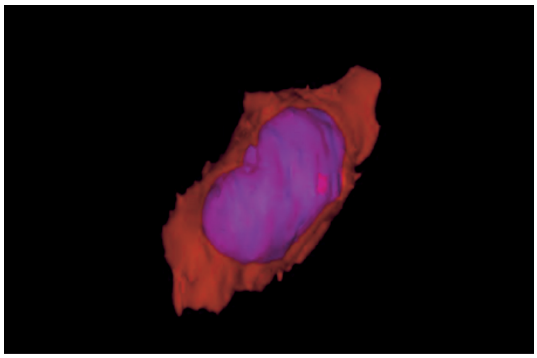

Fig. 10. A 3D image on Image Fiji of testicular venous smooth muscle cell 1. Red: cytoplasm, blue: nucleus

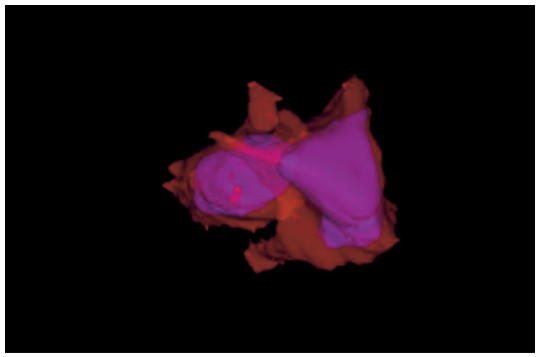

Fig. 11. A 3D image on Image Fiji of testicular venous smooth muscle cell 2. Red: cytoplasm, blue: nucleus

Discussion

Previous findings obtained from images using conventional SEM showed that the vascular walls of arterioles developed in transverse and longitudinal directions and arterioles were surrounded by spindle-shaped smooth muscle cells in a circular manner (Ushiwata and Ushiki 1990, Saino 2002a, 2002b, 2008, Tamagawa 2009, 2013, Okubo 2016). However, few morphological studies have been conducted on venules using SEM (Murakami 1971, Hoshi 1999). Murakami introduced a technique to obtain resin replicas of the microvascular bed and evaluated microvascular corrosion casts by SEM (Murakami 1971). Since its development by Murakami, the corrosion cast method for scanning electron microscopy is now regarded one of the most efficient means for clarifying the 3D features of the microcirculation of organs and tissues (Murakami 1971, Kohler 1983, Motta 1992). However, few morphological studies have been conducted on venules using TEM (Beacham 1976, Bizuneh 1991, Knop 2005). In contrast, smooth muscle cells were sparse in venules. Few vessels were identified as vascular smooth muscle cells due to the presence of microfilaments, and it was difficult to observe the overall morphology of venules from cross-sectional images alone. Difficulties were associated with clarifying the whole form of venules based only on cross-sectional images. However, the present results showed that the form of smooth muscle cells in venules was completely different from that in arterioles, which have many projections with short spindles. The activity of smooth muscles allows lymph vessels to slowly pump lymph fluid throughout the body independent of the heart. In contrast, smooth muscles in blood vessels are involved in vasoconstriction and vasodilation instead of fluid pumping. Given the characteristic form of the venules, venular smooth muscles likely have a larger role as a reservoir for blood rather than in maintaining the blood pressure by contracting like arteriolar smooth muscle cells to adjust the blood flow. However, compare with the lymphatic vessels, smooth muscle in venules may be necessary to prevent collapse of the lumen. Venules have a typical and unique smooth muscle cell shape, which suggests that they are not simply inert drainage ducts. However, further research is needed to clarify the functions of venules.

In the present study, 3D reconstruction techniques using STEM enabled 3D image acquisition with a wide field of view. This method appears to be useful for stereoscopically and minutely observing the shapes of some

tissues and a small number of undeveloped cells (Ponce 2012). 3D reconstruction techniques generally require time and effort; however, in STEM, it is relatively easier and more accurate to observe samples by considering the following points. Before making serial sections, it is important to grasp the position and size of the target to be observed and estimate thickness and the number of slices. If the thickness of the vascular wall is not determined, the number of segments will not be sufficient for the purpose of observation. In the case of an excess number of slices, observations become inefficient. Furthermore, when making serial sections of samples, it is necessary to make many slices by reducing the vertical thickness of sample blocks. Many sample slices may be placed on the slide glass, and subsequent dying and the preparation of microscope slides for observations may be simplified.

By performing the thinning process without photographing all serial slices at the time of observations, work efficiency increases and the amount of 3D reconstruction data may be reduced. However, since the amount of thinning data increases, the depth data on the z-axis direction becomes indistinct. Therefore, a trade-off with the amount of work needs to be considered. It may also be advantageous to estimate the total amount of thinning out from serial slice numbers. In the present study, we thinned out one slice at a time with 100 pieces of serial slices and photographed 50 images. When the thinning process was performed, it was impossible to accurately construct a 3D structure unless we corrected parameters by Image Fiji before performing the 3D reconstruction method and adjusting for the subtracted values with numerical values.

In conclusion, by performing 3D reconstruction techniques based on STEM images obtained in a wide field of view, it was possible to simultaneously compare multiple cells and elucidate the relationship between cells themselves and their intracellular structures. In the 3D reconstruction techniques used in the present study, 3D images were easily obtained. Therefore, this 3D reconstruction method may stereoscopically reveal all cell and tissue structures irrespective of blood vessels only, and may markedly contribute to future functional and morphological studies on cells and tissues in the body.

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Conflict of Interest

The authors declare that there are no conflicts of interest that may be perceived as prejudicing the impartiality of this work.

References

Balligand JL: Regulation of cardiac beta-adrenergic response by nitric oxide. Cardiovasc Res. 43: 607–620, 1999.

Beacham WS, Konishi A, Hunt CC: Observations of the microcirculatory bed in rat mesocecum using differential interference contrast microscopy in vivo and electron microscopy. Am J Anat. 146: 385–425,

1976.

Bizuneh M, Bohlen HG, Connors BA, Miller BG, Evan AP: Vascular smooth muscle structure and juvenile growth in rat intestinal venules. Microvasc Res. 42: 77–90, 1991.

Ham W, Cormack DH: The Circulatory System. In "Histology" 8th ed, pp 592–609, J. B. Lippincott Company, 1987.

Higuchi K, Kumaki K, Aizawa Y, Ushiki T: A KOH-collagenase digestion method for scanning electron microscopic studies of vascular smooth muscle fibers in the human heart. Ital J Anat Embryol. 106: 197–202, 2001.

Hoffpauir KB, Pope BA, Spirou GA: Serial sectioning and electron microscopy of large tissue volumes for 3D analysis and reconstruction: a case study of the calyx of Held. Nature protocols. 2: 9–22, 2007.

Hoshi O, Ushiki T: Scanning electron microscopic studies on the route of neutrophil extravasation in the mouse after exposure to the chemotactic peptide N-formyl-methionyl-leucyl-phenylalanine (fMLP). Arch Histol Cytol. 62: 253–260, 1999.

Knop N, Knop E: Ultrastructural anatomy of CALT follicles in the rabbit reveals characteristics of M-cells, germinal centres and high endothelial venules. J Anat. 207: 409–426, 2005.

Kohler T, Leiser R: Blood vessels of the bovine chorioidea. A scanning electron microscopic study. Acta Anat (Basel). 116: 55–61, 1983.

Lamont C, Vainorius E, Wier WG: Purinergic and adrenergic Ca^{2+} transients during neurogenic contractions of rat mesenteric small arteries. J Physiol. 549: 801–808, 2003.

Lands AM, Arnold A, McAuliff JP, Luduena FP, Brown TG Jr.: Differentiation of receptor systems activated by sympathomimetic amines. Nature. 214: 597–598, 1967.

Masu K, Saino T, Kuroda T, Matsuura M, Russa AD, Ishikita N, Satoh Y: Regional differences in 5-HT receptors in cerebral and testicular arterioles of the rat as revealed by Ca^{2+} imaging of real-time confocal microscopy: variances by artery size and organ specificity. Arch Histol Cytol. 71: 291–302, 2008.

Matsuura M, Saino T, Satoh Y: Response to ATP is accompanied by Ca^{2+} influx via P2X purinoceptors in the coronary arterioles of golden hamsters. Arch Histol Cytol. 67: 95–105, 2004.

Motta PM, Murakami T, Fujita H: Scanning electron microscopy of vascular casts: methods and applications. vol. xi. Boston: Kluwer Academic Publishers. 390 p, 1992.

Murakami T, Miyoshi M, Fujita T: Glomerular vessels of the rat kidney with special reference to double efferent arterioles. A scanning electron microscope study of corrosion casts. Arch Histol Jpn. 33: 179–198, 1971.

Nelson MT, Standen NB, Brayden JE, Worley JF 3rd: Noradrenaline contracts arteries by activating voltage-dependent calcium channels. Nature. 336: 382–385, 1988.

Ogura K, Yamada M, Hirahara O, Mita M, Erdman N, Nielsen C: Gigantic montages with a fully automated FE-SEM (serial sections of a mouse brain tissue). Microsc Microanal. 16 (Suppl 2): 52–53, 2010.

Okubo M, Satoh Y, Hirakawa M, Sasaki K, Masu K, J McHonde G, Ikeda-Kurosawa C, Kurosaka D, Saino T: Different effect of serotonin on intracellular calcium ion dynamics in the smooth muscle cells between rat posterior ciliary artery and vorticose vein. Biomed Res. 37: 101–115, 2016.

Ponce A, Mejía-Rosales S, José-Yacamán M: Scanning transmission electron microscopy methods for the analysis of nanoparticles. Methods in Molecular Biology. 906: 453–471, 2012.

Raat NJ, Wetzels GE, De Mey JG: Calcium-contraction relationship in rat mesenteric arterial smooth muscle. Effects of exogenous and neurogenic noradrenaline. Pflügers Arch Eur J Physiol. 436: 262–269, 1998.

Raymond JR, Hnatowich M, Lefkowitz RJ, Caron MG: Adrenergic receptors. Models for regulation of signal transduction processes. Hypertension. 15: 119–131, 1990.

Rosendorff C: Endothelin, vascular hypertrophy, and hypertension. Cardiovasc Drugs Ther. 10: 795–802, 1997.

Saino T, Matsuura M, Satoh Y: Application of real-time confocal microscopy to intracellular calcium ion dynamics in rat arterioles. Histochem Cell Biol. 117: 295–305, 2002a.

Saino T, Matsuura M, Satoh Y: Comparison of the effect of ATP on intracellular calcium ion dynamics between rat testicular and cerebral arteriole smooth muscle cells. Cell Calcium 32: 155–165, 2002b.

Saino T, Misaki T, Matsuura M, Shikanai T, Satoh Y: Dipyridamole inhibits intracellular calcium transients in isolated rat arteriole smooth muscle cells. Arch Histol Cytol. 71: 235–247, 2008.

Sawai T, Kamataki A, Uzuki M, Ishida K, Hanasaka T, Ochi K, Hashimoto T, Kubo T, Morikawa A, Ochi T, Tohyama K: Serial block-face scanning electron microscopy combined with double-axis electron beam tomography provides new insight into cellular relationship. Microscopy. 62: 317–320, 2013.

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez J-Y, White DJ,Hartenstein V, Eliceiri K, Tomancak P, Cardona A: Fiji: an open-source platform for biological-image analysis. Nat Methods. 9: 676–682, 2012.

Starling LM, Boullin DJ, Grahame-Smith DG, Adams CB, Gye RS: Responses of isolated human basilar arteries to 5-hydroxytryptamine, noradrenaline, serum, platelets, and erythrocytes. J Neurol Neurosurg Psychiatry. 38: 650–656, 1975.

Takahashi S, Hitomi J, Satoh Y, Takahashi T, Asakura H, Ushiki T: Fine structure of the mouse portal vein in relation to its peristaltic movement. Arch Histol Cytol. 65: 71–82, 2002.

Tamagawa Y, Saino T, Matsuura M, Satoh Y: The effects of diuretics on intracellular Ca^{2+} dynamics of arteriole smooth muscles as revealed by laser confocal microscopy. Acta Histochem Cytochem. 42: 121–128, 2009.

Tamagawa Y, Saino T, Matsuura M, Oikawa M, Satoh Y: Mechanism of spironolactone-induced Ca^{2+} increase in rat testicular arteriole smooth muscle cells revealed by real-time laser confocal scanning microscopy. Arch Histol Cytol. 74: 19–29, 2013.

Ushiwata I, Ushiki T: Cytoarchitecture of the smooth muscles and pericytes of rat cerebral blood vessels. A scanning electron microscopic study. J Neurosurg. 73: 82–90, 1990.

Vanhoutte PM: Endothelial adrenoceptors. J Cardiovasc Pharmacol. 38: 796–808, 2001.