The morphology, size and density of the touch dome in human hairy skin by scanning electron microscopy.

Running title: Morphology of touch dome in human hairy skin

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Abstract

The touch domes of mammalian hairy skin are mechanoreceptors characterized by the accumulation of Merkel cell-neurite complexes at the epidermal base. In this study, we examined the shape, size, and density of the touch dome of human skin of the forearm and the abdomen through scanning electron microscopy (SEM). Human skin samples were obtained from donated bodies, as well as a patient who underwent biopsy. Skin pieces were treated with a KOH-collagenase method for the separation of the epidermis from the dermis. The basal surface of the separated epidermis was then observed using SEM. The touch dome was clearly determined as a concave area bordered by a thick epidermal ridge, where neural components accumulated. The touch dome was rather independent from hair follicles, although they were sometimes located beside the touch dome. The average size and density of the touch dome were 0.06 mm^2 and 3.82 /cm^2 in the forearm, and 0.10 mm^2 and 1.30 /cm^2 in the abdomen, respectively. Our results suggested that the regional difference in

size and density of the touch dome might be related to the sensation's sensitivity as touch spots in human hairy skin.

Introduction

Mammalian hairy skin contains specialized tactile structures with accumulation of Merkel cells. These structures were first reported by Pinkus [1], who observed distinct disc-like structures in the human epidermis that were associated with dermal nerves and Merkel cells at the epidermal base. He named these structures “Haarscheiben” (hair discs) because of their close association with hair follicles, and he also showed the presence of similar structures in other mammals [2]. By using electron microscopy and physiological recording techniques in cats and primates, Iggo and Muir [3] further clarified that these discs were slowly adapting touch receptors, being innervated by myelinated sensory nerve fibers branching into unmyelinated terminals to end at Merkel cells. Therefore, these discs have been referred to as touch domes, touch corpuscles, tactile pads or Iggo-Pinkus domes. Hereafter, these structures will be referred simply as touch domes.

Previous investigators also demonstrated that the variety in the shape of the touch dome depends on the species. For example, rat touch domes are

observed as a clear round elevation surrounding a guard hair, while human touch dome have an irregular shape with an indistinct slight elevation [4-7]. Subsequently, many reports have been published on animal touch domes, while there have been few studies on human ones. This is probably due to the difficulty in identifying human touch domes by conventional light microscopy. To detect human touch domes more accurately and easily, we previously investigated the three-dimensional structure of human touch dome by light microscopy (LM) of serial sections and scanning electron microscopy (SEM) of KOH-collagenase treated tissues [8]. There, we demonstrated that the touch dome could be clearly identified by SEM as a concave region surrounded by thick epidermal ridges, showing the size of touch domes in human hairy skin. However, more detailed structure and density of these structures in the skin remain unelucidated. Thus, the aim of this study was to more precisely reveal the shape, size and density of the touch dome in human skin. For this purpose, we studied the touch domes in human skin by SEM of KOH-collagenase treated tissues and compared the three-dimensional ultrastructural images

with the two-dimensional section images through correlative electron microscopy. We also calculated the frequency and size of the touch domes in the forearm and the abdomen skin of three different cases and compared the results to discuss the regional and individual differences of the touch domes in human skin.

Methods

Biopsy sample

A skin biopsy sample of a 56-year-old female was obtained from a normal part of her left thigh skin, histologically diagnosed as benign skin tumors. After biopsy, the sample was fixed by immersion of 10% formalin in 0.1 M Phosphate buffer (PB, pH 7.4) and stored in the same fixative for one day at room temperature. The study was performed in accordance with the Declaration of Helsinki, after approval by the Medical Ethics Committee of Niigata University (acceptance number: 2527). Participant was informed in detail and written consent was collected.

Samples from donated bodies

Human skin was obtained from three Japanese cadavers: case 1 was a Japanese female who died at the age of 65, case 2 was a Japanese male who died at the age of 64 and case 3 was a Japanese female who died at the age of 73. All cadavers were from persons who donated their bodies to Niigata University School of Medicine, and consent for their use for medical education and research had been obtained. These bodies were fixed in 10 % formalin (approximately 15 liters) by vascular infusion from the radial artery and stored in a fixative containing ethanol (Solmix®, Japan Alcohol Trading, Tokyo, Japan) for nearly one year. Skin samples were removed from the extensor surfaces of the lower arm and the lateral surfaces of the abdomen in all cases and stored in 10% formalin in 0.1 M PB at room temperature before use.

SEM of KOH-collagenase treated tissues

For SEM, all skin blocks fixed in 10% formalin were cut into small pieces (about 3×3 mm), and further immersed in 2% glutaraldehyde solution in 0.1

M PB (pH 7.4) at 4 ºC for about 4 days, and subjected to a KOH-collagenase digestion method [8, 9] in order to separate the epidermis from the dermis. In brief, blocks were rinsed several times in the 0.1 M PB, placed in 30% KOH for 8–10 min at 62–63℃ and washed using the same buffer for more than one hour. After, tissues were further immersed in a collagenase solution (Type II; Sigma-Aldrich, MO; 1 mg/ml in 0.1 M PB, pH 7.4) for 3–12 h at 37 ºC and washed again using the same buffer several times. Under a dissection microscope and in the buffer, the epidermis was then carefully peeled from the dermis using sharp forceps. The epidermis was conductive-stained by treating it with 1% tannic acid solution for 2 h, followed by a wash step in PB for 30 min and posterior incubation in 1% osmium tetroxide solution for 2 h. They were then dehydrated in a graded series of ethanol, transferred to 3-metylburtyl acetate, and dried in a critical point-dryer with liquid CO_2. The dried tissues were mounted on a double-sided tape by placing the dermal side of the epidermis upward, supported with silver paste and coated with platinum-palladium. They were observed in an SEM (S-4300 SE/N and S-

3700N; Hitachi, Tokyo, Japan) at an accelerating voltage of 10 kV.

Epon-embedded semithin sections of KOH-collagenase treaded tissues

Several pieces epidermis treated with KOH-collagenase, from the biopsy sample, were used for correlative microscopic studies. After conventional SEM observations, the dried pieces were immersed in 70% ethanol for 15–20 min to hydrate the samples, dehydrated again in a graded series of ethanol, transferred into propylene oxide and embedded in epoxy resin (Epon 812, TAAB, Berkshire, UK) for polymerization during 24 h at 60 ºC.

These epoxy blocks were trimmed carefully under a dissecting microscope to determine the cutting position of the epidermis, and a series of semithin longitudinal sections of epidermis (0.3 μm thick) were cut with a Diamond knife using an ultramicrotome (Ultracut N; Reichert-Nissei, Tokyo, Japan). These sections were mounted on glass slides heated on a hot plate (60–80 ºC for 30 min), stained with 0.1% toluidine blue containing boric acid, on the same hot plate for 30 sec, and examined by light microscopy (LM). After sections of the touch dome were selected by LM, the sections were stained

with 1% uranyl acetate for 10 min followed by 1% lead citrate for 5 min and mounted on an aluminum stub with carbon tape, slightly coated with platinum-palladium (less than 10 nm). These were observed in an SEM using the BSE mode (SU3500; Hitachi, Tokyo, Japan) at an accelerating voltage of 7 kV. This allows to compare, for the same touch dome, section images and three-dimensional SEM images.

Statistical analyses of touch domes

The number of touch domes in both forearm and abdomen was analyzed statistically using SEM images obtained from the three cases of the donated bodies. The size of the touch dome was measured using imageJ, a public domain Java-based image processing program (Rasband WS, National Institutes of Health, Bethesda, MD, http://imagej.nih.gov/ij/, 1997-2012). The density of the touch domes was calculated as a result of the number of the domes in each epidermal sheet (3 × 3 mm). As a parameter for the area, we used the original size of the wet epidermis since epidermal sheets shrank at 60% of their normal size due to SEM preparation. The size of the touch domes

was also corrected by multiplying each measurement by its shrinkage factor. Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using Student's *t*-test (Microsoft Excel; Microsoft Corporation, Redmond WA) for comparison between groups and *p*-values of less than 0.05 were considered statistically significant.

Results

The touch dome of KOH-collagenase treated tissues

In KOH-collagenase treated specimens, the basal surface of the epidermis was clearly observed by SEM because the dermal components were removed away during this treatment (Fig. 1). At lower magnification, prominent structures of hairy skin were elongated epidermal ridges which ran in various directions to form a coarse network on the basal surface of the epidermis. These ridges were semicylindrical with smooth surfaces and their width was about 100–150 μm, although the ridges were shallow in one case (Fig. 3). In the mesh of the epidermal ridge network, numerous round depressions for dermal papillae were present with a diameter of about 10–50 μm. Ducts of sweat glands were observed beside the epidermal ridges. Hair follicles were also found in the mesh of the epidermal ridge network.

In addition, wide and deep concave areas bordered by the thick epidermal ridges were recognized in some places (Fig. 2-4). At a higher magnification, these areas were characterized by accumulation of unmyelinated nerve fibers with Schwann cells (Fig. 2 and 5), which were already identified as touch domes in our previous paper [8]. Hair follicles were sometimes associated with touch domes but were not in the center of the domes and rather beside them. There were also touch domes apparently independent from hair follicles.

In order to examine the ultrastructure of the touch domes more precisely, some samples with concaved areas were examined by correlative microscopy; we firstly observed the three-dimensional structure of touch domes by SEM followed by routine plastic section studies of the same samples by LM and BSE-mode SEM. By LM, the concaved areas on these semithin sections stained with toluidine blue were identified by delineation of the thick epidermis. BSE-mode SEM of the same sections after additional uranium-lead staining was useful for studying the ultrastructure of the touch domes at higher magnification. Because the images are similar to classical TEM

images of ultrathin sections, BSE-mode SEM clearly revealed the presence of Merkel cell-neurite complex covered by sheet-like processes of Schwan cells (Fig. 2d). Schwann cell bodies could be seen around nerve fibers.

The arrangement of Merkel cell-nerve complexes in the touch domes were further investigated by SEM at higher magnification (Fig. 5). Unmyelinated nerve branches, covered by Schwann cells, accumulated in the concave area of the touch dome. Some of the branches further extended into the base of the epidermis to have a wide attachment to the epidermis for Merkel cell-nerve complex formation. These nerve branches often innervated on more than two Merkel cells like *en-passant* endings.

Statistical analysis of the touch domes in the forearm and lateral abdomen

The size and density of touch domes were investigated to assess differences both individually and regionally. SEM images of touch domes in the forearm and lateral abdominal skin were obtained from three cases of donated bodies (Fig. 3, 4). These SEM images showed variations in morphology of epidermis and the touch domes in different samples; elongated epidermal ridges were

very shallow in both forearm and abdomen of case 1, while they were more prominent in case 2 and 3. Round depressions for dermal papillae were shallow and small in number in both forearm and abdomen of case 1 and in the forearm of case 2, while they were developed in the abdomen of case 2 and in both forearm and abdomen of the case 3.

The size and density of touch domes were then calculated in the three cases. Table 1 shows the calculated size and density of the touch domes from the forearm and the abdomen. Figure 6 also shows the results of statistical analysis of the touch domes.

The average size and density of the touch dome were 0.06 mm^2 and 3.82 /cm2 in the forearm, and 0.10 mm^2 and 1.30 /cm^2 in the abdomen, respectively. The size of the touch domes tended to be larger in the abdomen than in the forearm, and significant differences were seen in case 2 and 3 (Fig. 6a). In all cases, the density of the touch domes was higher in the forearm than in the abdomen (Fig. 6b).

Discussion

The present study showed that the touch domes in human skin can be clearly identified by SEM of KOH-collagenase treated epidermis, which was also shown in our previous paper [8]. The KOH-collagenase treatment removes collagen components without any severe damage to cellular elements enabling the direct observation of human touch domes from the dermal side by SEM. Thus, the present study confirmed our previous findings that human touch domes are characterized as a concave area bordered by thick epidermal ridges, even though the development of the epidermal ridges are variable among individuals. We also confirmed that the touch dome in human skin was rather independent from fair follicles, suggesting that, in humans, the touch dome functions as a touch spot and is independent from the structure that detects pressure upon the hair, unlike some animals, such as cats and rats, which have the touch domes in the center of a tylotrich follicle.

In this study we further analyzed the touch domes in human skin using correlative microscopy. SEM observations of semithin sections of the

epidermis were useful to investigate the internal structure of the touch domes. Because BSE images of semithin sections are comparable with conventional TEM images [10], we succeeded in correlating the three-dimensional morphology of touch domes with their two-dimensional section images. This technique is expected to be useful in the analysis of the precise relationship between the Merkel cells, nerve endings and Schwann cells in the three-dimensionally complicated structures of the human touch domes. Our SEM images suggest that terminal unmyelinated nerve branches form some territories in the human touch domes by innervating multiple Merkel cells with *en-passant* endings.

The size and density of human touch domes were reported by some previous investigators (Table 2). Kamide [4] studied human touch domes by methylene blue supravital staining and reported that the average size of the touch dome in trunk was $8.5\pm3.3\times10^{-2}$ mm^2 (n=33 from 1 case). Reinisch and Tschacheler [11] calculated the touch domes on the shoulder, trunk and extremities by immunohistochemistry and mentioned that the size of the

touch domes was 19.3±13.8 × 10^{-2} mm^2 (n=38 from 12 cases). We also previously reported that the size of the touch dome was 3.6±3.5 × 10^{-2} mm^2 (n=26 from 1 case). In this study, we calculated the size of touch domes in the forearm and abdomen from 3 cases and investigated the regional as well as the individual difference in size of the touch dome. Our results clearly showed that size of the touch dome does not change individually, but regionally. The difference in size of the touch domes between the present and previous papers may still remain fully unsolved, but technical differences might partly affect the size variation in these papers.

The density of human touch domes has been calculated by some previous investigators. Smith [12] observed the skin surface with naked eyes and estimated 1–2 touch domes per cm^2. Reinisch and Tschacheler [11] identified touch domes by immunostaining skin nerves and reported 1.56±0.55 × 10^2 touch domes per mm^2. These data are roughly the same as our present data confirming the density of the domes in the total skin samples. However, in this study, we analyzed the density of touch domes in forearm and abdominal

skin concluding that density was higher in the forearm than in the abdomen. This is the first to show regional differences in the density of the touch domes in human skin. This finding may be related to the difference in the sensitivity of the skin sensation in forearm and abdomen, which will linger for further studies.

Concluding remarks

The present study clarified the two- and three-dimensional ultrastructure of the touch domes using correlative techniques of SEM. The size of the touch domes was larger in the abdomen than that in the forearm. The density of touch domes was higher in the forearm than that in the abdomen. Recently, we are interested in the usefulness of capillaroscopy to identify non-invasively the touch domes in human skin. Since epidermal ridges surrounding a touch dome were thick, it was considered that the region appeared as a whitish area with poor blood vessels. Detecting the touch dome by capillaroscopy will be reported in future studies for clinical application, which is also expected to be

useful in revealing the structure and function of the human touch dome.

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Figure Legends

Figure 1. The basal surface of the epidermis observed by SEM of a KOH-collagenase treated tissue. Epidermal ridges are semicylindrical with smooth surfaces and run in various directions to form a coarse network. Small round depressions about 10–50 μm are present in the mesh of the epidermal ridge network. Ducts of sweat glands (#) are observed beside the epidermal ridges and hair follicles (*) with hair shafts are found in the mesh of the epidermal ridge network. Note a touch dome (arrowheads), which is observed as a wide and deep concave area bordered by the thick epidermal ridges. Scale bar = 500 μm.

Figure 2. The closer view of the touch dome in Fig. 1a. (a) The touch dome are clearly determined as a concave area surrounded by thick epidermal ridges One duct of the sweat gland (#) and two hair follicles (*) are present outside the thick epidermal ridges. (b) High magnification view of the boxed area in (a). Unmyelinated nerve fibers with Schwann cells (blue) are accumulated at

the base of epidermis. (c) LM image of the toluidine blue-stained semithin section through the dotted line in (a). The touch dome is identified by the thick epidermis sandwiched between slightly long epidermal ridges extending inward. (d) BSE-mode SEM of the boxed area in (c). Two Merkel cells (M) with nerve terminals (E) are covered by the sheet-like processes of Schwan cells (S). K; Keratinocyte. Scale bars (a, c) = 100 μm, (b) = 10 μm and (d) = 5 μm.

Figure 3. Basal surfaces of forearm (left) and lateral abdominal (right) epidermis observed from the dermal side by SEM of KOH-collagenase treated tissues. (a-f) Thick epidermal ridges are observed in all three cases although they are very shallow in (a) and (b). The round depressions for dermal papillae are well developed in case 3, but poor in case 1 (a, b). In case 2, the depressions are more developed in the abdomen than in the forearm. Arrowheads indicate the areas of the touch domes. *; hair follicle. #; sweat duct. Scale bars = 200 μm.

Figure 4. SEM of touch domes in forearms (left) and lateral abdomens (right) of case 1 (a, b), case 2 (c, d) and case 3 (e, f) observed from dermal side. (a-f) Closer views of boxed areas in Fig. 3a, b (a, b), Fig. 3c, d (c, d) and Fig. 3e, f (e, f). Although touch domes are varied in shape and size, they are observed as a concaved area surrounded by thick epidermal ridges. Scale bars = 100 μm.

Figure 5. Merkel cell-neurite complexes and nerve fibers (blue) of touch domes. (a) Closer view of boxed area of Fig.4b. (b) Closer view of boxed area of Fig.4f. Unmyelinated nerve fibers with Schwann cell investments attach to Merkel cell-neurite complexes. Scale bars = 10 μm.

Figure 6. Statistical analysis of the touch domes. (a) The size of touch domes tended to be larger in the abdomen than that in the forearm. Significant differences were seen in case 2 and 3. (b) A density of touch domes was higher in the forearm than that in the abdomen in all 3 cases.

Table 1. The density and size of touch domes in three donated bodies.

	Part	Observed area (mm^2)	Number of TD	Density ($\times 10^2$number / mm^2)	Average size per 1 TD ($\times 10^{-2} mm^2$)	Correction value of average size ($\times 10^{-2} mm^2$)
Case 1;	Forearm	450	22	4.89	4.3 ± 2.6	7.1 ± 4.4
65 y. o. female	Abdomen	459	6	1.31	5.8 ± 6.1	9.6 ± 10.2
Case 2;	Forearm	846	24	2.84	3.6 ± 1.8	6.0 ± 2.9
64 y. o. male	Abdomen	819	11	1.34	5.9 ± 3.3	9.8 ± 5.6
Case 3;	Forearm	639	28	4.38	4.2 ± 2.0	7.0 ± 3.3
73 y. o. female	Abdomen	882	11	1.25	6.9 ± 3.7	11.5 ± 6.1
Total of 3 cases	Forearm	1935	74	3.82	4.0 ± 2.0	6.7 ± 3.4
	Abdomen	2160	28	1.3	6.3 ± 3.9	10.4 ± 6.5

TD = touch dome

Table 2. Summary of the density and the size of the touch domes in the literature, including our study.

	Number of bodies	Part	Density ($\times 10^2$number / mm^2)	Average size per 1 TD ($\times 10^{-2} mm^2$)	Correction value of average size ($\times 10^{-2} mm^2$)	Observation device
Kamide, 1955	1	Trunk	NA	8.5 ± 3.3	-	LM
Smith, 1970	NA	NA	1 to 2	NA	-	Eye
Reinisch and Tschacheler, 2005	12	Shoulder, trunk, extremities	1.56 ± 0.55	19.3 ± 13.8	-	LM
Our study	3	Forearm	3.82 ± 1.07	4.0 ± 2.0	6.7 ± 3.4	SEM
		Abdomen	1.30 ± 0.05	6.3 ± 3.9	10.4 ± 6.5	
		Total of forearm and abdomen	2.50 ± 1.64	4.6 ± 2.8	7.6 ± 4.6	

TD = touch dome, NA = not available, LM = light microscope, SEM = scanning electron microscope

Fig.1

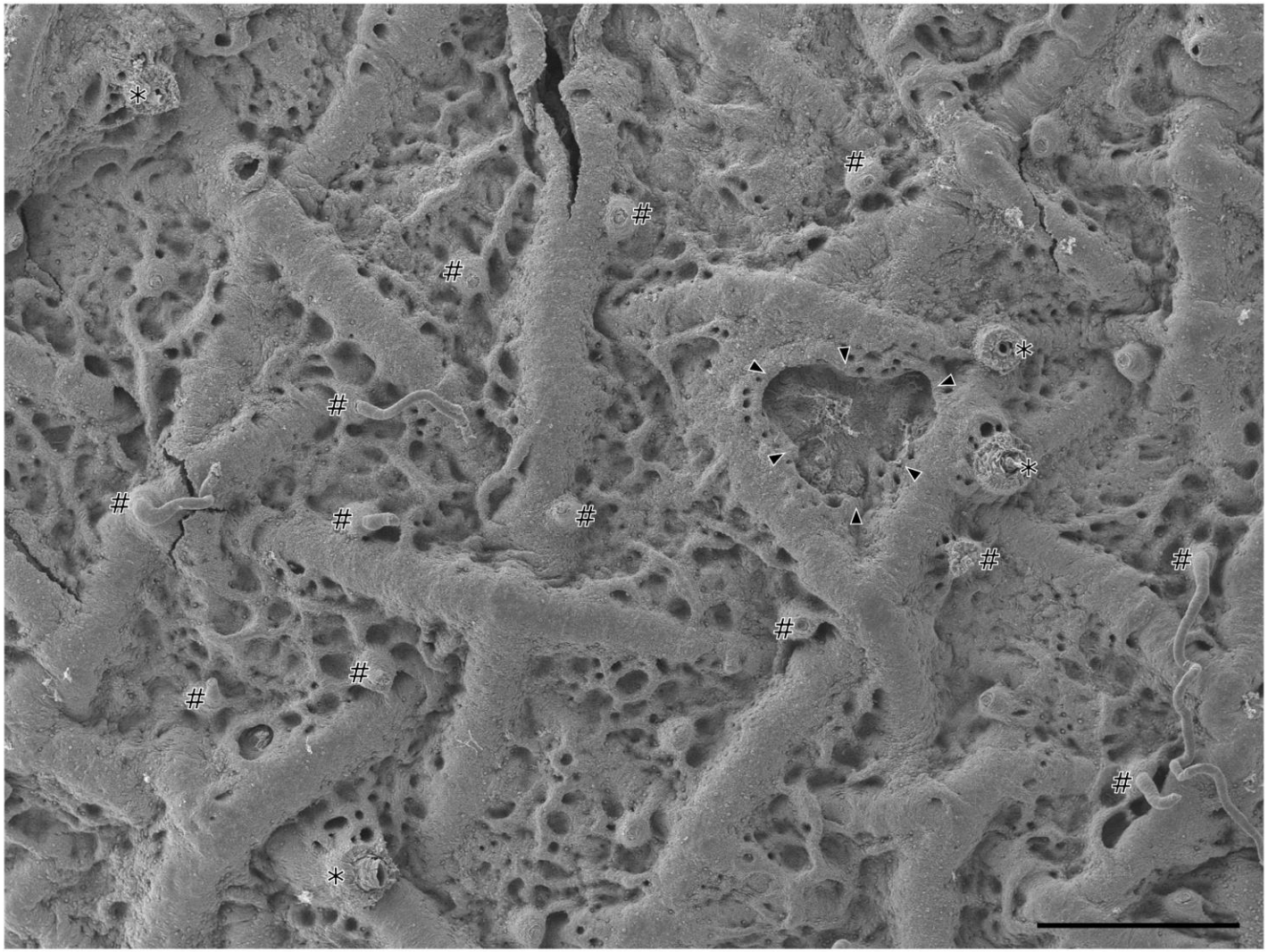

Fig.2

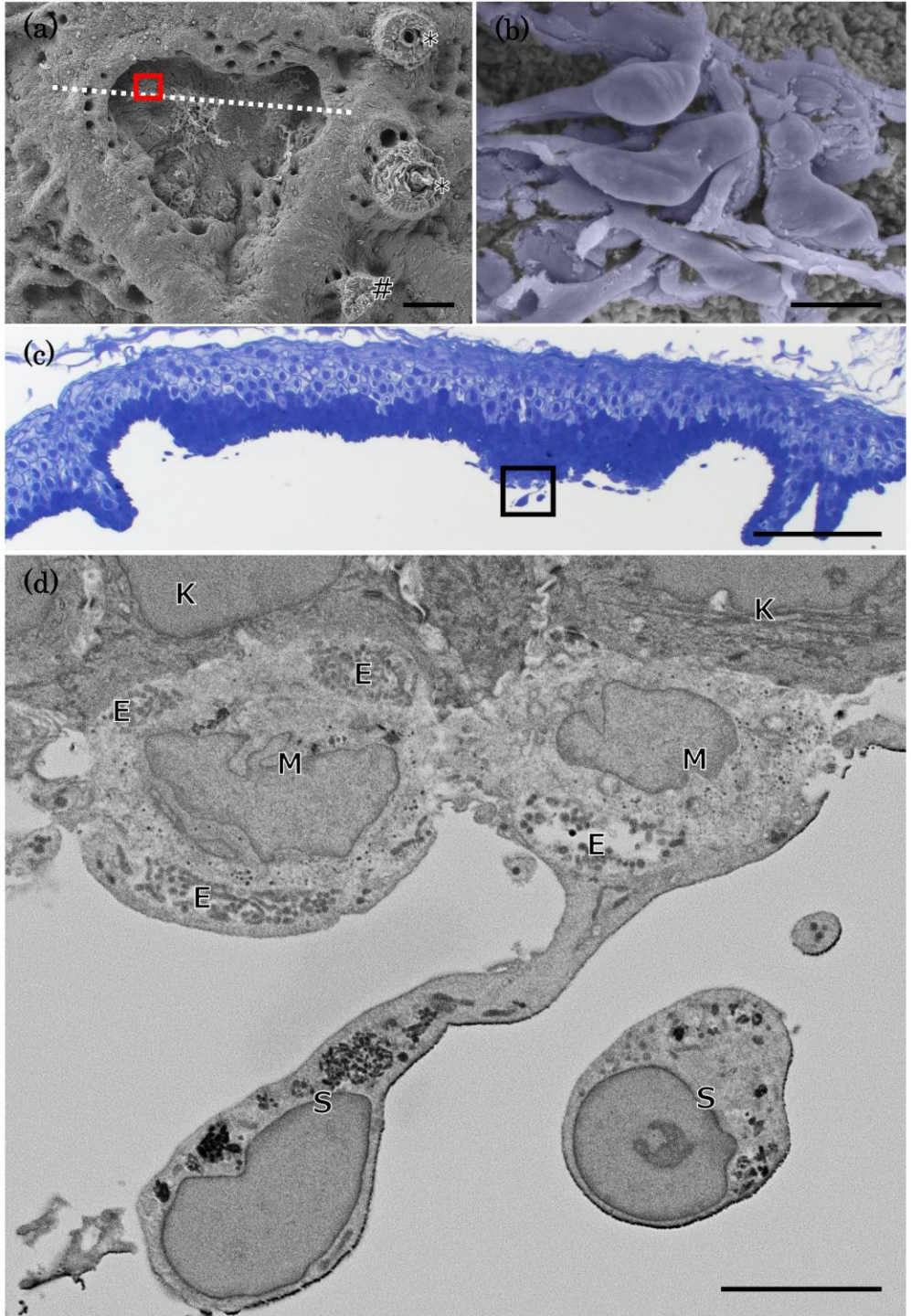

Fig.3

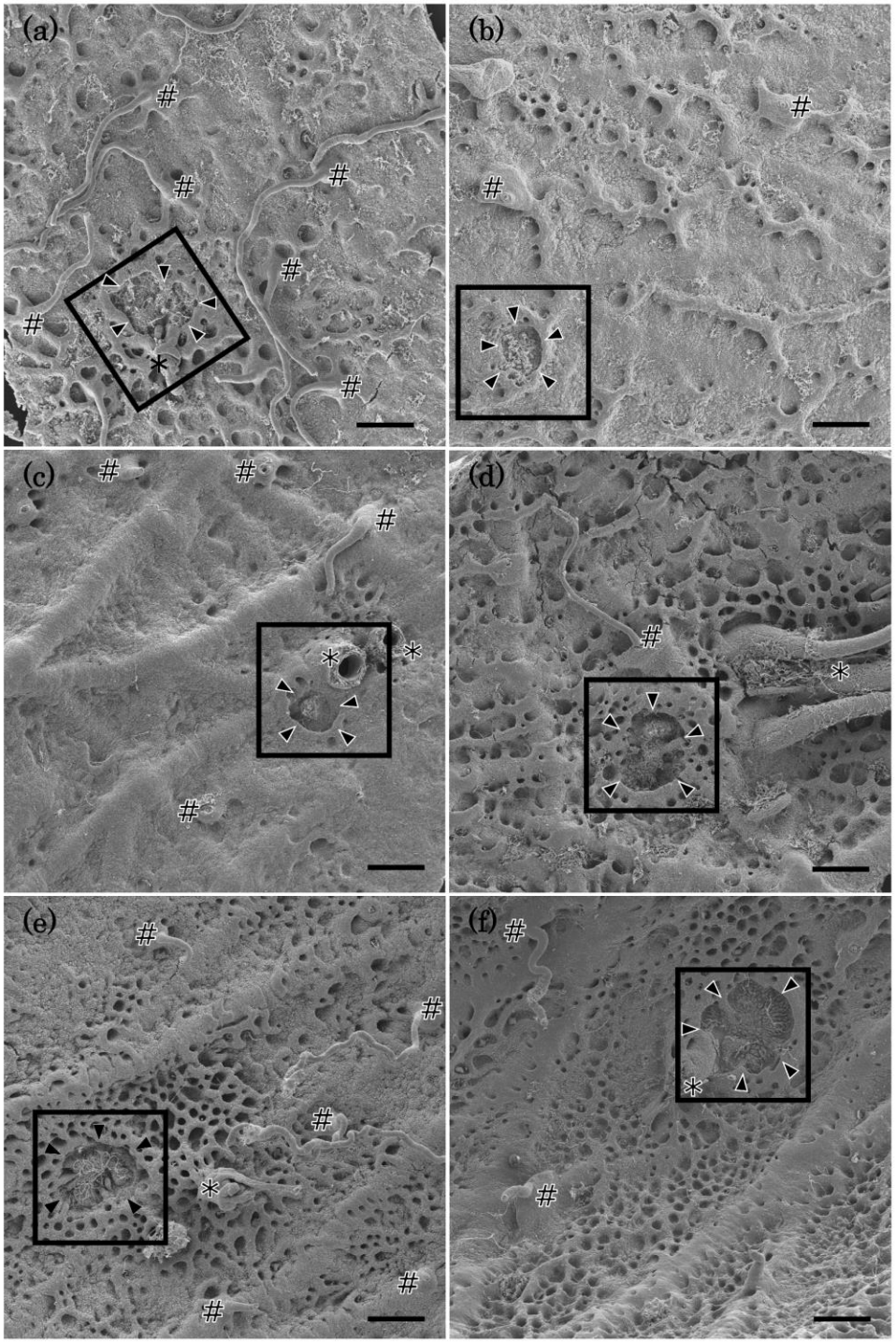

Fig.4

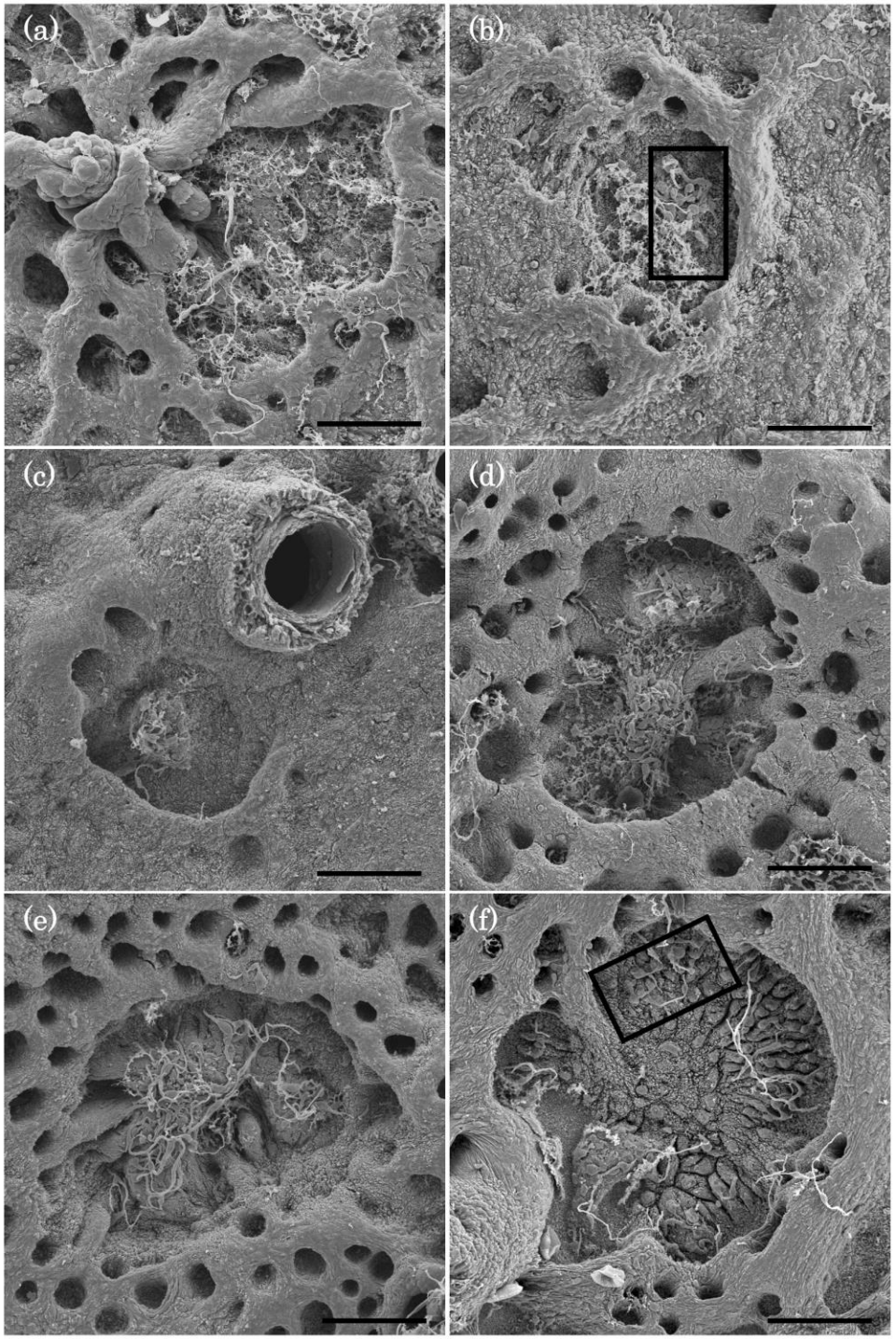

Fig.5