博 士 論 文

Incremental Lines in Human Cellular Cementum: a Histological Study

（ヒト有細胞セメント質の成長線の組織学的研究）

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Incremental lines in human cellular cementum: a histological study

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Abstract

Objectives: Human cellular cementum has incremental lines that demarcate individual cementum lamellae. The structural and functional details of the lines remain poorly understood. This study was designed to examine human cellular cementum by light microscopy, scanning electron microscopy, and contact microradiography and to elucidate the ultrastructure of incremental lines and their significance in cellular cementogenesis.

Methods: Longitudinal paraffin and ground sections of human mandibular molars were prepared. Paraffin sections were stained with hematoxylin, or hematoxylin and eosin, or impregnated with silver. Hematoxylin-stained sections were observed by scanning electron microscopy using NaOH maceration. Silver-impregnated sections were further stained with hematoxylin. Hematoxylin-stained ground sections were examined by contact microradiography.

Results: The incremental lines were found to be collagen fibril-poor layers. The outer area of each cementum lamella consisted of highly mineralized fibrils involved in constructing an alternating lamellar structure, whereas the inner area consisted of irregularly arranged, less highly mineralized, fibrils. The incremental lines corresponded with the innermost sites of the inner area.

Conclusions: On the basis of the obtained findings, we suggest cellular cementogenesis progresses as follows. (1) Cementoblasts alternate between low- to high-activity states.

(2) In the earliest low-activity stage, cementoblasts generate poorly mineralized, fibril-poor, incremental lines. (3) As cementoblasts recover activity, fibril-organization and mineralization advance in the cementum. (4) In the high-activity stage, cementoblasts reach full activity and construct the highly mineralized, alternating lamellar structure. (5) Cementoblasts again move into the low-activity stage. (6) The above processes are repeated, and the incremental lines and cementum lamellae are alternately generated.

250 words

Key words: Cellular cementum, Incremental lines, Contact microradiography, Scanning electron microscopy

1. Introduction

Cementum is a type of hard dental tissue that covers the root dentin and serves as one of the periodontal tissues. The end of each principal fiber is embedded in the cementum or alveolar bone as Sharpey's fibers. Through this mechanism, cementum fulfills the most important function, i.e., tooth fixation in the alveolar socket. Cementum is conventionally classified into acellular and cellular cementum based on the absence or presence of cementocytes [1].

Acellular cementum is 20–50 μm thick, covers the cervical root, and contains Sharpey's fibers, almost exclusively, as a fibrous constituent. Hence, its major function is tooth fixation. Cellular cementum is several tens to hundreds of micrometers thick, occasionally thicker than 1 millimeter, and covers the apical root. In addition to Sharpey's fibers, cellular cementum contains the intrinsic fibers that comprise the fibers of cementum proper, and these are arranged parallel to the cementum surface. Generally, cellular cementum has hematoxylin-stainable incremental lines that demarcate the cementum lamellae. The distributions of Sharpey's and intrinsic fibers differ in the individual cementum lamellae. Some lamellae contain no or only a few Sharpey's fibers, and such cementum lamellae do not work as a tooth-fixing device but instead work to adjust and disperse the masticatory force in cooperation with other periodontal tissues [1].

Many investigators have studied incremental lines under light microscopy,

scanning electron microscopy, and contact microradiography [2-9]. As a result, it is widely believed that the incremental lines, like the resting lines in bone, are highly mineralized and are generated between the resting and formative phases of cellular cementogenesis [4-9]. Nevertheless, their structural and functional details, i.e., their ultrastructure, mineralization degree, and signification in cellular cementogenesis, are still poorly understood. This study was designed to examine the incremental lines in human cellular cementum by light microscopy, scanning electron microscopy, and contact microradiography and to elucidate the issues in question. NaOH maceration was applied to sections before scanning electron microscopy to allow us to observe the collagen fibrils [10, 11].

2. Materials and Methods

2.1. Materials

The experimental materials were composed of 50 human mandibular molars that had been stored in 10% formalin in our laboratory. This study was approved by the Ethical Review Board for Life Science and Medical Research, Hokkaido University Hospital (Approval No. 021-0191).

2.2. Light microscopy

After demineralization with 5% formic acid, the teeth were fixed with 2.5% glutaraldehyde (pH 7.4), routinely embedded in paraffin, and longitudinally cut to 5-μm thickness. The sections were stained with hematoxylin, or hematoxylin and eosin, or impregnated with silver. After photographing, some silver-impregnated sections were further stained with hematoxylin or toluidine blue. Two microscopic images obtained from the same section were superposed, using the cementocytes as a guide, for comparison.

2.3. Scanning electron microscopy

Some hematoxylin-stained sections were treated with the 10% NaOH maceration method for 1-2 days [10, 11]. After conductive staining with 1% tannic acid and 1% OsO_4 [12], the portion including the section was cut out with a glass cutter. The

sections were dehydrated in a graded series of ethanol solutions, critical-point dried, and coated with platinum-palladium prior to examination with a Hitachi S-4800 scanning electron microscope at 10 KV.

2.4. Contact microradiography

The teeth were longitudinally cut with a diamond disk and polished to 50–70-µm thickness with emery paper. After etching with 1 N HCl for 30 s, the sections were stained with hematoxylin and photographed. Then contact microradiography was applied to the sections with ultrasoft X-ray microscopy equipment (Softex CSM-2) at 14 KV and 4 mA. The exposure time was 30 min. Images were recorded on Konica Minolta photo plate (HRP-SN-2). The light microscopic and radiographic images of the same section were superposed, using the cementocytes as a guide, for comparison.

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3. Results

3.1. Light microscopic observation

Sharpey's fiber-free and -poor regions of cellular cementum were examined. Many hematoxylin or toluidine blue (not shown) -stained incremental lines were seen to demarcate the cementum lamellae (Fig. 1). Generally, the outer area (periodontal ligament side) of each cementum lamella showed an alternating pattern of intensely and faintly stained lamellae, each of which was approximately 2-μm thick. The structure will henceforth be referred to as an alternating lamellar structure. The inner area (dentin side) did not show this structure. In other words, the alternating lamellar structure was seen inside of the incremental lines but not outside (Fig. 1).

In the silver-impregnated sections, non-impregnated, i.e., collagen fiber-poor lines, were observed. On image superposition, we could see that the lines were identical to the hematoxylin-stained incremental lines (Fig. 2). The silver-impregnated sections showed clearer fiber arrangement than the hematoxylin and eosin-stained sections. The fibers were organized into the alternating lamellar structure inside of the incremental lines, whereas the same types of fibers were irregularly arranged outside the incremental lines (Fig. 3)

3.2. Scanning electron microscopic observation

The hematoxylin-stained sections that had been used for the light microscopy were

examined by scanning electron microscopy (Fig. 4). The individual collagen fibrils were obvious and visible because interfibrillar substances were removed by NaOH maceration. In the light microscopic images, the incremental lines were identical to the fibril-poor, groove-like structures, which were not visible in the non-macerated specimens (not shown). As seen under light microscopy, the collagen fibrils were more irregularly arranged outside of the groove-like structures (i.e., incremental lines) than those inside (Fig. 4). The cementum lamellae were often detached at the incremental lines (Fig. 5). Where the alternating lamellar structure was present under light microscopy, two types of lamellae 2–3-μm in thickness—a lamella consisting of roughly longitudinally sectioned fibrils and a lamella consisting of roughly transversely sectioned fibrils—were stacked alternately to form the alternating lamellar structure (Fig. 6). At a higher magnification, the fibril groups showed periodic and synchronous changes in arrangement.

3.3. Contact microradiographic observation

Radio-dense or highly mineralized bands and less radio-dense or less highly mineralized bands appeared alternately (Fig. 7). The superposed images showed the incremental lines were always located at the boundaries between the two kinds of bands. Within each cementum lamella, a highly mineralized band was located in the outer area and a less highly mineralized band was located in the inner area. From the

inner to outer side, the radio-density increased gradually, and the boundaries between the two kinds of bands were not obvious (Fig. 7). In contrast, the boundaries between the two kinds of bands at the incremental lines, i.e., the boundaries between adjacent cementum lamellae, were more obvious. Highly mineralized lines were often found near the incremental lines. At a higher magnification, the highly mineralized lines were non-identical to the incremental lines but identical to the outermost site of the highly mineralized band (Fig. 7).

4. Discussion

4.1. Ultrastructure of incremental lines

The superposed light microscopic images showed that the incremental lines were lacking collagen fibers. The fibril poverty in the incremental lines was confirmed by scanning electron microscopy. Using contact microradiography, Selvig [4] and Fujii [6] reported that the incremental lines were homologous with acellular cementum. Cellular cementum often contains acellular cementum as one type of cementum lamellae [13]. Our study, however, verified that the incremental lines differed from acellular cementum.

Matsuo and Yajima [8] and Matsuo [9] observed cellular cementum by scanning electron microscopy following NaClO and HCl treatment and found collagen-fibril-poor groove-like structures very similar to those in our study. Regarding the appearance of the groove-like structures, they suggested the following. Cellular cementum has fibril-poor and poorly mineralized linear structures. The disorganizing- and demineralizing-actions of NaClO and HCl cause the outflow of interfibrillar amorphous substances from the linear structures, causing groove-like structures to appear. Similarly, we considered that the fibril-poor, incremental lines may have appeared as groove-like structures via the removal of interfibrillar substances. However, Matsuo and Yajima [8] and Matsuo [9] reported that these groove-like structures did not correspond with the incremental lines in their studies. The disagreement of opinions

will be discussed later.

The separation of the cellular cementum was always seen at the incremental lines, and therefore the incremental lines were considered to be structurally weak sites where fibril continuation is interrupted. The weakness may be compensated for by interfibrillar substances. On the basis of this and previous studies [6, 7], we believe the incremental lines may contain proteoglycans and/or glycoproteins. However, we failed to identify the substances. The nature of the interfibrillar substances should be detected in more detail by histochemistry and immunohistochemistry.

4.2. Degree of mineralization of incremental lines

From the precise superposition of light microscopic and radiographic images, we concluded that the incremental lines were more poorly mineralized than the surrounding cementum. The conclusion disagrees with a widely believed opinion that incremental lines are more highly mineralized [2, 3, 5, 6, 8, 9]. In previous studies, the superposition of light microscopic and radiographic images was not conducted, or if conducted, the superposition did not seem to be as strict as that in our study. Matsuo and Yajima [8] and Matsuo [9] compared light microscopic, scanning electron microscopic, and contact microradiographic images of the same sections and concluded that the highly mineralized lines were identical to the outermost sites of highly mineralized bands (i.e., the outermost sites of the cementum lamellae). This conclusion

agrees with ours. However, as mentioned in the previous section, they considered the incremental lines to be identical to the highly mineralized lines but not identical to the groove-like structures. We believe that the groove-like structures in our study were identical to the incremental lines. The groove-like structures (i.e., incremental lines) and highly mineralized lines are contiguous with each other. Owing to heavy structural alteration by NaClO and HCl treatment, their radiographic and scanning electron microscopic images were probably inaccurately superposed in their studies [8, 9].

Mizuki [14] and Watanabe [15] examined human mandibular alveolar bone by light microscopy, scanning electron microscopy, and contact microradiography and showed that the resting lines lacked collagen fibrils and appeared as groove-like structures after NaClO and/or HCl treatment under scanning electron microscope. Although the resting lines were structurally similar to the incremental lines in our study, the authors reported that the resting lines were highly mineralized. To compare the two types of lines accurately, further investigations will be required.

4.3. Signification of incremental lines in cellular cementogenesis

The alternating lamellar structure has been ultrastructurally studied [7-9, 16-18], and this structure is established to conform to the twisted plywood model proposed for compact bone [16-19]. The twisted plywood model is considered to be a highly elaborate, pressure-resistant structure found in both bone and cellular cementum.

Some reports [17-18] have suggested that cementoblasts and osteoblasts move periodically and synchronously while secreting collagen fibrils, and thereby generate the twisted plywood model structure. In our study, the alternating lamellar structure was an obvious feature of the outer area of the cementum lamellae but not the inner area. Regarding structural polarity, Chen [7] suggested that cellular cementogenetic cementoblasts enter alternate resting and formative stages. In the resting stage, because of their low activity, cementoblasts generate the fibril-poor incremental lines. As they recover their activity in the formative stage, fibril arrangement becomes more organized in the cementum. After cementoblasts reach full activity, they generate the alternating lamellar structure. They then move into the resting phase once more.

On the basis of the findings in this study, we propose that there should be modification of the opinion of Chen [7]. (1) Cellular cementogenesis proceeds through alternating low- and high-activity stages. (2) In the earliest low-activity stage, because they are at their lowest fibril-secreting and -mineralizing activity, cementoblasts generate fibril-poor and poorly mineralized cementum, which results in the incremental lines. (3) As cementoblasts recover their activity, fibril organization and mineralization advance in the cementum. (4) In the high-activity stage, when cementoblasts recover full activity, they construct the highly mineralized, alternating lamellar structure. (5) Then, cementoblasts return to the low-activity stage. The transition may occur sharply because the degree of mineralization and fibril

organization reduces sharply at the incremental lines. (6) The above processes are repeated, and the incremental lines and cementum lamellae are alternately generated (Fig. 8).

Conclusions

Incremental lines of human cellular cementum were examined by light microscopy, scanning electron microscopy, and contact microradiography. Regarding the structure and genesis of the incremental lines, our findings were in basic agreement with those of previous studies. However, in contrast to the generally agreed consensus, our data led us to the conclusion that the incremental lines were more poorly mineralized than the surrounding cementum. We are confident of our conclusion because the light microscopic and radiographic images were strictly superposed in our study.

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Author contribution

Shizuka Takahashi: Main researcher involved in all procedures in this work, especially light microscopic and contact microradiographic observations.

Tsuneyuki Yamamoto, Tomomi Takahashi: Performed sample preparations for scanning electron microscopy (T.Y.) and light microscopy (T.T.)

Yasutaka Yawaka: Participated in the discussion and preparation of the manuscript.

All of the above authors have read and agreed to the submission of the manuscript.

Ethical statement

This study was approved by the Ethical Review Board for Life Science and Medical Research, Hokkaido University Hospital (Approval No. 021-0191).

Conflict of interest

We declare no conflicts of interest.

References

[1] Yamamoto T, Hasegawa T, Yamamoto T, Hongo H, Amizuka N. Histology of human cementum: Its structure, function, and development. Jpn Dent Sci Rev. 2016; 52:63-74.

[2] Soni NN, van Huysen G, Swenson HM. A microradiographic and X-ray densitometric study of cementum. J Periodontol 1962; 33:372-78.

[3] Yamamoto H, Masuda M, Toeda K, Suzuki K. Microradiographic and histopathological study of the cementum. Bull Tokyo Med Dent Univ 1962; 9:141-50.

[4] Selvig KA. The fine structure of human cementum. Acta Odont Scand 1965; 23:423-41.

[5] Furseth R, Johansen E. A microradiographic comparison of sound and carious human dental cementum. Arch Oral Biol 1968; 13:1197-206.

[6] Fujii Y. Mineralization pattern of human cementum (in Japanese). Shigaku 1969; 57:570-92.

[7] Chen M. Observations on the structure of matrix fibers in human cementum (in

Japanese). J Stomatol Soc (Jpn) 1987; 54: 635-75.

[8] Matsuo A, Yajima T. Fibrous components and lamellar structures in cementum (in Japanese). J Jpn Assoc Periodont 1990; 32:140-9.

[9] Matsuo A. Study of the lamellar structure of cementum, its degree of mineralization and fibrous components by light and scanning electron microscopy and by contact microradiography (in Japanese). Higashi Nippon Dent J 1993; 12:193-217.

[10] Ohtani O. Three-dimensional organization of the connective tissue fibers of the human pancreas: a scanning electron microscopic study of NaOH-treated tissues. Arch Histol Jap 1987; 50: 557-66.

[11] Ushiki T, Ide C. Three-dimensional organization of the collagen fibrils in the rat sciatic nerve as revealed by transmission and scanning electron microscopy. Cell Tissue Res 1990; 260:175-84.

[12] Murakami T. A revised tannin-osmium method for non-coated scanning electron microscopic specimens. Arch Histol Jap 1974; 36:189-93.

[13] Schroeder HE. Human cellular mixed stratified cementum: a tissue with alternation layers of acellular extrinsic- and -cellular intrinsic fiber cementum. Schweiz Monatsschr Zahnmed 1993; 103:550-60.

[14] Mizuki H. Histological observations on the structure of the bundle bone at the inner wall of human alveolar bone (in Japanese). J Stomatol Soc (Jpn) 1979; 46: 65-104.

[15] Watanabe I. Observations on the structure of resting line and reversal line in the alveolar bone of human mandible (in Japanese). J Stomatol Soc (Jpn) 1985; 52: 397-437.

[16] Yamamoto T, Domon T, Takahashi S, Islam N, Suzuki R. Twisted plywood structure of an alternating lamellar pattern in cellular cementum of human teeth. Anat Embryol 2000; 202: 25-30.

[17] Yamamoto T, Li M, Liu Z, Guo Y, Hasegawa T, Masuki H, Suzuki R, Amizuka N. Histological review of the human cellular cementum with special reference to an alternating lamellar pattern. Odontology 2010; 98: 102-9.

[18] Yamamoto T, Hasegawa T, Hongo H, Amizuka N. Alternating lamellar structure in

human cellular cementum and rat compact bone: Its structure and formation. J Oral Biosci 2019; 61:105-14.

[19] Giraud-Guille MM. Twisted plywood architecture of collagen fibrils in human compact bone osteons. Calcif Tissue Int 1988; 42: 167-80.

Figure legends

Fig. 1

Light micrograph of cellular cementum containing no Sharpey's fibers. In all figures, the periodontal ligament side, i.e., outer side, is at the top. Incremental lines (arrowheads) demarcate cementum lamellae. In each cementum lamella, the outer area shows an alternating lamellar structure, i.e., intensely and faintly stained lamellae appear to be stacked alternately (**), whereas the inner area does not (*). Hematoxylin and eosin. Scale bar = 50 µm.

Fig. 2

Silver-impregnated (left), superposed (middle), and hematoxylin-stained images (right) of the same section. Non-impregnated lines (arrowheads) are identical to hematoxylin-stained incremental lines (white arrows) in the superposed image (black arrows). Scale bar = 50 µm.

Fig. 3

In each cementum lamella, the outer area consists of an alternating lamellar structure (**), whereas the inner area consists of irregularly arranged fibers (*). Arrows indicate incremental lines. Silver impregnation. Scale bar = 20 µm.

Fig. 4

a: Light micropraph of incremental lines (arrowheads) and cementum lacunae (allows). Hematoxylin. Scale bar = 20 µm. **b:** Scanning electron micrograph of the structures in **a**. Arrowheads and arrows indicate the same incremental lines and cementum lacunae as those in a. Scale bar = 20 µm. **c:** Magnification of the box in **b**. Incremental line is seen as a collagen fibril-poor, groove-like structure. Fibrils are irregularly arranged outside of the incremental line (**), whereas fibrils are more regularly and densely arranged inside of the line (*). Scale bar = 2 µm.

Fig. 5

a: Scanning electron micrograph of detached cementum lamellae. Scale bar = 20 µm. **b:** Magnification of the box in **a**. Detachment occurred at the incremental lines between irregularly arranged (**) and regularly arranged fibrils (*) (see Fig. 3 and 4c). Scale bar = 5 µm.

Fig. 6

a: Scanning electron micrograph of alternating lamellar structure. Scale bar = 5 µm. **b:** Magnification of the box in **a**. Alternating lamellar structure is constructed with alternating roughly longitudinally sectioned (L) and roughly transversely sectioned fibril groups (T). On close examination, from the bottom to top, transversely sectioned

fibrils (1), fibrils with sections facing the right (2), longitudinally sectioned fibrils (3), and fibrils with sections facing the left (4) are repeated in order. The periodic arrangement is explained by the twisted plywood model proposed by Giraud-Guille [19]. Scale bar = 2 μm.

Fig. 7.

a: Radiographic (left) and light microscopic (right) images of the same section are connected. In the light microscopic image, incremental lines (arrowheads) demarcate cementum lamellae. Alternating lamellar structure is seen in the outer area (**) of cementum lamellae but not in the inner area (*). In the radiographic image, a highly mineralized area (**) corresponds to the outer area and a less highly mineralized area (*) corresponds to the inner area. Highly mineralized lines (arrows) were seen near the incremental line. Scale bar = 50 μm. **b:** Magnification of the box in **a**. Highly mineralized lines (arrows) are identical to the outermost sites of the highly mineralized, outer areas. Incremental lines (arrowheads) are identical to the innermost sites of the less mineralized inner area. Scale bar = 10 μm.

Fig. 8.

Schematic diagram depicting cellular cementogenesis. While cementoblasts advance through cementogenesis, they undergo low- and high-activity stages. **a:** Earliest

low-activity stage. Cementoblasts (CB) form the fibril-poor incremental line (*) on the established alternating lamellar structure. **b:** Low-activity stage. Cementoblasts form cementum, which is still composed of irregularly arranged fibers (**). **c:** High-activity stage. Cementoblasts form the alternating lamellar structure. Cementoblasts repeat the cycle from **a** to **c**.

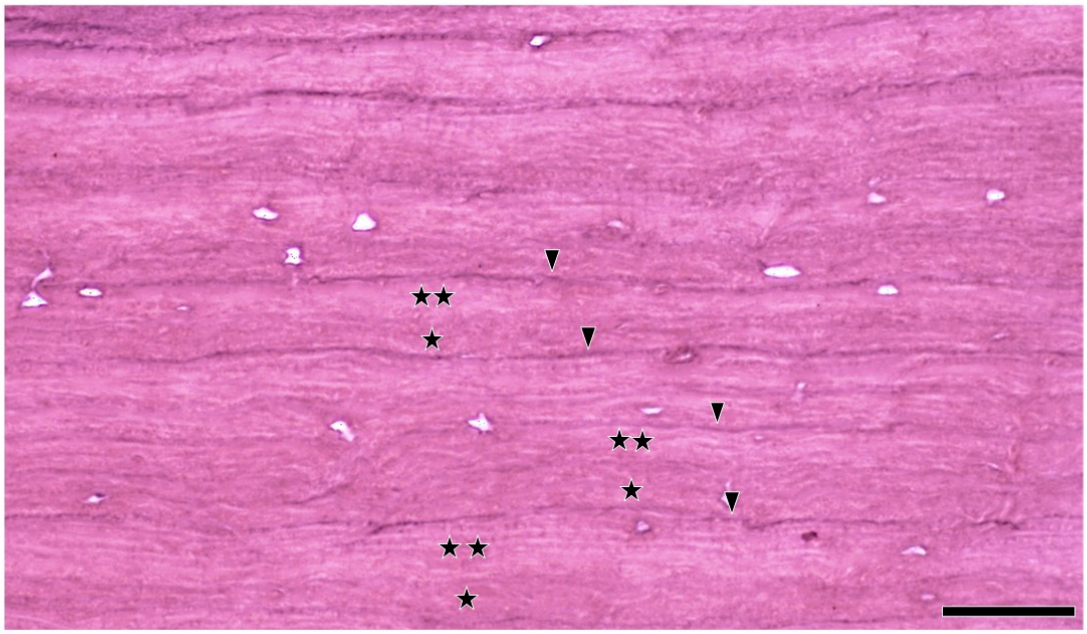
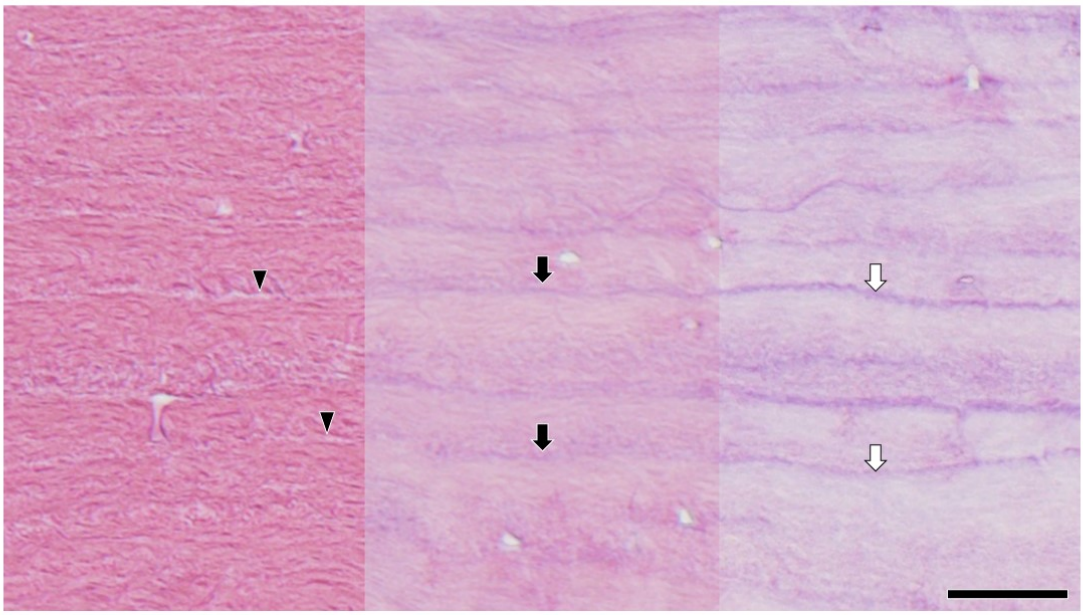


Fig.1



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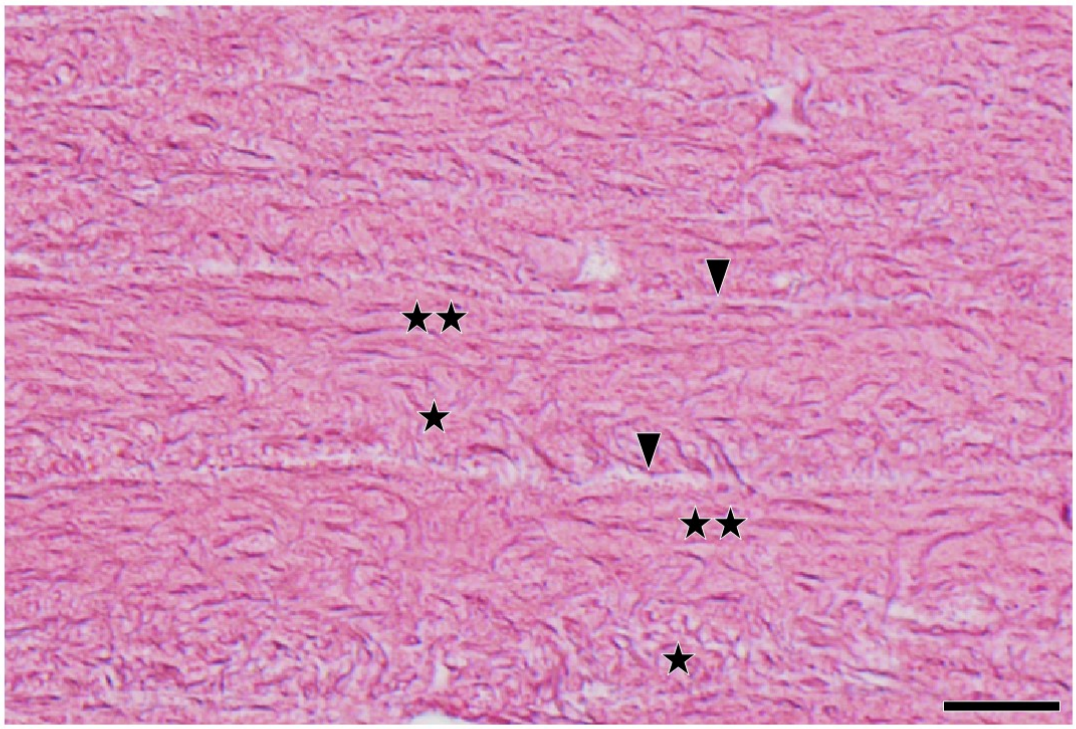

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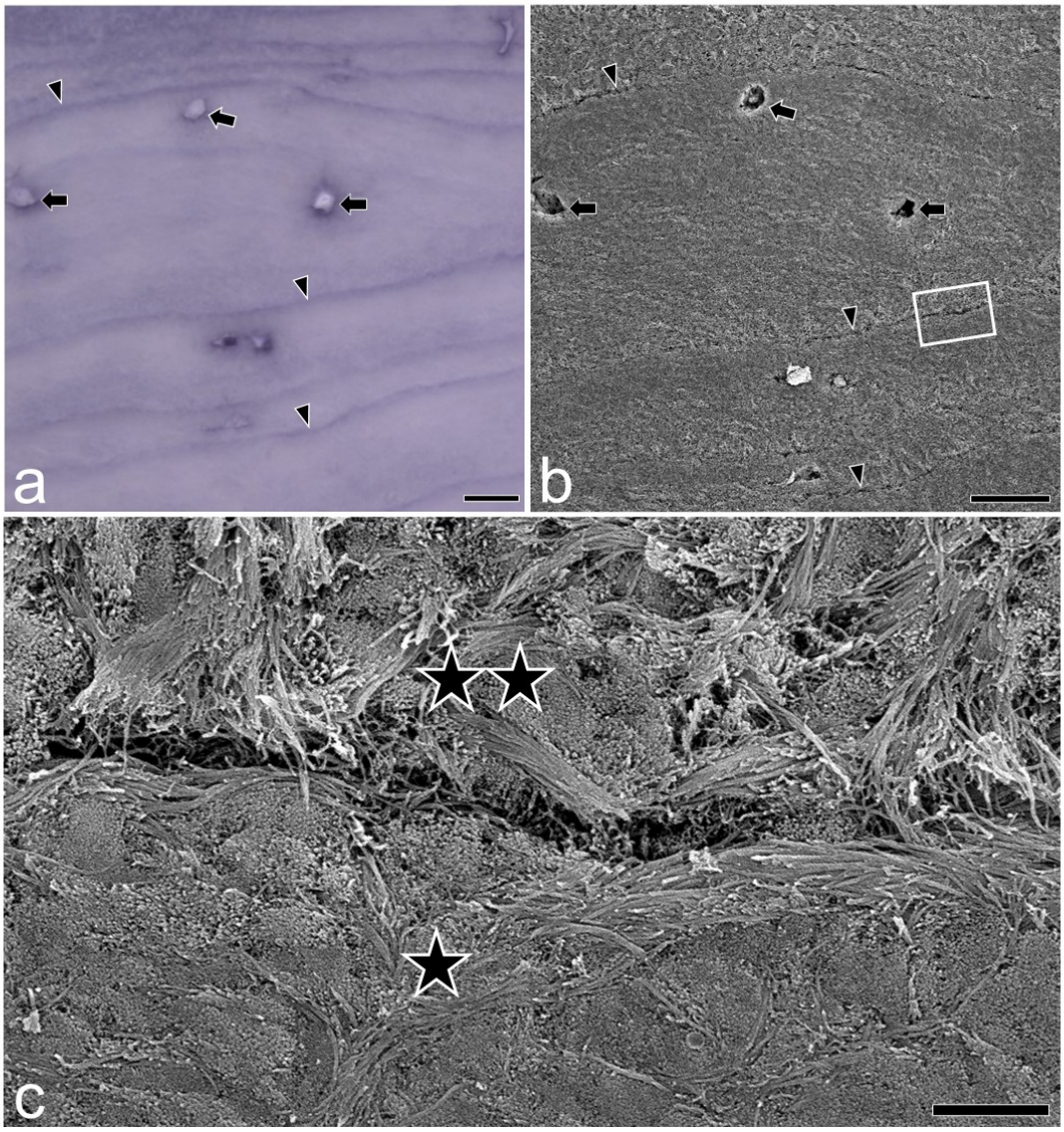

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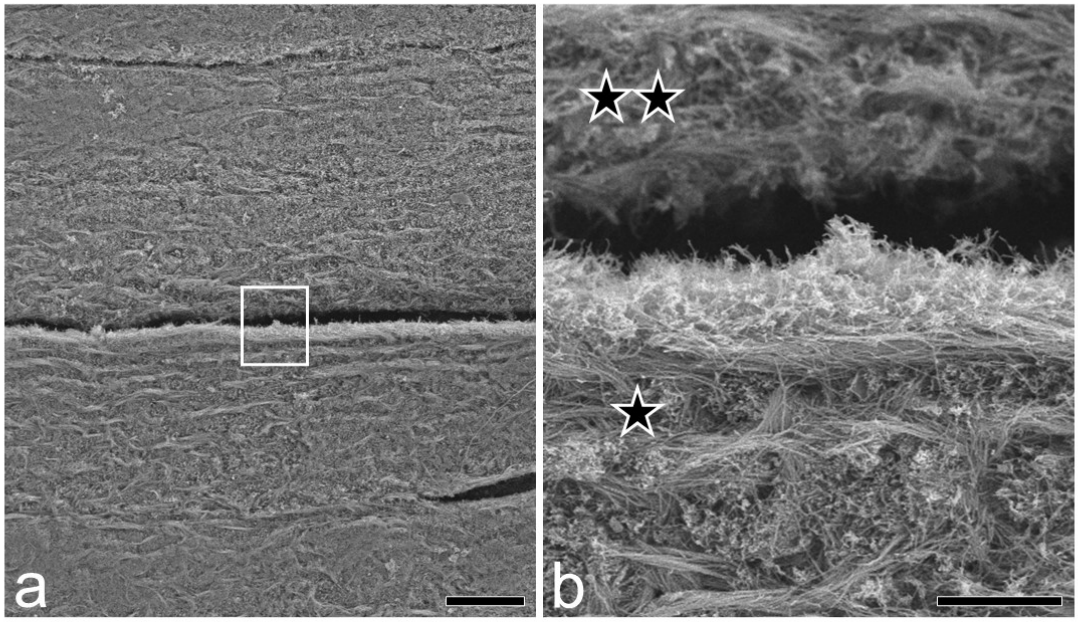

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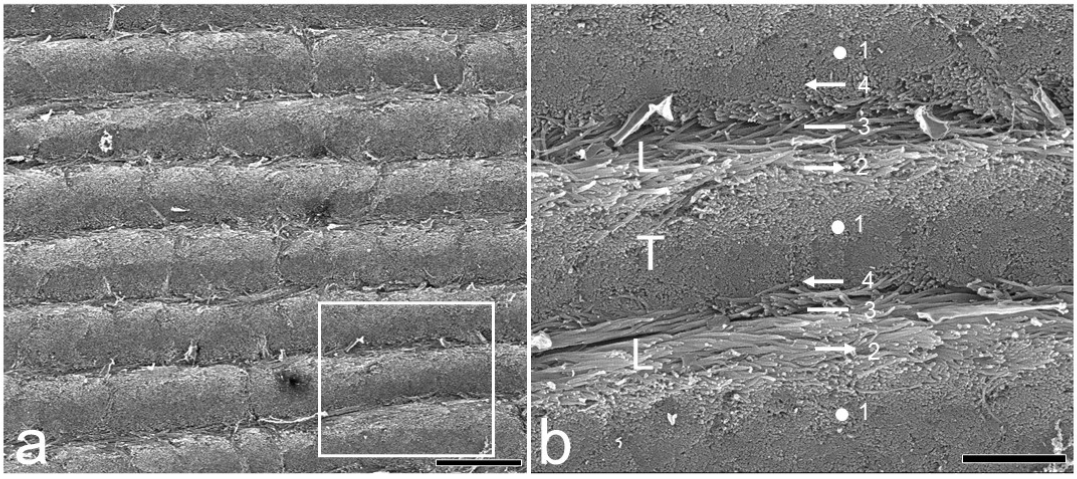

Fig.6

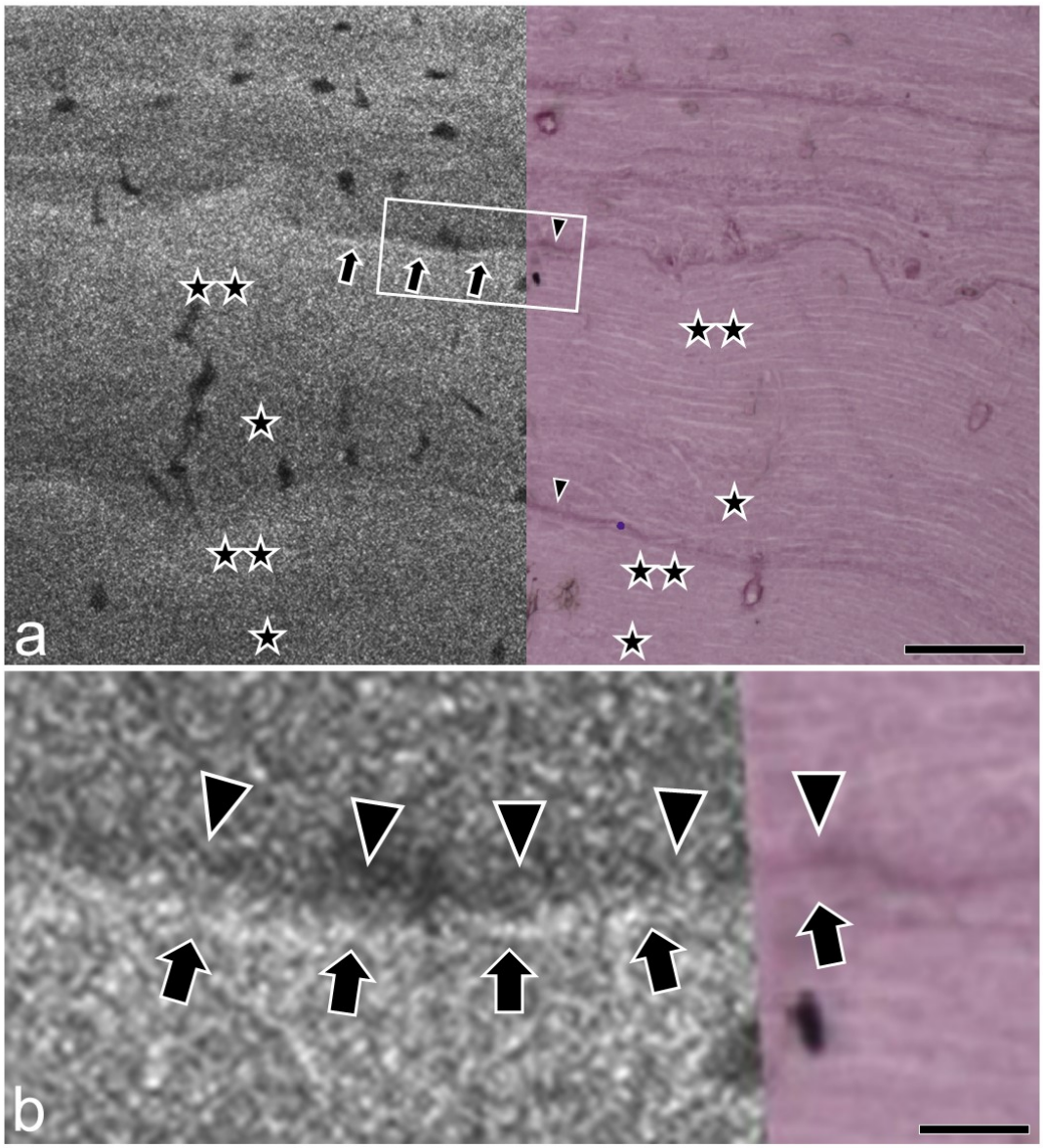

Fig.7

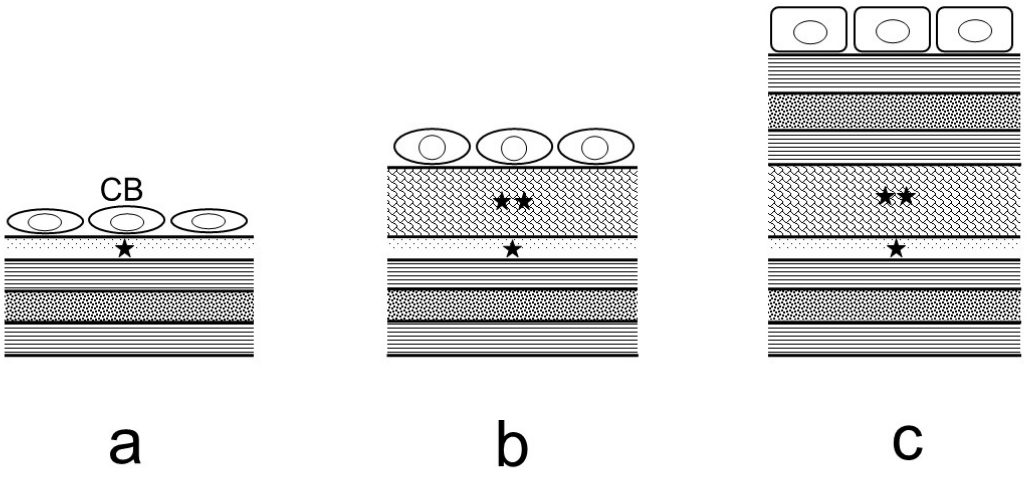

Fig.8