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Review

Alternating lamellar structure in human cellular cementum and rat compact bone: Its structure and formation

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ABSTRACT

Background: Human cellular cementum and compact bone exhibit an alternating lamellar structure, in which intensely and faintly stainable lamellae are stratified in an alternating manner. Many investigators, including our group, have accumulated considerable data regarding lamellar structure. In this review, we summarize the alternating lamellar structure, based on available data, and introduce our hypothesis regarding its formation.

Highlight: We implemented 10% and 24% NaOH maceration methods for scanning electron microscopy. The 10% NaOH maceration method was used for detailed examination of the collagen fibril arrangement, whereas the 24% NaOH maceration method was used for examination of cell morphology in the absence of collagen fibrils. The following findings were obtained: (1) sections of cementum and bone showed two types of alternating lamellae—those comprising longitudinally and nearly longitudinally arranged fibril arrays, and those comprising transversely and nearly transversely arranged fibril arrays; (2) the fibril arrays appeared to shift arrangement in a regular and periodic manner, such that the alternating lamellar structure appeared in sections; (3) where the alternating lamellar structure was being formed, osteoblasts and cementoblasts extended slender processes alongside newly deposited fibrils.

Conclusion: Our data showed that the alternating lamellar structure was consistent with the twisted plywood model previously proposed for osteonal lamellae. For the formation of this structure, there have been two major hypotheses: a self-assembly hypothesis and a cellular control hypothesis. Our data support the latter; osteoblasts and cementoblasts move their processes synchronously and periodically to control fibril arrangement, thereby forming the alternating lamellar structure.

Contents

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1. Introduction

Cementum is a type of dental mineralized tissue that covers the root and supports the tooth in the alveolar socket, in a manner involving cooperation with principal fibers and alveolar bone. Cementum is conventionally classified into two types: acellular and cellular. In general, acellular cementum is thinner and covers the cervical root, whereas cellular cementum is thicker and covers the apical root. The major organic components of cementum include collagen fibers, which are divided into Sharpey's fibers and intrinsic fibers. Sharpey's fibers comprise the embedded ends of principal fibers, and are also referred to as extrinsic fibers. Intrinsic fibers comprise fibers of cementum proper, which are arranged in parallel with the cementum surface, or perpendicularly to the extrinsic fibers. Acellular cementum contains only extrinsic fibers, no intrinsic fibers, whereas the ratio of intrinsic and extrinsic fibers differs by region in cellular cementum [1–3].

In extrinsic fiber-poor and fiber-free regions of cellular cementum, an alternating lamellar structure can be observed in histological sections; intensely and weakly stainable lamellae, each of which are approximately 2–3 μm thick, are stratified in an alternating manner [2–8], similar to that present in compact bone. The alternating lamellar structure of bone has been studied for more than 100 years by many investigators, and a large quantity of data has been accumulated. More recently (1987), Chen [4] was the first to perform detailed investigation of the alternating lamellar structure of human cellular cementum. Based on her findings and the prior data regarding bone structure, Chen suggested that the alternating lamellar structure of cementum, similar to that of bone, was caused by alternating changes in the arrangement of intrinsic fibers.

In the 1990's, we began our ongoing efforts to investigate the alternating lamellar structures of cementum [2,3,7–9]. We have principally used scanning electron microscopy, because it is generally superior to transmission electron microscopy for the purpose of observing fibril arrangement. However, despite the use of scanning electron microscopy, fibril arrangement is difficult to investigate in hard tissue (e.g., cementum and bone) because individual fibrils remain masked with interfibrillar substances after demineralization. Hence, we implemented a 10% NaOH maceration method that effectively removes cells and interfibrillar substances without damaging fibril structures. This method has been used to examine the fibril arrangement in various types of connective tissue [10–16]. Based on the data obtained using this method [3,8,9], we confirmed that the alternating lamellar structure of cementum conformed to the twisted plywood model that had been proposed by Giraud-Guille [17]. Next, we investigated how the alternating lamellar structure was formed. Because it was difficult to obtain intact human teeth due to ethical issues, rat compact bone (femora) was primarily used for this purpose [18]. Based on our previous studies regarding the development of periodontal tissue [2,7,19,20], we assumed that osteoblasts controlled the fibril arrangement by cellular processes and formed the alternating lamellar structure, in a manner similar to that of fibroblasts and cementoblasts. By solely performing routine scanning electron microscopy, however, it is difficult to directly observe osteoblast processes, due to the presence of surrounding collagen fibrils. Hence, we implemented a 24% NaOH maceration method, described by Takahashi-Iwanaga and Fujita [21], that effectively removes collagen fibrils without damaging cell morphology.

The purpose of this review is to summarize the alternating lamellar structure, based on the existing data in the literature, and to introduce our hypothesis for its formation.

2. Alternating lamellar structure

2.1. Human cellular cementum

In extrinsic fiber-poor and fiber-free, cellular cementum, an alternation of two types of lamellae—intensely and faintly stainable lamellae, each approximately 2 μm thick—is present (Fig. 1A). Alternating lamellar structure tends to be more clearly visible at the dentin side of the hematoxylin-stainable incremental lines, rather than at the periodontal ligament side of the lines (Fig. 1B).

In sections of specimens treated by the 10% NaOH maceration method (Fig. 2), individual collagen fibrils (i.e., intrinsic fibers) are clearly observed, because interfibrillar substances have been removed. The collagen fibrils are arranged in parallel with the cementum surface and show alternation of two types of lamellae: those comprising longitudinally and nearly longitudinally arranged fibril arrays, and those comprising transversely and nearly transversely arranged fibril arrays. For convenience, this review will refer to the former as longitudinal lamellae, and the latter as transverse lamellae. In accordance with the findings by Chen [4], the two types of lamellae exhibit different staining affinities and therefore appear as alternating lamellar structure under a light microscope. Longitudinal and transverse lamellae correspond to intensely and faintly stainable lamellae in paraffin sections, respectively; notably, the staining correlation is reversed in ground sections [4].

When traced to the periodontal ligament side or opposite side, fibril arrays are observed to change their arrangement in a regular and periodic manner in a plane parallel to the cementum surface (i.e., they appear to rotate periodically) (Fig. 2). This suggests a basis for alternation of the two types of lamellae in sections.

2.2. Rat compact bone

The internal basic lamellae of rat femora also show an alternating lamellar structure comparable with that in the cellular cementum (Fig. 3A and B). In brief, fibril arrays are arranged in parallel with the inner bone surface. When traced to the marrow side or opposite side, the fibril arrays appear to rotate continuously and periodically (Fig. 3C), similar to those of the cellular cementum; consequently, transverse and longitudinal lamellae appear in an alternate manner in each section of bone.

2.3. Twisted plywood model

The plywood model was initially proposed for osteonal lamellae by Gebhardt [22]. In the Gebhardt model (Fig. 4A), collagen fibril arrays are organized into distinct lamellae, each of which exhibits

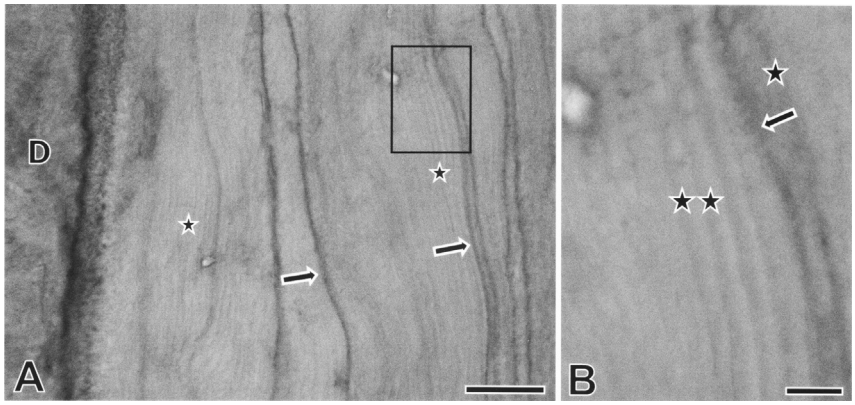

Fig. 1. Human cellular cementum without extrinsic fibers, shown in a hematoxylin and eosin-stained section. **A:** Arrows indicate incremental lines. Intensely and faintly stainable lamellae are stratified in an alternating manner (asterisks). D, dentin. Bar = 50 μm. **B**: Magnification of the box in **A**. The presence of the alternating lamellar structure is more obvious at the dentin side (double asterisks) of the incremental line (arrow) than at the periodontal ligament side (asterisk). Bar = 10 μm.

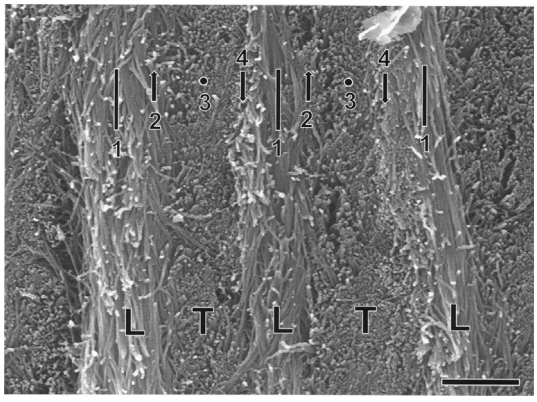

Fig. 2. Alternating lamellar structure in cellular cementum of a longitudinally cut, human mandibular molar. The specimen has been processed using a 10% NaOH maceration method. Two types of lamellae are stratified in an alternating manner: transverse lamellae (T), comprising transversely and nearly transversely cut fibril arrays, and longitudinal lamellae (L), comprising longitudinally and nearly longitudinally cut fibril arrays. The fibril arrays can generally be divided into four types: 1, longitudinally cut fibril arrays; 2, obliquely cut fibril arrays facing upward; 3, transversely cut fibril arrays; 4, obliquely cut fibril arrays facing downward. When traced from left to right, fibril arrays appear to rotate counterclockwise; consequently, transverse and longitudinal lamellae appear in an alternating manner within the section. Bar = 2 μm.

parallel arrays. Between consecutive lamellae, the fibril orientation changes abruptly, with an angle near 90°. The Gebhardt model, which is now known as the orthogonal plywood model, provides an explanation for the alternating lamellar structure that appears under a light microscope. Concurrently, the model provides an explanation for the herringbone pattern that accompanies the alternating lamellar structure (Fig. 4B). Chen [4] also observed the herringbone pattern in the cellular cementum, and concluded that the orthogonal plywood model could thus be applied to the alternating lamellar structure of the cementum. Specifically, she understood the extrinsic fiber-poor and fiber-free cellular cementum to comprise two types of alternating intrinsic fiber arrays: one fiber array parallel to the root axis, and the other fiber array perpendicular to the root axis.

Giraud-Guille [17] refined the orthogonal plywood model and proposed the twisted plywood model (Fig. 5), because the orthogonal plywood model could not explain a nested arc pattern (Fig. 6) that appeared more frequently than the herringbone pattern. In the twisted plywood model, bone lamellae comprise parallel and equidistant planes; in each plane, fibril arrays lie parallel. The array direction rotates continuously between consecutive planes by a constant angle. Rotation of the arrays by an angle of 180° corresponds to the periodicity of the lamellae (Fig. 5A). When the stack of planes is transversely sectioned, transversely, obliquely, and longitudinally cut arrays appear in a periodic manner, thereby forming the alternating lamellar structure observed in each section (Fig. 5B). When the stack of planes is obliquely sectioned, nested arcs appear in each section (Fig. 6A). Cellular cementum also often shows a nested arc pattern (Fig. 6B). Currently, the twisted plywood model is widely accepted for the alternating lamellar structure in both basic and osteonal lamellae.

By using the 10% NaOH maceration method, we visualized the periodic change in fibril array arrangement and the nested arc pattern in both cementum and bone; this provided validation of the twisted plywood model. We found no typical herringbone pattern in either cementum or bone. The nested arc pattern is also present in figures provided by Chen (Figs. 8 and 30 of [4]), although she regarded this as the herringbone pattern. Overall, we concluded that the alternating lamellar structure of cellular cementum conforms to the twisted plywood model, rather than to the orthogonal plywood model.

In addition to the two plywood models described above, a third plywood model has been proposed: an oscillating plywood model [23–25], in which collagen fibril arrays tilt by a constant angle, but return to the previous position without undergoing rotation. Thus far, the prevailing opinion is that the three types of plywood models may coexist within alternating lamellar structure in bone. However, we have not yet confirmed the validity of the third plywood model in cellular cementum or bone, using the 10% NaOH maceration method.

Notably, alternating lamellar structure has been suggested to resist tensile and compressive strains in bone [26]. The apical region of the root experiences extreme occlusal and masticatory forces from various directions. Accordingly, thickened cellular cementum may develop and form alternating lamellar structure, facilitating resistance and dispersal of these forces.

2.4. Other models

According to the orthogonal and twisted plywood models, the two types of lamellae are homogenous in structure; both exhibit similar collagen density and contain parallel collagen fibrils. Notably, the fibril arrangement comprises the sole difference between the two neighboring lamellae. By using scanning electron microscopy, Marotti [27] and Marotti *et al.* [28] examined polished

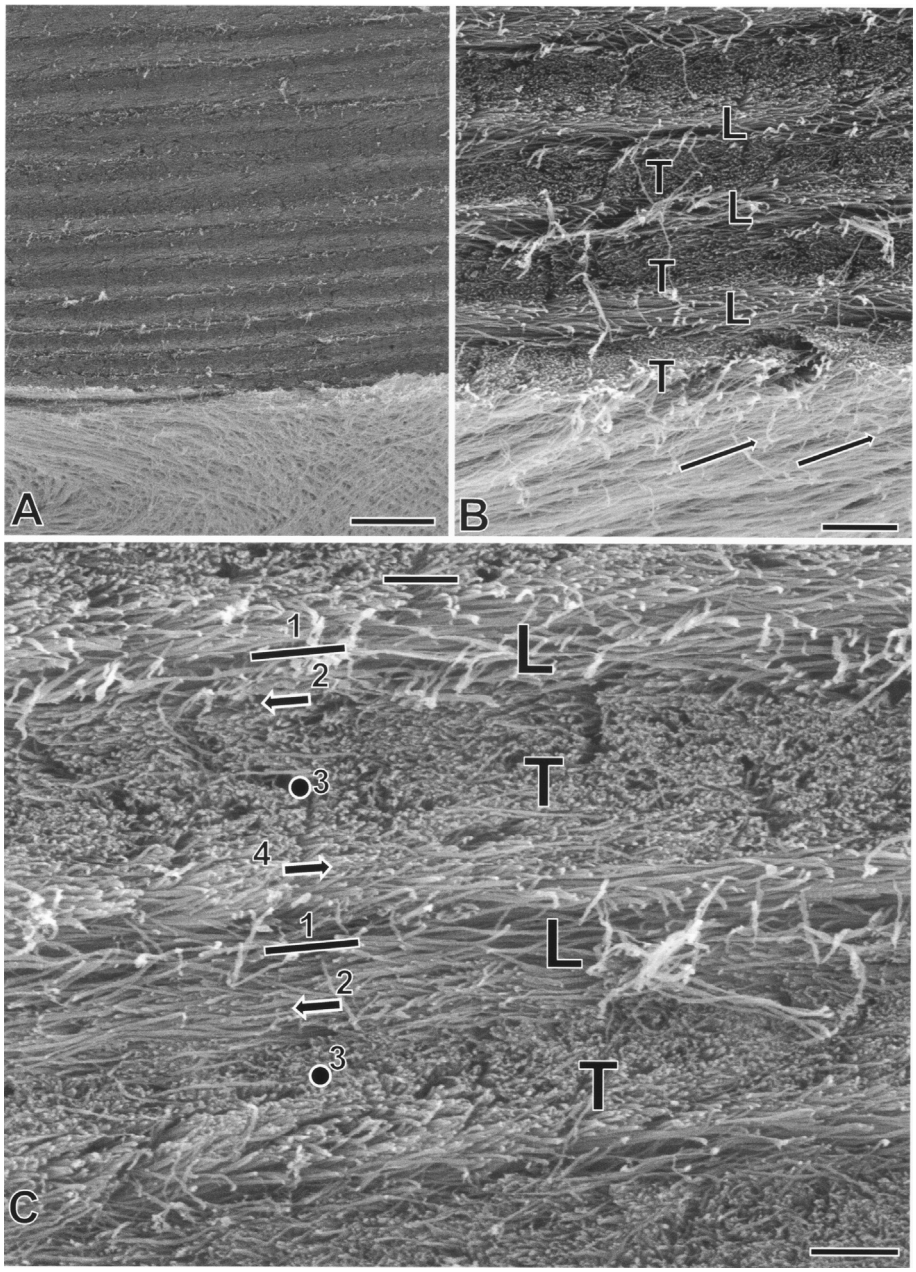

Fig. 3. Internal basic lamellae of a longitudinally cut rat femur. The specimen has been processed using a 10% NaOH maceration method. **A:** Overview of lamellae. Bar = 10 μm. **B:** Longitudinal (L) and transverse (T) lamellae are stratified in an alternating manner, which can be observed in the cellular cementum (see Fig. 2). In the inner bone surface, fibrils with a transverse lamellar organization are arranged in a specific direction (arrows). Bar = 2 μm. **C:** Under magnification, four types of fibril arrays can generally be recognized: 1, longitudinally cut fibril arrays; 2, obliquely cut fibril arrays facing the left side; 3, transversely cut fibril arrays; 4, obliquely cut fibril arrays facing the right side. When traced from top to bottom, the fibril arrays appear to rotate clockwise, causing alternation of transverse (T) and longitudinal (L) lamellae within the section. Bar = 1 μm.

and HCl-etched cubic specimens of human compact bone; they found that each osteonal lamella displayed a similar appearance in both transverse and longitudinal planes of the cube. This finding cannot be explained by either orthogonal or twisted plywood models, because the transverse and longitudinal lamellae shift to the other type at an edge between two planes in each of the two plywood models (Figs. 4A and 5C). Hence, they concluded that the alternating lamellar structure comprised two types of qualitatively different lamellae—dense (fibril-rich) and loose (fibril-poor) lamellae—both exhibiting an interwoven fibril arrangement [27,28]. The Marotti model, however, cannot explain the nested arc pattern. To verify the Marotti model, we conducted follow-up experiments using cellular cementum [8,9]. We prepared two types of specimens. The first type was prepared by polishing and etching in accordance with the approach of Marotti [27] and Marotti *et al.* [28]; the second type was prepared using a freezing microtome after demineralization, then treated by the 10% NaOH maceration method. In specimens of the first type, the transverse lamellae experienced more severe damage than the longitudinal lamellae; consequently, the transverse lamellae exhibited a loose lamella-like appearance, whereas the longitudinal lamellae exhibited a dense lamella-like appearance. In cubic specimens of the second type, in which the edge was sharpened with a microtome knife, the transverse and longitudinal lamellae shifted to the other type at the edge (for further details, see Ref. [9]). Hence, we suspect that polishing and etching caused surface deformation of specimens

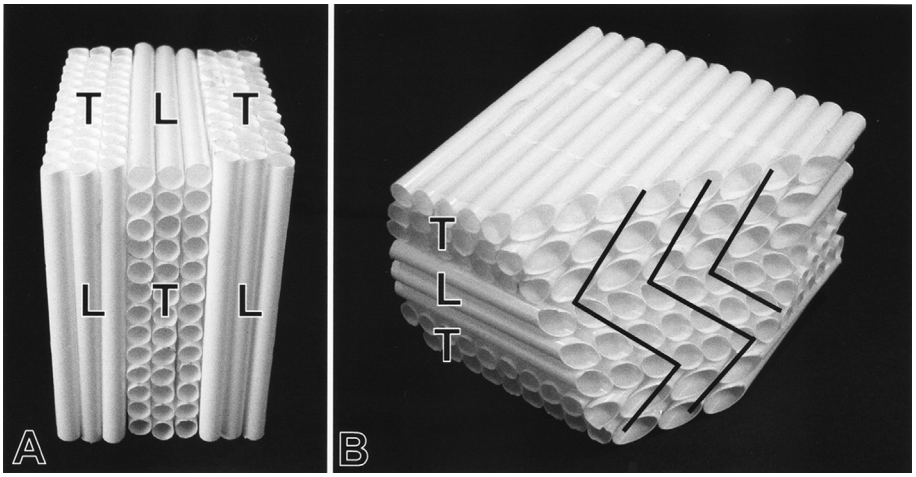

Fig. 4. A: Gebhardt model (i.e., orthogonal plywood model) based on the diagrams of Chen [4] and Giraud-Guille [17]. The model contains nine plates, each of which comprises parallel arrays of drinking straws, similar to collagen fibril arrays. Three continuous plates form a single unit. The direction of the arrays differs at an angle of 90° between two neighboring units. As a result, transverse lamellae (T), comprising transversely cut arrays, and longitudinal lamellae (L), comprising longitudinally cut arrays, appear in an alternating manner within the section. In a cubic specimen, transverse and longitudinal lamellae abruptly shift to the other type at the edge between two planes. **B:** When the model is obliquely cut, a herringbone pattern appears ("zigzag" lines). T, transverse lamellae. L, longitudinal lamellae.

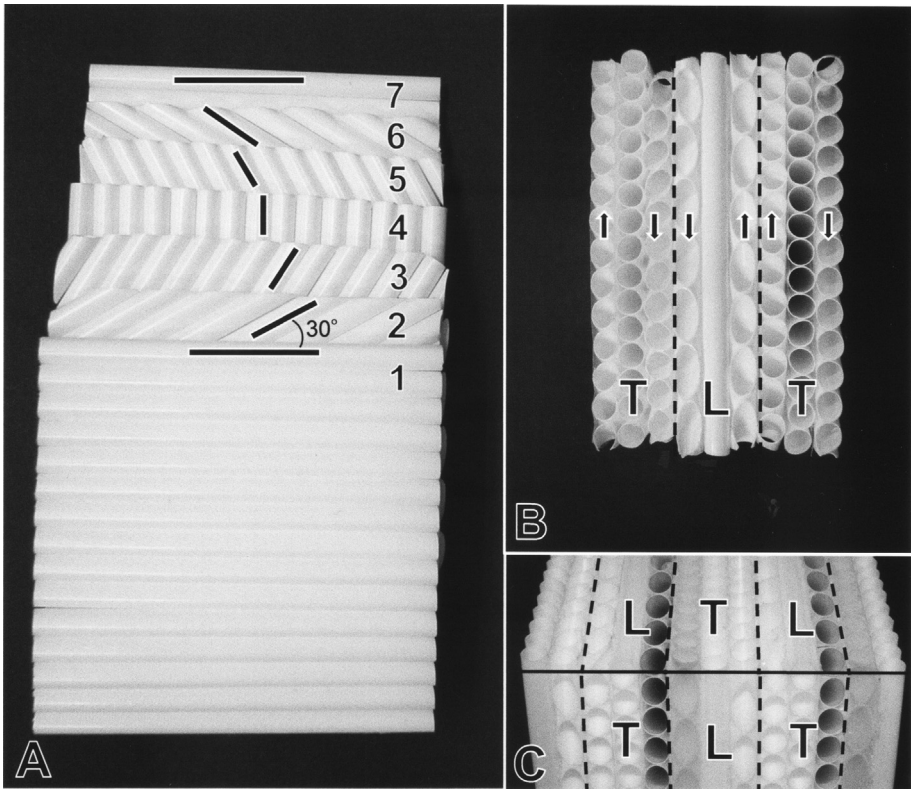

Fig. 5. Twisted plywood model based on the diagrams of Giraud-Guille [17] and Yamamoto *et al.* [8,9]. **A:** Each plate (numbered) comprises parallel arrays of drinking straws, similar to collagen fibril arrays. The direction of the arrays differs at an angle of 30° between two neighboring plates. Six continuous plates correspond to a single period, 180° rotation. **B:** When the model is cut at right angles to the plates, transverse lamellae (T), comprising transversely and nearly transversely cut arrays, and longitudinal lamellae (L), comprising longitudinally and nearly longitudinally cut arrays, appear in an alternating manner within the section. Arrows indicate the direction of sections in the arrays. **C:** In a cubic specimen, transverse (T) and longitudinal (L) lamellae abruptly shift to the other type at the edge (lined) between two planes.

(including dull edges), which led to incorrect conclusions by Marotti [27] and Marotti *et al.* [28].

Ascenzi and Lomovtsev [29] proposed that osteonal lamellae exhibit two types of lamellae: one type comprises fibrils that are predominantly parallel to the osteon axis, while the other comprises fibrils that are predominantly perpendicular to, or at a ± 45° angle, to the osteon axis. This model, however, does not explain the nested arc pattern. Overall, we consider the twisted plywood model to be the most reliable explanation of alternating lamellar structure.

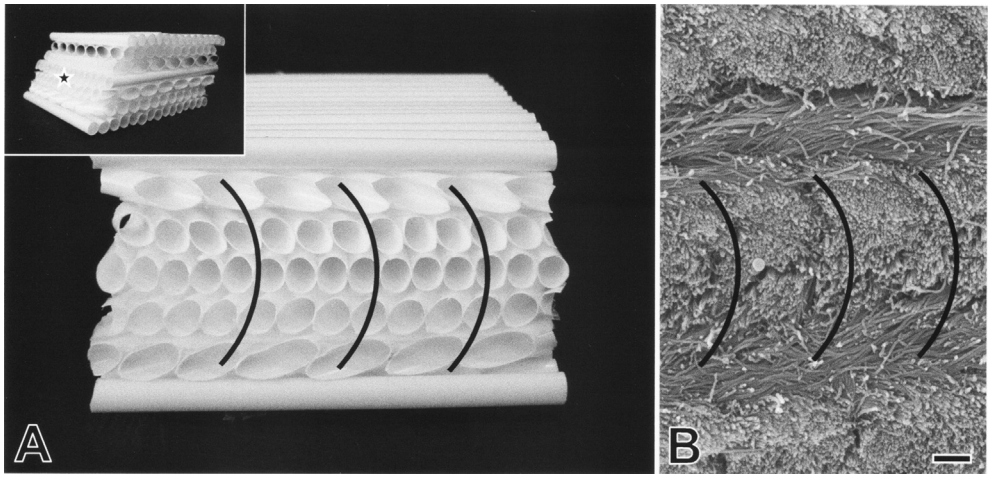

Fig. 6. A: When the twisted plywood model is obliquely cut (asterisk in the inset), a nested arc pattern appears (curved lines). **B:** Arc pattern (curved lines) in cellular cementum. Bar = 1 μm.

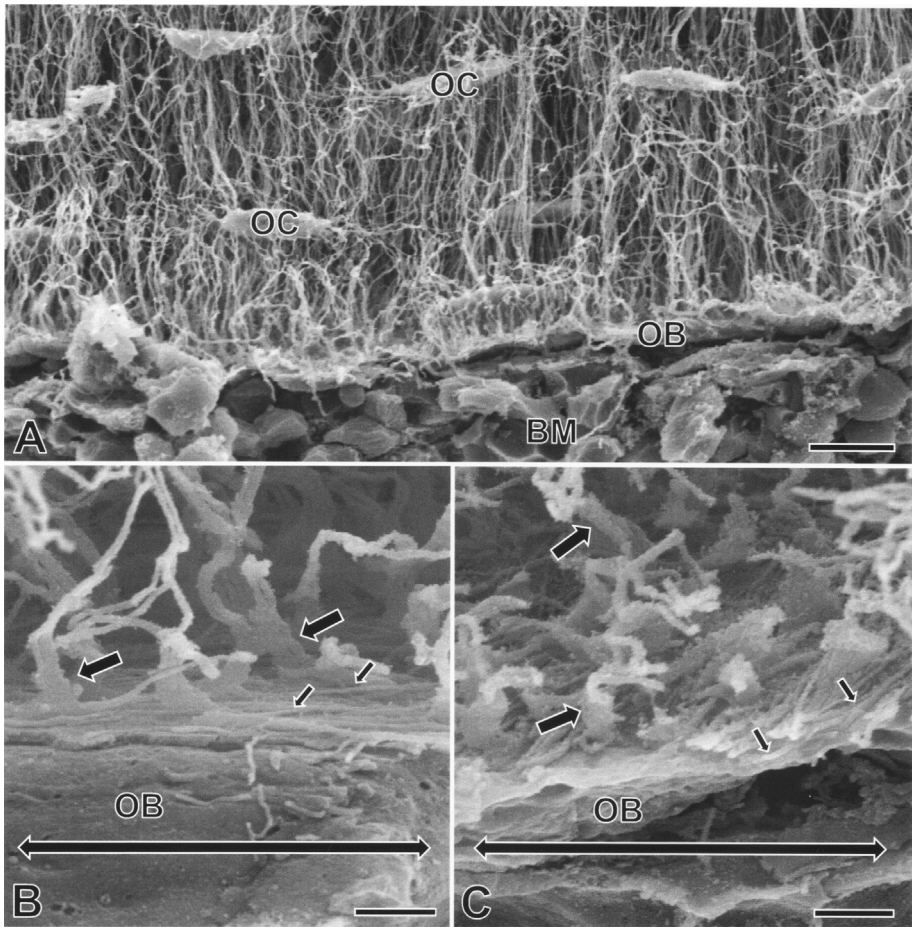

Fig. 7. Region corresponding to the internal basic lamellae of a longitudinally cut rat femur. The specimen has been processed using a 24% NaOH maceration method. **A:** Collagen fibrils have been completely removed and osteocytes (OC) are exposed. Osteocytes extend numerous fine processes that contact with those of other osteocytes. OB, osteoblasts. BM, bone marrow. Bar = 10 μm. **B and C:** Magnification of osteoblasts (OB). Two types of cell processes can be observed on the cell surface facing bone. Type I processes (large arrows) make contact with osteocyte processes. Type II processes (small arrows) extend along the cell surface. Type II processes are arranged in parallel with the long axis of bone (double-headed arrows) in **B** and at right angles to the long axis of bone in **C**. Bars = 2 μm.

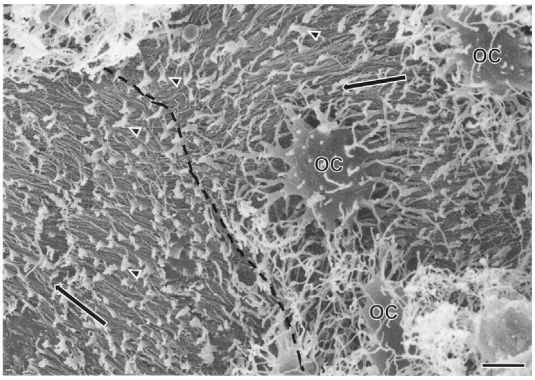

Fig. 8. Osteoblasts on the internal basic lamellae of a rat femur. The specimen has been processed using a 24% NaOH maceration method. Cell surfaces facing the internal basic lamellae are exposed manually with a fine needle. Some osteocytes (OC) are shown embedded. Numerous type II processes can generally be divided into two groups; in each group, processes are arranged in a specific direction (arrows). A dotted line demarcates the two groups. Arrowheads indicate type I processes. Bar = 5 μm.

3. Formation of alternating lamellar structure

3.1. Scanning electron microscopy

Fig. 7 shows internal basic lamellae that have been treated by the 24% NaOH maceration method. Collagen fibrils have been completely removed, such that osteocytes can be observed with nearly intact morphology. These osteocytes extend many fine processes that contact with those of other osteocytes, thereby forming a network within the bone. Magnification of the bone-osteoblast interface, where formation of alternating lamellar structure is ongoing, reveals that osteoblasts exhibit two types of processes on the cell surface facing bone. Processes of the first type (type I processes) extend into bone and contact with osteocyte processes. Processes of the second type (type II processes) are arranged in parallel with the inner bone surface. Osteoblasts form groups; in each group, type II processes are extended in a definite direction, which differs in a group- or region-dependent manner (Figs. 7B and C, and 8).

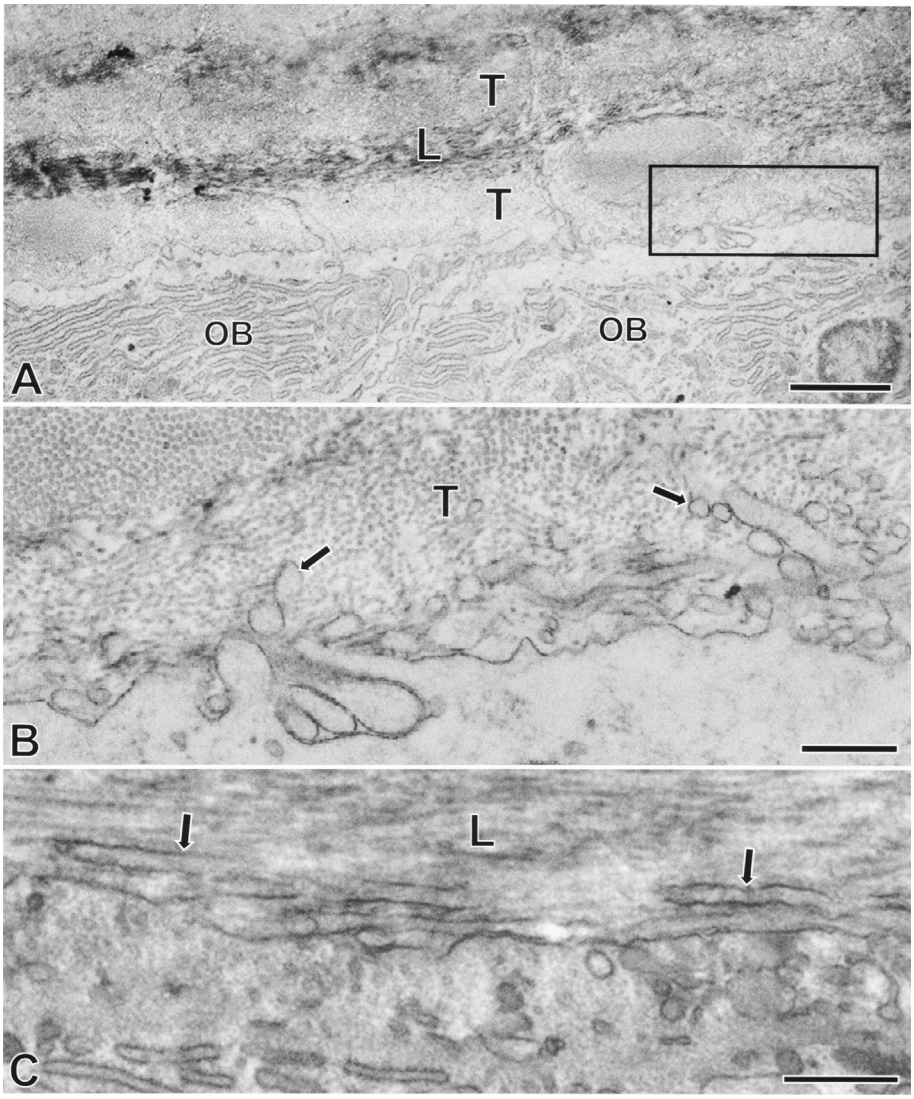

Fig. 9. Bone-osteoblast interface in a longitudinally cut rat femur. **A:** Transverse (T) and longitudinal lamellae (L) are stratified in an alternating manner in the bone. OB, osteoblasts. Bar = 2 μm. **B:** Magnification of the box in **A**. Where transverse lamella (T) formation is ongoing, vesicular cellular elements (arrows) can be observed among transversely cut collagen fibrils. Bar = 0.5 μm. **C:** Where longitudinal lamella (L) formation is ongoing, slender cellular elements (arrows) can be observed in parallel with collagen fibrils. Bar = 0.5 μm.

3.2. Transmission electron microscopy

Where transverse lamella forms at the bone-osteoblast interface, vesicular cellular elements can be observed among transversely cut fibrils (Fig. 9A and B); in contrast, where longitudinal lamella forms (Fig. 9C), slender cellular elements can be observed in parallel with longitudinally cut fibrils. Based on findings in scanning electron microscopy analyses (Figs. 7 and 8), the vesicular and slender cellular elements are assumed to correspond to the transversely and longitudinally cut type II processes, respectively; thus, fibrils and type II processes always coexist in close proximity with parallel orientation, where formation of the alternating lamellar structure is ongoing.

3.3. Hypotheses

3.3.1. Self-assembly hypothesis

There are two major hypotheses regarding formation of the alternating lamellar structure. Here, the first is referred to as the self-assembly hypothesis. According to this hypothesis, collagen molecules assemble naturally and form alternating lamellar structure without manipulation by cells or osteoblasts. This hypothesis is based on *in vitro* observations, in which collagen fibrils form alternating lamellar structure-like structures in the absence of cells under strictly controlled conditions [30–32].

We have identified two simple problems with the self-assembly hypothesis. First, the specific *in vitro* conditions are markedly

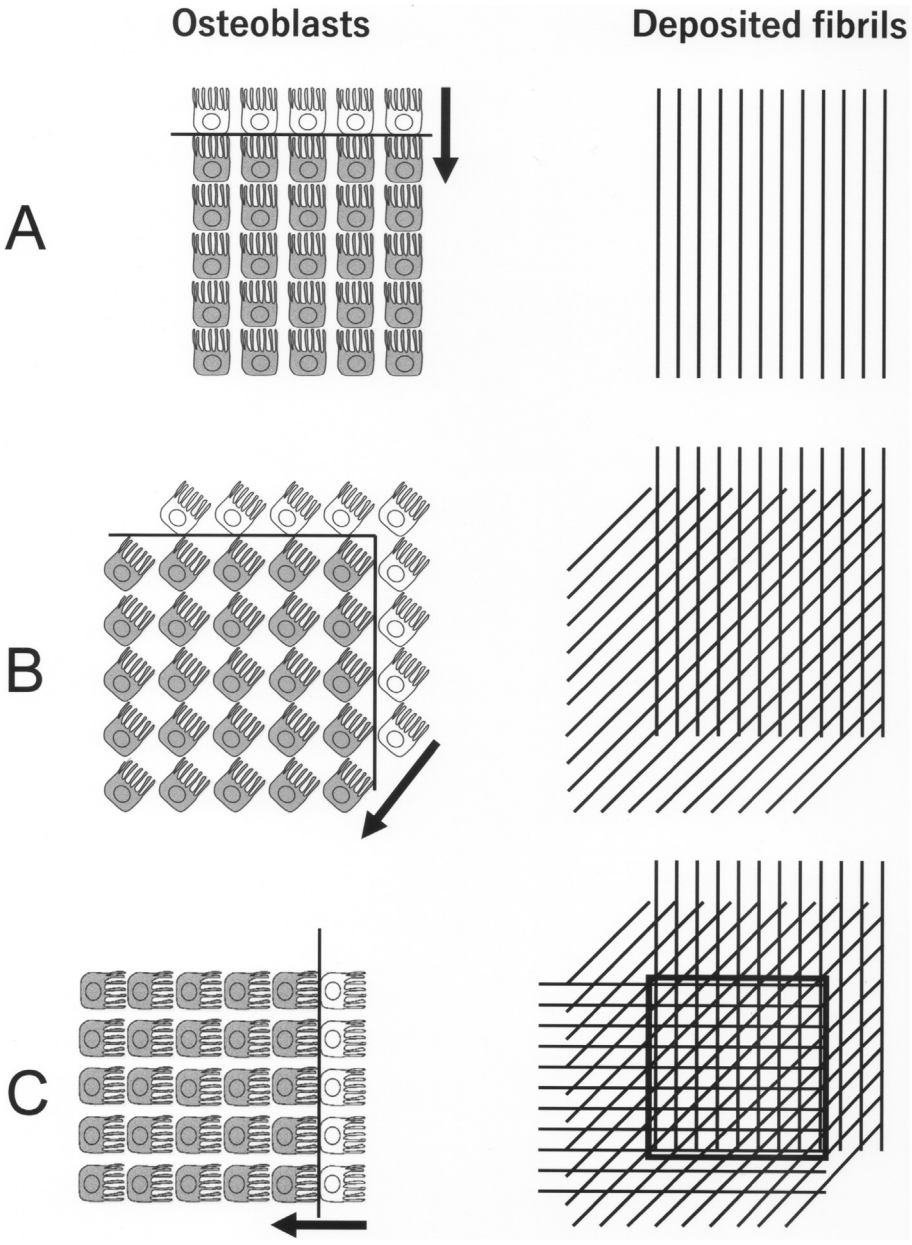

Fig. 10. Diagrams depicting the formation of the twisted plywood structure. The figure and legend were reproduced from Ref. [18] with the permission of the publisher. Twenty-five osteoblasts form a group, in which they behave similarly. The osteoblasts migrate in a preferred direction (arrows), secreting collagen fibrils in parallel with type II processes. **A:** The osteoblasts migrate downward by a specific distance (here, one cell-length for convenience of depiction). Accordingly, fibrils are vertically arranged in the first layer. **B:** The osteoblasts modify the arrangement of the processes and migrate in a new (oblique) direction. Fibrils in the second layer overlap obliquely with the fibrils in the first layer. **C:** The osteoblasts again modify the arrangement of the processes and migrate in a new direction (toward the left). As a result, a half-period of the twisted structure forms in a square.

different from *in vivo* physiological conditions. In particular, the *in vitro* conditions are such that collagen molecules can freely bind to other collagen molecules. In contrast, there are numerous collagen-binding molecules (e.g., several types of proteoglycans and glycoproteins) that may interfere *in vivo*; this would likely inhibit self-assembly by collagen molecules [25]. Second, collagen fibrils are generated throughout the body. If the self-assembly hypothesis is correct, formation of the alternating lamellar pattern should not be limited to cellular cementum and bone; it would be expected to form in collagen fibril-rich tissue such as tendon, cartilage, and dermis. Therefore, the self-assembly hypothesis seems insufficient to address these concerns.

3.3.2. Cellular control hypothesis

According to the second hypothesis, referred to here as the cellular control hypothesis, cells or osteoblasts control the fibril arrangement and actively form the alternating lamellar pattern. Jones *et al.* [33] reported that osteoblasts were arranged in parallel (or almost parallel) association with underlying collagen fibrils, which indicated a strong correlation between cell alignment and fibril orientation. Ascenzi and Benvenuti [34] suggested that the orientation of the collagen depended on the orientation of the cells from which it originated. Ziv *et al.* [35] suggested that lamellar structure is created by groups of osteoblastic cells that produce collagen fibrils and associated proteins, which are then secreted into the extracellular space; moreover, the osteoblasts guide the three-dimensional orientations of these fibrils. Notably, the investigators in the prior studies [33–35] suggested that osteoblasts controlled fibril arrangement, but did not speculate how they might be involved in formation of alternating lamellar structure.

Previously, we investigated the formation of alternating lamellar structure within the context of human cellular cementogenesis [3,7]. Where alternating lamellar structure formation was ongoing, cementoblasts exhibited finger-like processes on the cementum-facing side. Cementoblasts formed groups; in each group, cementoblasts all extended finger-like processes in a specific direction. Notably, the finger-like processes were always arranged alongside newly deposited collagen fibrils. Other investigators have also suggested that fibroblasts and cementoblasts secrete fibrils alongside finger-like processes, thus controlling fibril arrangement during the initial formation of tendon [36–39], cornea [40], and cellular cementum [41]. Based on these findings, we thus propose a mechanism by which cementoblasts form the alternating lamellar structure: cementoblasts move their finger-like processes in a synchronous and periodic manner, thereby causing alternating changes in fiber arrangement. This dynamic sequence produces the alternating lamellar structure [3,7]. Regarding differences in appearance of the lamellar structure in different parts of cellular cementum, we agree with Chen's view [4]. In brief, the incremental lines are resting lines, formed by cementoblasts currently in a resting phase. When cementoblasts resume cementum formation, they do not exhibit sufficient fibril-arranging activity; consequently, lamellar structure becomes obscure at the periodontal ligament side of the incremental lines. As the cementoblasts recover full activity, the fibrils become organized and form the typical alternating lamellar structure, which can be observed at the dentin side of the incremental lines. In general, however, human tissues are not necessarily processed appropriately to view such changes, because of various constraints. Hence, we used rat compact bone, which enabled us to verify the above hypothesis in more appropriately processed materials.

Where formation of alternating lamellar structure is ongoing, osteoblasts formed groups; in each group, the osteoblasts extended slender, type II processes in a specific direction. Newly deposited collagen fibrils were always present alongside the processes. These situations were comparable with those observed in cellular cementogenesis. Hence, we hypothesize that osteoblasts, in a manner similar to that of cementoblasts, form the alternating lamellar structure as follows: osteoblasts behave similarly within a given group; in the group, they secrete collagen fibrils and guide the fibril arrangement in a specific direction by using type II processes. Osteoblasts change the direction of these processes in a synchronous and periodic manner, forming the alternating fibril arrangement. This dynamic sequence results in the formation of alternating lamellar structure. As suggested by Hosaki-Takamiya *et al.* [42], if osteoblasts do not move from the initial position, collagen fibrils may become entangled just beneath the osteoblasts. During formation of the alternating lamellar structure, osteoblasts may continue to migrate. Fig. 10 summarizes our view.

We admit that our hypothesis includes some questionable aspects related to cell movement. Notably, type I processes extend into bone and make contact with osteocyte processes. If these osteoblasts then undergo migration, the contact must be repeatedly broken and re-generated. Skedros *et al.* [25] agreed with the concept of cellular control of fibril arrangement, but doubted whether cell migration could occur because the osteoblasts are confined to circular or cylindrical groups during the formation of osteonal lamellae. Moreover, Skedros *et al.* suggested that osteoblasts controlled fibril alignment by using type II processes without cell rotation or cell movement.

Our hypothesis is based on static photographs that do not provide direct evidence of cell movement. Although we are confident in this hypothesis, we are currently establishing another experimental system to investigate osteoblast movement during the formation of alternating lamellar structure.

4. Conclusion

Human cellular cementum and compact bone are known to exhibit an alternating lamellar structure, in which intensely and faintly stainable lamellae stratify in an alternating manner. We have investigated this alternating lamellar structure by using scanning electron microscopy with 10% and 24% NaOH maceration methods. The 10% NaOH maceration method enables removal of interfibrillar substances and detailed examination of collagen fibril arrangement. The 24% NaOH maceration method enables removal of collagen fibrils and detailed examination of cell morphology in the absence of collagen fibrils. Our data revealed that regular and periodic changes in collagen fibril arrangement led to the formation of alternating lamellar structure in both cementum and bone, and validated the twisted plywood model. Regarding the formation of the alternating lamellar structure, there are two major hypotheses: a self-assembly hypothesis and a cellular control hypothesis. The self-assembly hypothesis states that collagen molecules assemble naturally without cellular control, thereby forming the alternating lamellar structure. Although some questionable aspects remain to be resolved, we support the cellular control hypothesis, in which osteoblasts and cementoblasts actively control fibril arrangement and formation of the alternating lamellar structure.

Ethical approval

The animal protocols used in this study were approved by the Hokkaido University Guidelines for Animal Experimentation (approval No.10-0081 and 15-0041).

Conflict of interest

The authors have no conflict of interest to disclose.

CRediT authorship contribution statement

Tsuneyuki Yamamoto: Conceptualization, Methodology, Investigation, Supervision, Writing - original draft. **Tomoka Hasegawa:** Investigation, Writing - review & editing. **Hiromi Hongo:** Investigation, Writing - review & editing. **Norio Amizuka:** Conceptualization, Project administration, Writing - review & editing.

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