C. R. Palevol 15 (2016) 23–31

Contents lists available at ScienceDirect

Comptes Rendus Palevol

www.sciencedirect.com

General Palaeontology, Systematics and Evolution (Vertebrate Palaeontology)

A literature review of the spatial organization of lamellar bone

Revue bibliographique sur l'organisation spatiale de l'os lamellaire

Jessica Mitchell*, Anneke H. van Heteren

Steinmann-Institut für Geologie, Mineralogie und Paläontologie, Universität Bonn, Nußallee 8, 53115 Bonn, Germany

ARTICLE INFO

Article history:
Received 28 November 2014
Accepted after revision 8 April 2015
Available online 11 July 2015

Handled by Jorge Cubo Gracia

Keywords:
Lamellar bone
Collagen fiber orientation
Heterogeneous lamellae

ABSTRACT

Identification and interpretation of bone tissue types is the primary goal of histological analysis. Lamellar bone, a fundamental tissue, is generally easily identifiable in polarized microscopy. It is important, however, to understand the formation and structure of the tissues that are being studied. Lamellae are widely accepted to form a plywood-like structure, but this hypothesis has been and continues to be contested. Here, we discuss the common interpretations provided by the scientific community as to the spatial organization of lamellar bone. The two major competing hypotheses, lamellae that have alternating tissue compositions versus lamellae that alternate in fiber orientation, are described. In addition, recent research has led to a confounding array of interpretations of lamellae, with several authors viewing the thickness, orientations and composition of lamellae differently. We conclude that a blended approach is needed, as the varying methods are not easily comparable to one another. With an integrated approach, lamellae can be better understood, improving interpretations, including histological ones.

Mots clés :
Os lamellaire
Orientation des fibres de collagène
Lamellae hétérogènes

RÉSUMÉ

L'identification et l'interprétation des types de tissu osseux constituent le but principal de l'analyse histologique. L'os lamellaire, un tissu fondamental, est généralement facilement identifiable au microscope polarisant. Il est cependant important de comprendre la formation et la structure des tissus qui sont étudiés. Il est largement admis que les « lamellae » forment une structure de type contreplaqué, mais cette hypothèse a été et continue à être contestée. Dans cet article sont discutées les interprétations courantes fournies à la communauté scientifique quant à l'organisation spatiale de l'os lamellaire. Les deux hypothèses majeures en présence sont, d'une part, que les lamellae ont des compositions de tissu alternant, d'autre part, qu'elles alternent dans l'orientation des fibres. En outre, une recherche récente a conduit à un ensemble déconcertant d'interprétations des lamellae, avec des auteurs envisageant différemment l'épaisseur, les orientations et la composition des lamellae. Nous en concluons qu'une approche combinée est nécessaire, sachant que les diverses méthodes d'étude ne sont pas facilement comparables les unes aux autres. Avec une approche intégrée, les lamellae peuvent être mieux comprises, en perfectionnant les interprétations incluant celles de type histologique.

* Corresponding author at: Bereich Paläontologie, Steinmann-Institut, Nußallee 8, 53115 Bonn, Germany.
E-mail address: jessica.mitchell@uni-bonn.de (J. Mitchell).

http://dx.doi.org/10.1016/j.crpv.2015.04.007
1631-0683/

1. Introduction

Lamellar bone is one of the main tissue types of bone and is found throughout vertebrates. In recent years, much has come to light about the origin and structure of the lamellae. However, with new methods and approaches come re-interpretations, and a decisive explanation of the organization and composition of lamellae has not yet been established. And, although the theory of bone lamellation has been discussed for over a century with several different hypotheses proposed, a consensus is still lacking.

Histologically, lamellar bone can be either a primary or secondary tissue. In polarized light microscopy, lamellae are typically easily identifiable with organized and parallel alternating dark and light layers and elongated osteocyte lacunae. Both in primary and secondary osteons, lamellar bone centripetally infills the vascular spaces. Secondary osteons form by first resorbing bone then redepositing new lamellae (Fig. 1A, B, D, E). They are elliptical, have a cement line, separating the osteon from surrounding bone, and are oriented parallel to the main loading direction, which means they can alter the original vascular organization. Primary osteons, on the other hand, do not alter the original vascular organization and form directly on the surface of bone. Circumferential layers are lamellae with usually very elongated and thin osteocyte lacunae, which are deposited on the endosteal or periosteal edge of the cortex (Fig. 1C). Trabeculae (Fig. 1F) are frequently remodeled with scalloped regions of lamellae. Researchers rely on these observations to make assumptions about the collagen fiber organization (e.g., Ascenzi and Bonucci, 1967, 1968; Skedros et al., 2009). Even though there is support for these interpretations from the literature, alternative hypotheses have been proposed, as well as new techniques applied, which provide more complex ideas as to how collagen fibers are organized within lamellae.

When studying fossil material, however, diagenetic and fossilization processes can alter, damage, or obscure the ability to effectively observe lamellae as they were in life. We, therefore, need comparisons with modern bone to be able to say anything relevant about fossil bone. Unfortunately, however, it is not possible to make proper assessments about lamellar organization in fossil bone, if there is no consensus on its organization in modern bone. Here, we review the recent literature on lamellar bone organization with the different methods used and discuss the problems and potential solutions.

1.1. Lamellar bone formation

Lamellar bone forms from dynamic osteogenesis (Ferretti et al., 2002; Marotti et al., 1999), a process that involves osteoblasts moving away from the osteogenic surface as they deposit osteoid. Osteoblasts secrete a collagenous matrix in an organized and parallel fashion on a preexisting hard surface (e.g., cartilage or bone) (Prondvai

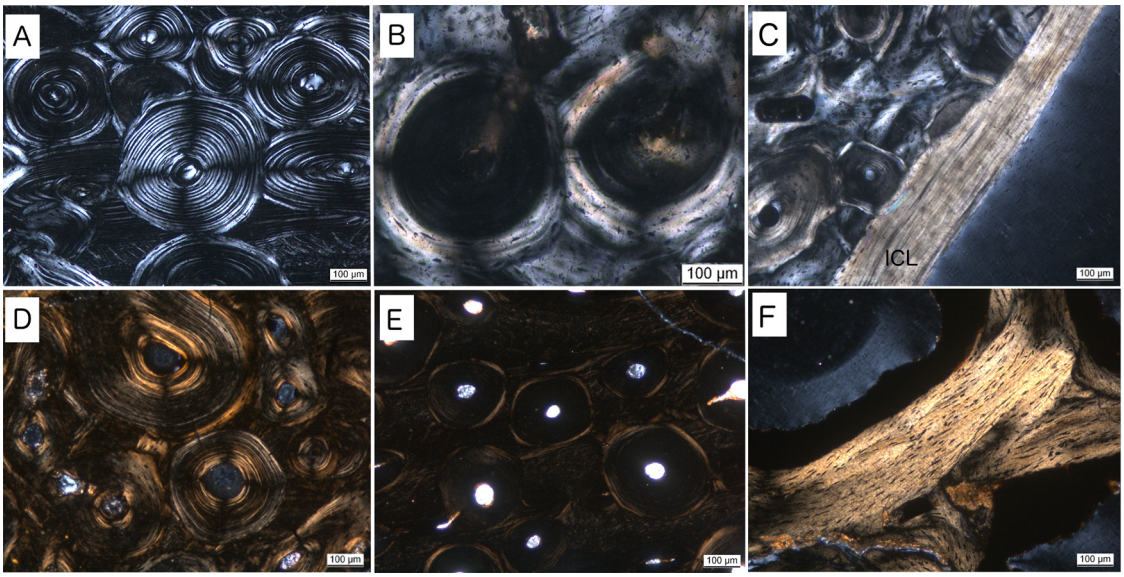

Fig. 1. Polarizing light microscopic images of lamellae in recent (A–C) and fossil (D–F) bone. **A**. Elephant humerus showing the distinctive alternating dark and light lamellar layers in secondary osteons. **B**. Elephant showing secondary osteons with almost all lamellae appearing dark, except the cement line. **C**. Giraffe tibia showing very bright inner circumferential layers (ICL) (also known as endosteal lamellae). **D**. Alternating pattern of bright and dark layers in secondary osteons of a long bone of nodosaurid *Hungarosaurus*. **E**. Mostly dark secondary osteons with a bright cement line in a sauropod humerus *Alamosaurus*. Secondary osteons that are predominantly dark are common in many fossil sections and can be in some cases attributed to diagenetic effects. **F**. Bright secondary lamellae forming trabeculae of an indet. nodosaurid.

Fig. 1. Images au microscope polarisant de lamellae dans des os récents (A–C) et fossiles (D–F). **A**. Humérus d'éléphant montrant les feuillets de lamellae distincts, alternativement noirs et blancs dans les ostéones secondaires. **B**. Ostéones secondaires d'os d'éléphant montrant que presque tous les feuillets apparaissent sombres, excepté la ligne de cimentation. **C**. Tibia de girafe montrant les feuillets internes circulaires très brillants (CL) (connus aussi en tant que lamellae endostéennes). **D**. Réseau à alternance de feuillets brillants et sombres dans des ostéones secondaires d'un os long de nodosauridé *Hungarosaurus*. **E**. Ostéones secondaires sombres pour la plupart, avec une ligne de cimentation brillante dans un humérus de sauropode *Alamosaurus*. Les ostéones secondaires, sombres de manière prédominante, s'observent couramment dans de nombreuses coupes d'os d'animaux fossiles et sont à rapporter, dans de nombreux cas, à des effets diagénétiques. **F**. Lamellae secondaires brillantes formant des trabécules chez un nodosauridé indéterminé.

et al., 2014). Some osteoblasts stop secreting osteoid and become buried in the newly forming bone, thus becoming osteocytes. This differs from woven bone, which is formed from static osteogenesis, where the osteoblasts deposit osteoid in all directions, trapping themselves in the matrix (Marotti, 2010). In addition to this difference, lamellar bone is also much more slowly deposited, and according to Currey (2002), it is less mineralized than woven bone.

At the ultrastructural level, lamellae are composed of ordered collagen fiber bundles and apatite crystals; collagen fibrils form helical structures and within these structures include embedded apatite platelets that are elongated, with the long axis parallel to the long axis of the collagen fiber (Landis et al., 1993, 1996). The apatite platelets can vary in size (Landis et al., 1993). Dumont et al. (2011) showed that sauropod dinosaurs had larger apatite platelets than recent mammals, which may be caused by diagenetic or scaling effects. Shinoda and Okada (1988) have shown with Pb (lead) staining that there is a diurnal pattern of lamella formation in Wistar rats, chipmunks and rabbits, both in primary and secondary lamellar bone. Bromage et al. (2009) show, however, that lamellae are formed following a long period rhythm (analogous to the striae of Retzius in teeth) in Wistar rats, macaques, patas monkeys, sheep and humans. Although this seems contradictory, Wistar rats, and presumably other small mammals, have fast rhythmic growth and a striae of Retzius repeat interval of one day (Bromage et al., 2009, 2011). These rhythmic patterns suggest a more or less constant and cyclical rate of bone deposition that is dependent on species. Further research in this field is needed, and it could be quite beneficial if a relationship between formation time, or these rhythmic patterns, and the sub-structure of individual lamellae can be uncovered.

1.2. Lamellar bone organization

Most research and textbooks defining or referring to lamellar bone assume and accept the Gebhardt model (Gebhardt, 1906), or some variation thereof (Ascenzi and Bonucci, 1967, 1968; Francillon-Vieillot et al., 1990; Frank, 1968; Frank et al., 1955; Giraud-Guille, 1988; Reid, 1986; Smith, 1960; Wagermaier et al., 2006; Weidenreich, 1930; Weiner et al., 1997) when interpreting histological and microstructural aspects of lamellae. The basic model proposes that collagen fibers in alternating layers, or lamellae, are oriented at different angles, similar to a plywood structure (see Fig. 2A). However, since its conception, the Gebhardt model has been contested, and alternative interpretations have been presented (Ardizzoni, 2001; Boyde and Hobdell, 1968; Engström and Engfeldt, 1953; Marotti, 1993; Marotti and Muglia, 1988; Marotti et al., 2013; Rouiller, 1956; Rouiller et al., 1952; Ruth, 1947; Vincent, 1957; Ziegler, 1908). These alternative hypotheses are based on the concept that lamellae are actually heterogeneous (e.g., alternating collagen-poor and collagen-rich layers; see Fig. 2B). And within the last few years, more complex interpretations have arisen using methods such as serial surface view (SSV) (Faingold et al., 2013; Reznikov et al., 2013, 2014) and synchrotron X-ray phase nanotomography (Varga et al., 2013).

2. Lamellar bone organization as observed using various techniques

2.1. Light microscopy

When studying bone in polarized light microscopy, birefringence is assumed to be an indicator of collagen fiber orientation, since collagen is an anisotropic material. When the fibers are perpendicular to the light source, they appear the brightest and when the fibers are parallel to the light source, they appear dark (Bromage et al., 2003). Intermediate levels of brightness indicate intermediate orientations. When rotating the stage, if the bone remains dark or isotropic, we assume the bone is of a woven nature (collagen fibers are unorganized, preventing the light from reaching the eye) or that the collagen fibers are oriented longitudinally.

Gebhardt (1906) primarily used light microscopy to come up with the first models of lamellar bone organization. The simplest model is the orthogonal plywood structure, in which the lamellae are oriented at approximately 90 degrees relative to one another (Fig. 2A, Gebhardt, 1906). The extinction pattern in polarized light microscopy is uniform (when all fibers are at a 45 degree angle to the section plane) or banded (when the fibers in some lamellae are perpendicular and in others parallel to the section plane).

These observations can be readily observed in modern (Fig. 1A–C) and fossil (Fig. 1D–F) bone. The distinctive banded or alternating pattern can be observed in secondary osteons of Fig. 1A and D. A more uniform pattern can be observed with either mostly dark (isotropic) lamellae (Fig. 1B and E) or bright lamellae as observed in the inner circumferential layer on Fig. 1C or the trabeculae on Fig. 1F. However, with respect to fossil bone, diagenetic and fossilization effects have to be taken into consideration. In many cases, diagenetic staining can make the entire section, both primary and secondary tissues, appear uniformly dark or isotropic looking. Thus, it cannot be ruled out that diagenetic effects have altered the bone or prevented accurate microscopic interpretations.

Boyde and Hobdell (1968) argued that studies, such as those of Gebhardt (1906) that rely on polarized light microscopy, should be questioned with respect to their reliability to determine collagen fiber orientation. It is well known that differential birefringence can also be attributed to section thickness (Bromage et al., 2003), and it is very important that the same thickness is maintained in thin-section preparation (Lamm, 2013). This can be problematic with the diagenetic staining previously mentioned. A section with diagenetic staining may need to be polished more to observe the histology, but this may mean the section is much thinner. In the same way, different densities have different optical properties (refractive index, in particular). It is feasible to assume if lamellae had different concentrations of collagen, mineral and ground substance (i.e., noncollagenous organic matrix), the optical properties could be different, erroneously

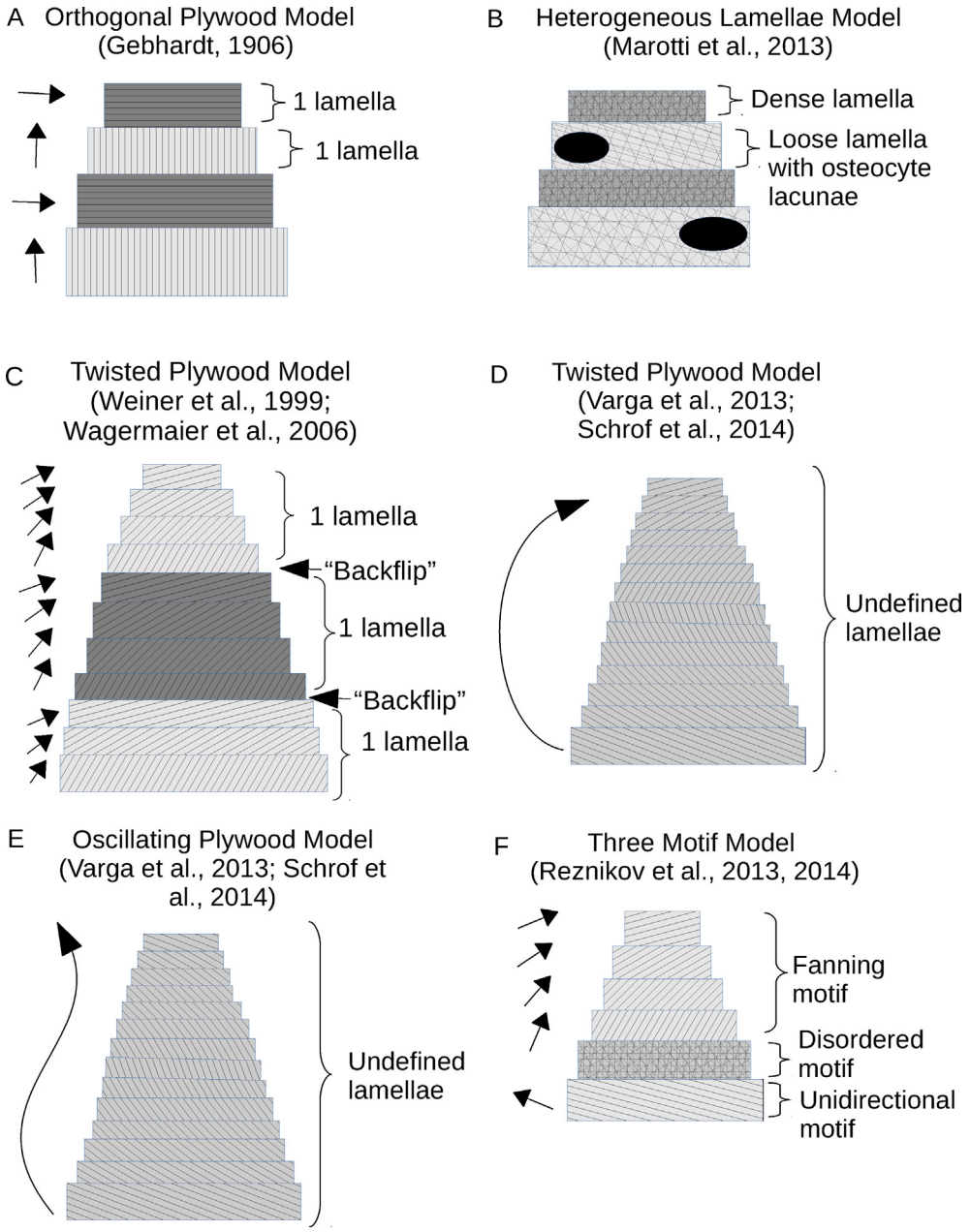

Fig. 2. Interpretation of proposed models using simplified osteonal diagrams. Arrows indicate the orientation of collagen fibers. **A.** The orthogonal plywood model, which assumes two distinct orientations of lamellae. Note that the longitudinally oriented fibers would appear dark in a polarizing microscope, and the transversally oriented fibers would appear bright in transverse view (based on Gebhardt, 1906). **B.** The heterogeneous lamellae model, which depicts the unorganized collagen-rich dense lamellae and the collagen-poor loose lamellae, which also contain osteocyte lacunae (black ovals) (based on Marotti et al., 2013). **C.** Twisted plywood model with distinctive sub-layers of lamellae that fan out. Several lamellae maintain a similar range of orientations except for the "backflip", which drastically changes angle. The number of sub-lamellae can be varied (based on Weiner et al., 1999 and Wagermaier et al., 2006). **D.** Twisted plywood model with a smooth transition from one orientation of collagen fibers to the next. Lamellae, however, are not distinguished (based on Varga et al., 2013 and Schrof et al., 2014). **E.** Oscillating plywood model, which is similar to D, but with a slight difference in collagen fiber orientations. **F.** Three motif model, which illustrates the concept of three sub-layers (fanning, disordered, and unidirectional) (based on Reznikov et al., 2013, 2014).

Fig. 2. Interprétation de modèles proposés utilisant des diagrammes d'ostéones simplifiés. Les flèches indiquent l'orientation des fibres de collagène. **A.** Modèle orthogonal de contreplaqué, qui suppose deux orientations différentes des lamellae. À noter que les fibres orientées longitudinalement apparaissent sombres au microscope polarisant, et que les fibres orientées transversalement apparaîtraient brillantes en vue transversale (d'après Gebhardt, 1906). **B.** Modèle à lamellae hétérogènes, qui dépeint les lamellae denses, inorganisées, riches en collagène et les lamellae lâches, pauvres en collagène, qui contiennent aussi des lacunes d'ostéocytes (ovales noirs) (d'après Marotti et al., 2013). **C.** Modèle de contreplaqué enroulé, avec des sous-feuillets de lamellae qui s'étalent en éventail. Certaines lamellae conservent la même fourchette d'orientations, avec quelques exceptions, celles-ci changeant alors drastiquement d'angle. Le nombre de sous-lamellae peut varier (d'après Weiner et al., 1999 et Wagermaier et al., 2006). **D.** Modèle de contreplaqué enroulé, avec transition floue d'une orientation des fibres de collagène à la suivante. Les lamellae ne sont cependant pas distinguées (d'après Varga et al., 2013 et Schrof et al., 2014). **E.** Modèle de contreplaqué oscillant, similaire à D, mais avec une légère différence dans les orientations des fibres de collagène. **F.** Troisième modèle de motif qui illustre le concept de trois sous-feuillets (en éventail, désordonné et unidirectionnel) (d'après Reznikov et al., 2013, 2014).

suggesting a difference in orientation of collagen fibers. The dark lamellae could easily be caused by more disorganized layers or a different density (compositional difference) than the bright layers. The reversal or cement line (well visible on Fig. 1B and E), which usually appears as the brightest aspect of secondary osteons in polarizing light, has been interpreted as having high amounts of sulfur but less calcium and phosphorus than surrounding bone using back-scattered electron imaging (BSE) and X-ray microprobe analysis (Schaffler et al., 1987). With similar techniques (BSE and energy dispersive X-ray spectroscopy), Skedros et al. (2005), on the other hand, found that cement lines were actually highly calcified. Although authors do not necessarily agree on its exact composition, most affirm that it is a collagen-poor layer (Currey, 2002). This is important, because it shows that it is not well-established how compositional differences affect polarized light observations, that is, it is not clear which proportions of collagen, mineral and ground substance produce the 'bright' and 'dark' birefringence patterns.

2.2. SEM and TEM

The orthogonal plywood structure in transmission electron microscopy (TEM) is characterized by a herringbone pattern in an oblique section, or, very rarely, longitudinal segments alternating with short perpendicular segments, when the section plane is parallel to one of the fiber orientations (Giraud-Guille, 1988). The Weiner research group (Liu et al., 2000; Weiner and Wagner, 1998; Weiner et al., 1997, 1999; Ziv et al., 1996) also supports the Gebhardt model and, using scanning electron microscopy (SEM) and TEM in their analyses, proposed additional structures beyond the orthogonal structure of the Gebhardt model. The twisted or rotated plywood structure (Fig. 2C) is slightly more complex and is found in the same bone specimens, sometimes even the same osteons, as the orthogonal structure (Giraud-Guille, 1988; Weiner and Wagner, 1998; Weiner et al., 1991). They suggest that each lamellar unit has five sub-layers. The sub-layers are not of equal thickness and the relative thickness of the sub-layers varies between taxa.

In the twisted plywood model, the fibers appear as arcs in an oblique section and as line segments of oscillating length in transverse section. Each lamellar unit is composed of several sub-layers. The fibers are offset by 30 degrees, with a 120-degree backflip to the original orientation every fifth layer, which is observed as a 60-degree change in fiber orientation (180–120 degrees). In polarized light microscopy, the extinction pattern is banded, with relatively narrow dark bands.

SEM and TEM have both been used to support the Gebhardt model, but also to uphold an alternative hypothesis, which suggests that lamellae are heterogeneous, alternating in composition, not orientation. Boyde and Hobdell (1968), for instance, concluded in SEM studies of mammalian lamellar bone that lamellae contained layers of higher density, amorphous material, which they termed "interlamellar" bone. These interlamellar layers were similar to the perilacunar bone (bone surrounding osteocyte lacunae) and peritubular dentine, being more resistant to resorption from osteoclasts than surrounding mineralized collagen fibers (Boyde and Hobdell, 1968). They further argued that "dark" lamellae, as interpreted in light microscopy, could be the interlamellar bone. However, they did not completely disagree with the idea of oriented collagen fiber bundles, noting that, within a lamella, collagen fiber bundles oriented themselves at different angles.

The most recent publications supporting heterogeneous lamellae are also based on SEM and TEM studies, examining transverse and longitudinal sections of human lamellar bone (Marotti, 1993, 1996; Marotti and Muglia, 1988; Marotti et al., 1994a,b, 2013). They propose that lamellae are heterogeneous, with alternating dense (collagen-rich) and loose (collagen-poor) lamellae (Fig. 2B), and that osteocytes only occur in the loose lamellae (e.g., Marotti, 1993; Marotti et al., 2013).

Marotti et al. argue that no single orientation of collagen fibers can be observed in a lamella. Comparing longitudinal and cross sections of a secondary osteon, they argue that one would expect to see a striped pattern in one view and a stippled pattern in the other view, if the fibers in a lamella were truly oriented in one direction. Their results, however, show no such change in pattern between longitudinal and cross sectional views (Figs. 1 and 3 in Marotti et al., 2013). However, one could argue that this pattern would only be visible if the orientation was orthogonal with collagen fibers oriented perfectly transversally and longitudinally.

Marotti et al. (2013) note that the loose lamellae, which is the only type that contains the osteocyte lacunae, can be easily split and appear to be similar to the osteocytic collagen surrounding osteocytes in woven bone. Their description of osteocytic collagen is similar to the interlamellar layers of Boyde and Hobdell (1968). The dense lamellae, on the other hand, cannot be split as easily, and TEM observations show collagen fiber bundles intertwined, with bundles of fibers splitting off in arrays of around 30 degree intervals (Fig. 6 in Marotti et al., 2013). In polarized light microscopy, they consider the dense lamellae to be the bright lamellae, and the loose lamellae to be the dark lamellae (Marotti, 1993).

Interestingly, Marotti et al. suggest, due to the interwoven nature of the observed collagen fibers, that lamellar bone should be considered a type of woven bone (Marotti et al., 2013). However, Ferretti et al. (2002) and Marotti (2010) describe woven and lamellar bone as derived from two different types of osteogenesis, static and dynamic, respectively. Thus, classifying lamellar bone as a type of woven bone would mean disregarding the separate origins of these bone tissues.

They also suggest that the changing orientations of lamellae observed by other researchers are actually the changing orientations of collagen fiber bundles *within* a lamella. They state the magnification of TEM analyses from the Giraud-Guille and Weiner studies (e.g., Giraud-Guille, 1988; Weiner et al., 1999) was too high, and the interpretation of a plywood structure (see below) was on the wrong scale (Marotti et al., 2013).

2.3. X-ray diffraction and nanotomography

Wagermaier et al. (2006) had similar findings to the Weiner research group using X-ray diffraction, where

they also found a twisted plywood pattern. According to Wagermaier et al. (2006), in humans, collagen fibrils change orientation by 5–25 degrees approximately every micrometer and change back to their original orientation (so a change of more than 30 degrees in the opposite direction, called a "backflip") with a periodicity of 5–7 μm, which corresponds to a single lamella (see Fig. 2C).

Using synchrotron X-ray phase nanotomography, Varga et al. (2013) propose two additional types of plywood structures that they observed in human bone in addition to twisted plywood (Fig. 2D): oscillating plywood (Fig. 2E) and irregular oscillating plywood. Varga et al. (2013) found no evidence for the orthogonal plywood structure. However, their definition of twisted plywood is not the same as that of Wagermaier et al. (2006) and Weiner et al. (1997). In their (Varga et al., 2013) definition of twisted plywood, there is no backflip and the fibril orientations change continuously and in a smooth manner in the same direction, and their data show that the fibrils change in a smooth manner across lamellae (Fig. 2D). Consequently, it is not clear how the smooth changes in fibril orientation relate to the dark and light bands as seen in polarized light microscopy. Using the same definition as Varga et al. (2013) (i.e., a continuous change of fibril orientations without backflip), Schrof et al. (2014) found twisted and oscillating plywood in the same osteons.

2.4. Serial surface view and SEM

Recently, a few authors have used serial surface view (SSV) in addition to SEM. Serial surface view allows very thin layers (nanometer scale) to be serially removed using an electron beam, exposing a new layer. Each layer is subsequently scanned by the electron microscope, providing a detailed three-dimensional image at high magnifications. Using this technique, these authors have described the presence of disorganized sub-layers (Faingold et al., 2013; Reznikov et al., 2013). It is unclear if these sub-layers could correlate with the interlamellar layers of Boyde and Hobdell (1968) or the transition zone from Weiner et al. (1997), for example. Based on Reznikov et al.'s (2013) finding in rats that these layers are less mineralized, Faingold et al. (2013) propose that the disorganized layers may simply be more compliant during polishing and have been misinterpreted as layers with fibrils oriented parallel to the cutting plane. Although this seems reasonable enough at first sight, Faingold et al. (2013) found in their sample that the disorganized layers occur at a distance of less than 1 μm, whereas lamellae have a thickness of approximately 5 μm. In addition, several of the constant fiber orientations in Faingold et al. (2013) have standard deviations (SD) of more than 25° and would certainly have been interpreted as being disorganized by Reznikov et al. (2013), who consider an SD (= dispersion) of 22.1° typical for disorganized sub-layers.

Here, we would like to tentatively propose an alternative explanation for the appearance of disorganized sub-layers. They might be an artifact caused, ironically, by the high precision with which the measurements are made. The "disorganized" layers almost always occur at intervals of rapid orientation change of the fibrils (Fig. 9 in Faingold et al., 2013; Figs. 2 and 3 in Reznikov et al., 2013; Figs. 6, 7 and 11 in Reznikov et al., 2014). Slices cut at these intervals are likely to also contain fibrils of the previous and the next orientation, increasing the standard deviation of the measured orientation. This effect would be even stronger if the cut is not exactly parallel to the growth front, which due to the curvature of the lamellae, the fact that bone is a living substance, and inaccuracies during preparations, it will almost never be. This explanation is also consistent with the loss of anisotropy observed in some lamellae by Schrof et al. (2014), which they suggest might be due to the difference in lamellar thickness and their own measurement resolution.

3. Discussion

Although it is clear that there is no consensus among researchers, a notable trend of twisted or oscillating patterns is observed using different approaches. This could be an extension of the nanoscale organization of collagen molecules. Experiments have shown (e.g., Giraud-Guille, 1987) that concentrated solutions of collagen fibrils will naturally, and without cellular aid, organize themselves into twisted or helicoidal patterns; this pattern is also commonly observed in other biological materials such as insect cuticles and plant cell walls (Bouligand, 1972). This suggests that the helicoidal structure does not need a functional reason for its development; rather it is the nature of the molecules to form this structure. This lends favor to the twisted plywood model, and variations thereof, but does not actually provide insight into how collagen fibers change orientation between lamellae nor does it address the influence of mineralization on the structure.

3.1. Variability in Lamellae

Before assessing phylogenetic relationships, the intra- and inter-variability of lamellae and their organization should be analyzed to see if there is a significant difference in different bones or bone tissues (e.g., primary versus secondary lamellar bone). As noted in the supplementary material, Appendix 1, lamella thickness has been measured by several researchers, with mixed findings. For human bones, Frost (1962) obtained measurements averaging 7 μm in width; Reid (1986) reached measurements of two to three micrometers. Yet, both authors had slightly larger values for secondary osteons or interstitial lamellae compared to circumferential lamellae. Other authors have lamellar subdivisions, with different size classes. Ardizzoni (2001), for instance, found dense lamellae to be around 1 μm in secondary osteons, but the loose lamellae varied, decreasing in size as they approached the Haversian canal (from around 7 to 2 μm). Weiner et al. (1997) stated that a 'lamellar unit', consisting of a thin, thick and transition zone, was around 3 μm for rats. Pazzaglia et al. (2012) had measured lamellar thickness to be around 9 μm, with great variation. Curiously, in their SEM images (Fig. 4 of Pazzaglia et al., 2012), they note interlamellar lines, which they interpret as being collagen-poor. These layers, however, look very similar to the collagen-rich dense lamellae from Fig. 2 of Marotti et al. (2013). These diverse

interpretations could be related to the method used to measure (polarized microscopy, electron microscopy, X-ray, etc.), the bone or bone tissue used, number of measurements made, as well as the species or even the specimen's age. Hence, a thorough study with a consistent approach is needed.

Even within different secondary osteons from one individual, there appear to be differences, which may partly explain the different results obtained by different researchers. Studies of Ascenzi and Bonucci (1967, 1968, 1972) and Ascenzi et al. (2003, 2008) showed that different secondary osteons had different mechanical constraints (i.e., some had greater tensile strength, others greater compressive strength). These osteon types were based on collagen fiber orientation determined from polarized light microscopy. They showed that secondary osteons with more longitudinally oriented collagen fibers (those that stayed dark in polarized light) had greater tensile strength (Ascenzi and Bonucci, 1967), and the modulus of elasticity was greatest in osteons with more transversally oriented fibers (those that appear brightest in polarized microscopy) (Ascenzi and Bonucci, 1968). They also used X-ray diffraction techniques to corroborate their interpretations of lamellae orientation (Ascenzi et al., 2003). However, these studies do not necessary invalidate the idea that lamellae can be compositionally heterogeneous. Hengsberger et al. (2002) point out that the different mechanical properties of different lamellae, as found by their studies using atomic force nanoindentation, could have been derived from either differently oriented collagen fibers or different mineral densities. In their model, both longitudinally oriented collagen fibers, as well as higher mineral densities, would result in more resistance, whereas transversely oriented collagen fibers and lower mineral densities would be weaker. Assuming mechanical properties result from collagen fiber orientation may, therefore, be too assumptive in some cases. Thus, a mechanical study of lamellae should consider possibilities other than collagen fiber orientation, when interpreting the results.

It should also be noted that when bone is deposited, the mineralized component is formed at a later stage, and that older lamellae are more mineralized than young lamellae (Engström and Engfeldt, 1953). Akkus et al. (2003) noted in their study on human femora that primary lamellar bone increased in mineral content over a period of around two decades, then tapers off once "fully mineralized". They also noted similarly high mineralization content in secondary osteons in older samples. Thus, it is also important to consider the ontogenetic stage of bone development when assessing bone.

3.2. Phylogenetic considerations

Unfortunately, most studies on lamellae focus on humans or rats, which make assumptions about whether a phylogenetic influence is present difficult to answer. Weiner et al. (1997) describe a slightly different pattern in rats compared to humans, with respect to the twisted plywood model, as, in their study, rats have thinner sublayers. They (Weiner et al., 1997) also note that the changes in orientation of the fibers appear to be more gradual in bovine bone.

Based on the analyses of Wagermaier et al. (2006) and Weiner et al. (1997), it may tentatively be hypothesized that the incremental orientation changes between adjacent fiber bundles decrease with increasing body size, or with increasing growth period. This hypothesis is also supported by the observation of Faingold et al. (2013) that the lamellar organization of collagen fibrils in the horse is morphologically similar to that in the human.

With the SSV method, Reznikov et al. (2014) found the situation to be slightly different in humans. Where the rats had organized and disorganized layers, in humans, the material was not organized in layers. Rather, the organized material was organized in rods, which were enveloped by disorganized material (see Fig. 2F). More work on different species needs to be done to know if there is indeed a phylogenetic signal.

3.3. Future work

The many interpretations of the organization of lamellae are difficult to associate to one another. A more thorough approach is needed and we propose that the following considerations should be made when examining the spatial organization of lamellae.

A possible way to resolve some issues with respect to the ambiguity or discrepancy of whether observations are made within or between lamellae is to compare the position of osteocyte lacunae. As Marotti et al. (2013) point out in their interpretations, the osteocyte lacunae occur in only one type of lamella (which they call loose lamellae). Most other studies do not mention osteocyte lacunae. It would be interesting to know how osteocyte lacunae are positioned in the twisted or the oscillating plywood model; this would provide a common point of comparison. However, it should be noted that osteocytes are not necessary in the formation of lamellar bone, as anosteocytic fish have been observed to produce bone lamellae as well (Atkins et al., 2014). But for amniotes, at least, osteocyte lacunae could very well be a decent way to find correlations between methods.

The ratio of collagen to mineral and ground substance appears to be an important point to consider, as variability in these components exists and can influence observations made in microscopic analyses. It would be important that the analysis be at the appropriate scale so the composition between lamellae is compared rather than within a single lamella, for instance. Different lamellar bone types (e.g., secondary osteons, circumferential layers, etc.) should be assessed to determine if significant differences occur. It would be interesting to know how the organization of lamellae is affected by mineral/collagen proportions. It has been shown that collagen with the embedded apatite is mechanically improved with increased flexibility and load bearing capabilities compared to collagen alone (Landis and Silver, 2002; Landis et al., 2006). However, it is not clear if the increasing mineralization causes any structural changes at the histological level.

The three-dimensional organization is also an important consideration. With only a two dimensional plane of

observation, it is very difficult to impossible to fully assess the true nature of collagen fiber orientations. Methods like SEM and TEM are useful if transverse and longitudinal sections are used. They do not seem, however, to suffice in resolving the problem of the organization of lamellae because interpretations of collagen content of similar images (e.g., Marotti et al., 2013; Pazzaglia et al., 2012) can be different. An additional approach, like X-ray diffraction, is necessary to provide support. The newer methods like SSV can obtain very fine detail of changes in three dimensions. But to be beneficial, from a histological perspective of lamellar bone, this method needs to be applicable on the micrometer scale. As it is used now, the detail is so high that no clear correlation to lamellae organization studied with other microscopic methods can be stated.

It would be useful, especially when studying fossil bone, if the orientation and composition of lamellae could be correlated to the observations of lamellae in polarized microscopy (bright, dark layers). However, the organization and composition of lamellae seem to be quite varied, and an accurate interpretation in polarizing microscopy may not be feasible. Finally, a multi-species analysis using the same element and lamellar tissue type would be interesting to see if any variations are indeed related to phylogeny.

4. Conclusion

It is clear from the present review that bone lamellae are complex features in bone histology. The large number of studies with the huge variability in interpretation of lamellae leads to more confusion than answers. Understanding the spatial organization and composition of lamellae is important because we interpret lamellar bone based on some underlying assumptions, which if not fully correct or understood can lead to misinterpretation of data.

In general, there needs be a consistent, multi-methodological approach across a variety of taxa to obtain a comprehensive understanding of what the different interpretations mean. With a better understanding of the organization of lamellae, improved interpretations can be made with respect to the mechanical, physiological, phylogenetic and ecological aspects of bone development.

Authors' contributions

JM drafted much of the manuscript and compiled the figures. AHvH conceived the idea for the manuscript and wrote sections thereof. Both authors read and approved the final version of the manuscript.

Acknowledgements

This work was supported in part by a post-doctoral research grant from the Humboldt Foundation to AHvH. The authors would like to thank P.M. Sander and two anonymous reviewers for valuable comments on an earlier version of the manuscript.

Appendix 1. Supplementary material

Supplementary material associated with this article can be found in the online version available at http://dx.doi.org/10.1016/j.crpv.2015.04.007.

References

Akkus, O., Polyakova-Akkus, A., Adar, F., Schaffler, M.B., 2003. Aging of microstructural compartments in human compact bone. J. Bone Miner. Res. 18, 1012–1019.
Ardizzoni, A., 2001. Osteocyte lacunar size–lamellar thickness relationships in human secondary osteons. Bone 28, 215–219.
Ascenzi, A., Bonucci, E., 1967. The tensile properties of single osteons. Anat. Rec. 158, 375–386.
Ascenzi, A., Bonucci, E., 1968. The compressive properties of single osteons. Anat. Rec. 161, 377–391.
Ascenzi, A., Bonucci, E., 1972. The shearing properties of single osteons. Anat. Rec. 172, 499–510.
Ascenzi, M.G., Ascenzi, A., Benvenuti, A., Burghammer, M., Panzavolta, S., Bigi, A., 2003. Structural differences between "dark" and "bright" isolated human osteonic lamellae. J. Struct. Biol. 141, 22–33.
Ascenzi, M.G., Gill, J., Lomovtsev, A., 2008. Orientation of collagen at the osteocyte lacunae in human secondary osteons. J. Biomech. 41, 3426–3435.
Atkins, A., Dean, M.N., Habegger, M.L., Motta, P.J., Ofer, L., Repp, F., Shipov, A., Weiner, S., Currey, J.D., Shahar, R., 2014. Remodeling in bone without osteocytes: billfish challenge bone structure–function paradigms. Proc. Natl. Acad. Sci. U S A 111, 16047–16052.
Bouligand, Y., 1972. Twisted fibrous arrangements in biological materials and cholesteric mesophases. Tissue Cell 4, 189–217.
Boyde, A., Hobdell, M.H., 1968. Scanning electron microscopy of lamellar bone. Z. Zellforsch. Mik. Ana. 93, 213–231.
Bromage, T.G., Goldman, H.M., McFarlin, S.C., Warshaw, J., Boyde, A., Riggs, C.M., 2003. Circularly polarized light standards for investigations of collagen fiber orientation in bone. Anat. Rec. Part B 274, 157–168.
Bromage, T.G., Juwayeyi, Y.M., Smolyar, I., Hu, B., Gomez, S., Chisi, J., 2011. Enamel-calibrated lamellar bone reveals long period growth rate variability in humans. Cells Tissues Organs 194, 124–130.
Bromage, T.G., Lacruz, R.S., Hogg, R., Goldman, H.M., McFarlin, S.C., Warshaw, J., Dirks, W., Perez-Ochoa, A., Smolyar, I., Enlow, D.H., Boyde, A., 2009. Lamellar bone is an incremental tissue reconciling enamel rhythms, body size, and organismal life history. Calcif. Tissue Int. 84, 388–404.
Currey, J.D., 2002. Bones: Structure and Mechanics. Princeton University Press, Princeton, N.J., pp. 12–13.
Dumont, M., Kostka, A., Sander, P.M., Borbely, A., Kaysser-Pyzalla, A., 2011. Size and size distribution of apatite crystals in sauropod fossil bones. Palaeogeogr. Palaeoclimatol. Palaeoecol. 310, 108–116.
Engström, A., Engfeldt, B., 1953. Lamellar structure of osteons demonstrated by microradiography. Experientia 9, 19.
Faingold, A., Cohen, S.R., Reznikov, N., Wagner, H.D., 2013. Osteonal lamellae elementary units: lamellar microstructure, curvature and mechanical properties. Acta Biomater. 9, 5956–5962.
Ferretti, M., Palumbo, C., Contri, M., Marotti, G., 2002. Static and dynamic osteogenesis: two different types of bone formation. Anat. Embryol. 206, 21–29.
Francillon-Vieillot, H., de Buffrénil, V., Castanet, J., Géraudie, J., Meunier, F.J., Sire, J.Y., Zylberberg, L., de Ricqlès, A., 1990. Microstructure and mineralization of vertebrate skeletal tissues. In: Carter, J. (Ed.), Skeletal Biomineralization: Patterns, Processes and Evolutionary Trends. Van Nostrand Reinhold, New York, pp. 471–530.
Frank, R., 1968. Ultrastructural relationship between the odontoblast, its process and the nerve fibre. In: Symons, N.B.B. (Ed.), Dentine and Pulp. Their Structure and Reactions. Churchill Livingstone, Edinburgh, pp. 115–145.
Frank, R., Frank, P., Klein, M., Fontaine, R., 1955. L'os compact humain normal au microscope électronique. Arch. Anat. Microsc. Morphol. Exp. 44, 191–206.
Frost, H.M., 1962. Interlamellar thickness in human bone. Clin. Orthop. Relat. R. 24, 198–205.
Gebhardt, W., 1906. Über funktionell wichtige Anordnungsweisen der feineren und größeren Bauelemente des Wirbeltierknochens. II. Spezieller Teil. Der Bau der Haversschen Lamellensysteme und seine funktionelle Bedeutung. Arch. Entwickl. Mech. Org. 20, 187–322.
Giraud-Guille, M.-M., 1987. Cholesteric twist of collagen in vivo and in vitro. Mol. Cryst. Liq. Cryst. 153, 15–30.

Giraud-Guille, M.M., 1988. Twisted plywood architecture of collagen fibrils in human compact bone osteons. Calcif. Tissue Int. 42, 167–180.
Hengsberger, S., Kulik, A., Zysset, P., 2002. Nanoindentation discriminates the elastic properties of individual human bone lamellae under dry and physiological conditions. Bone 30, 178–184.
Lamm, E.T., 2013. Preparation and sectioning of specimens. In: Padian, K., Lamm, E.-T. (Eds.), Bone Histology of Fossil Tetrapods Advancing Methods, Analysis, and Interpretation. University of California Press, Berkeley, pp. 55–160.
Landis, W.J., Silver, F.H., 2002. The structure and function of normally mineralizing avian tendons. Comp. Biochem. Phys. A 133, 1135–1157.
Landis, W.J., Silver, F.H., Freeman, J.W., 2006. Collagen as a scaffold for biomimetic mineralization of vertebrate tissues. J. Mater. Chem. 16, 1495–1503.
Landis, W.J., Song, M.J., Leith, A., McEwen, L., McEwen, B.F., 1993. Mineral and organic matrix interaction in normally calcifying tendon visualized in three dimensions by high-voltage electron microscopic tomography and graphic image reconstruction. J. Struct. Biol. 110, 39–54.
Landis, W.J., Hodgens, K.J., Arena, J., Song, M.J., McEwen, B.F., 1996. Structural relations between collagen and mineral in bone as determined by high-voltage electron microscopic tomography. Micros. Res. Techniq. 33, 192–202.
Liu, D., Wagner, H., Weiner, S., 2000. Bending and fracture of compact circumferential and osteonal lamellar bone of the baboon tibia. J. Mater. Sci.-Mater. M. 11, 49–60.
Marotti, G., 1993. A new theory of bone lamellation. Calcif. Tissue Int. 53, 47–56.
Marotti, G., 1996. The structure of bone tissues and the cellular control of their deposition. Ital. J. Anat. Embryol. 101, 25–79.
Marotti, G., 2010. Static and dynamic osteogenesis. Ital. J. Anat. Embryol. 115, 123–126.
Marotti, G., Muglia, M.A., 1988. A scanning electron microscope study of human bony lamellae. Proposal for a new model of collagen lamellar organization. Ital. J. Anat. Embryol. 93, 163–175.
Marotti, G., Muglia, M.A., Palumbo, C., Zaffe, D., 1994a. The microscopic determinants of bone mechanical properties. Ital. J. Miner. Elect. M. 8, 167–175.
Marotti, G., Muglia, M.A., Palumbo, C., 1994b. Structure and function of lamellar bone. Clin. Rheumatol. 13 (Suppl. 1), 63–68.
Marotti, G., Ferretti, M., Palumbo, C., Benincasa, M., 1999. Static and dynamic bone formation and the mechanism of collagen fiber orientation. Bone 25, 156.
Marotti, G., Ferretti, M., Palumbo, C., 2013. The problem of bone lamellation: an attempt to explain different proposed models. J. Morphol. 274, 543–550.
Pazzaglia, U.E., Congiu, T., Marchese, M., Spagnuolo, F., Quacci, D., 2012. Morphometry and patterns of lamellar bone in human Haversian systems. Anat. Rec. 295, 1421–1429.
Prondvai, E., Stein, K.H., de Ricqlès, A., Cubo, J., 2014. Development-based revision of bone tissue classification: the importance of semantics for science. Biol. J. Linnean Soc. 112, 799–816.
Reid, S.A., 1986. A study of lamellar organisation in juvenile and adult human bone. Anat. Embryol. 174, 329–338.
Reznikov, N., Almany-Magal, R., Shahar, R., Weiner, S., 2013. Three-dimensional imaging of collagen fibril organization in rat circumferential lamellar bone using a dual beam electron microscope reveals ordered and disordered sub-lamellar structures. Bone 52, 676–683.
Reznikov, N., Shahar, R., Weiner, S., 2014. Three-dimensional structure of human lamellar bone: the presence of two different materials and new insights into the hierarchical organization. Bone 59, 93–104.
Rouiller, C., 1956. Collagen fibres in connective tissue. In: Bourne, G.H. (Ed.), The Biochemistry and Physiology of Bone, first ed. Academic Press, New York, pp. 104–143.
Rouiller, C., Huber, L., Kellenberger, E., Rutishauser, E., 1952. La structure lamellaire de l'ostéone. Acta Anat. 14, 9–22.
Ruth, E.B., 1947. Bone studies. I. Fibrillar structure of adult human bone. Am. J. Anat. 80, 35–53.
Schaffler, M.B., Burr, D.B., Frederickson, R.G., 1987. Morphology of the osteonal cement line in human bone. Anat. Rec. 217, 223–228.
Schrof, S., Varga, P., Galvis, L., Raum, K., Masic, A., 2014. 3D Raman mapping of the collagen fibril orientation in human osteonal lamellae. J. Struct. Biol. 187, 266–275.
Shinoda, H., Okada, M., 1988. Diurnal rhythms in the formation of lamellar bone in young growing animals. P. Jpn. Acad. B-Phys. 64, 307–310.
Skedros, J.G., Holmes, J.L., Vajda, E.G., Bloebaum, R.D., 2005. Cement lines of secondary osteons in human bone are not mineral-deficient: new data in a historical perspective. Anat. Rec. A 286, 781–803.
Skedros, J.G., Mendenhall, S.D., Kiser, C.J., Winet, H., 2009. Interpreting cortical bone adaptation and load history by quantifying osteon morphotypes in circularly polarized light images. Bone 44, 392–403.
Smith, J., 1960. The arrangement of collagen fibres in human secondary osteones. J. Bone Joint Surg. Br. 42, 588–605.
Varga, P., Pacureanu, A., Langer, M., Suhonen, H., Hesse, B., Grimal, Q., Cloetens, P., Raum, K., Peyrin, F., 2013. Investigation of the three-dimensional orientation of mineralized collagen fibrils in human lamellar bone using synchrotron X-ray phase nanotomography. Acta Biomater. 9, 8118–8127.
Vincent, J., 1957. Correlation entre la microradiographie et l'image en lumière polarisée de l'os secondaire. Exp. Cell Res. 12, 422–424.
Wagermaier, W., Gupta, H., Gourrier, A., Burghammer, M., Roschger, P., Fratzl, P., 2006. Spiral twisting of fiber orientation inside bone lamellae. Biointerphases 1, 1–5.
Weidenreich, F., 1930. Das Knochengewebe. Handb. Mikrosk. Anat. Menschen 2, 391–520.
Weiner, S., Wagner, H.D., 1998. The material bone: structure–mechanical function relations. Annu. Rev. Mater. Sci. 28, 271–298.
Weiner, S., Arad, T., Sabanay, I., Traub, W., 1997. Rotated plywood structure of primary lamellar bone in the rat: orientations of the collagen fibril arrays. Bone 20, 509–514.
Weiner, S., Arad, T., Traub, W., 1991. Crystal organization in rat lamellar bone. FEBS Lett. 285, 49–54.
Weiner, S., Traub, W., Wagner, H.D., 1999. Lamellar bone: structure–function relations. J. Struct. Biol. 126, 241–255.
Ziegler, D., 1908. Studien über die feinere Struktur des Röhrenknochens und dessen Polarisation. Dtsch. Z. Chir. 85, 248–262.
Ziv, V., Wagner, H.D., Weiner, S., 1996. Microstructure-microhardness relations in parallel-fibered and lamellar bone. Bone 18, 417–428.