Annu. Rev. Mater. Sci. 1998. 28:271–98

THE MATERIAL BONE: Structure-Mechanical Function Relations

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KEY WORDS: biomineralization, biomechanics, lamellar bone, mineralized collagen, bone mineral

ABSTRACT

The term bone refers to a family of materials, all of which are built up of mineralized collagen fibrils. They have highly complex structures, described in terms of up to 7 hierarchical levels of organization. These materials have evolved to fulfill a variety of mechanical functions, for which the structures are presumably fine-tuned. Matching structure to function is a challenge. Here we review the structure-mechanical relations at each of the hierarchical levels of organization, highlighting wherever possible both underlying strategies and gaps in our knowledge. The insights gained from the study of these fascinating materials are not only important biologically, but may well provide novel ideas that can be applied to the design of synthetic materials.

INTRODUCTION

Bone refers to a family of materials each with a somewhat different structural motif, but all having in common the basic building block, the mineralized collagen fibril. This family of materials also contains other members, which for historical reasons, have different names. The better known examples are dentin, the material that constitutes the inner layers of teeth; cementum, the thin layer that binds the roots of teeth to the jaw; and mineralized tendons. Here we review the structure-mechanical function relations of these materials, highlighting wherever possible the basic underlying themes. We emphasize the mechanical functions, although some of these materials also fulfill other

0084-6600/98/0801-0271$08.00

functions. Many bones, for example, are also the major reservoirs of calcium and phosphate necessary for a wide variety of metabolic functions.

The diversity of structures within this family reflects the fine-tuning or adaptation of the structure to its function. This diversity thus provides us with an invaluable research handle that when used in a comparative way can provide key insights into the relations between structure and function. This approach, powerful as it may be, needs to be used with caution. Biological structures are not necessarily perfectly adapted to function, although the millions of years devoted to the fine-tuning of this relation has produced some remarkable solutions to tough structural-mechanical problems. An exciting prospect for the study of such biological materials is to identify these "novel" solutions and possibly apply some to solve problems in synthetic materials. It is also important not to assume that each member of the family fulfills one or only several functions. This may well be the case, but the contrary may also occur—the overall design strategy is to achieve an all-purpose material (the concretes of the biological world) that will function adequately, if not optimally, under many conditions. Finally, these biological materials are no different from many synthetic materials that are required, for example, to function well under compression, but also on occasion need to withstand serious challenges from impacting and/or bending stresses. Thus the term function in our title is fraught with difficulty; unfortunately, so is the term structure.

The basic building block of the bone family of materials is the mineralized collagen fibril. It is composed of the fibrous protein collagen in a structural form that is also present in skin, tendon, and a variety of other soft tissues. The collagen constitutes the main component of a three-dimensional matrix into which, and in some cases onto which, the mineral forms. The mineral in this family of materials is dahllite, also known as carbonated apatite (Ca_5 (PO_4, CO_3)$_3$ (OH)) (1). The third major component is water. The major components are intimately associated into an ordered structure, the mineralized collagen fibril. Their proportions, however, can vary considerably between family members. The manner in which the building blocks are organized into higher order structures can also vary, and in fact, this is the basis for differentiating between the members of the bone family of biological materials. To further complicate matters, some of these materials are composed of two different organizational motifs that are juxtaposed, and in turn the whole structure may be folded into even larger suprastructures (reviewed in 2, 3). Thus there is no meaning to the term the structure of bone, but rather the structures of these materials have to be understood both in terms of the differences between family members, and most importantly according to hierarchical levels of organization. Figure 1 illustrates the 7 levels of hierarchical organization of the family of mineralized collagen based materials as we envisage it.

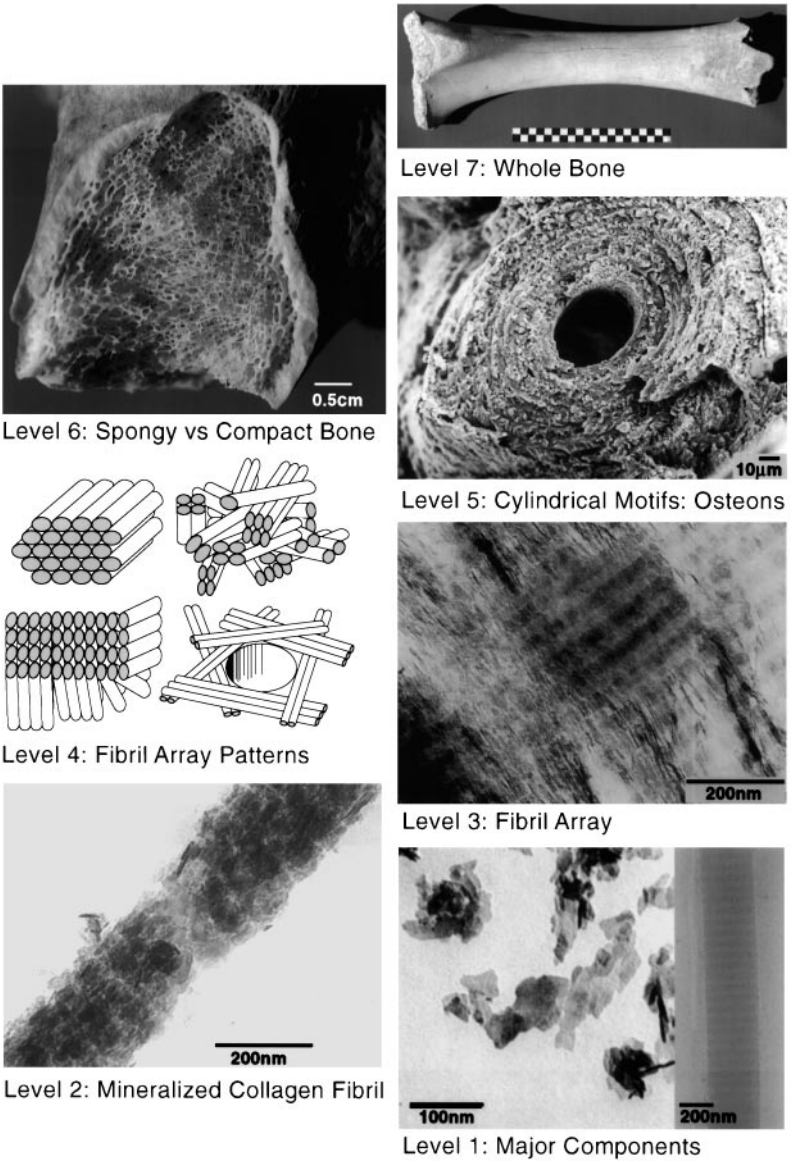


Figure 1 The 7 hierarchical levels of organization of the bone family of materials. Level 1: Isolated crystals from human bone (*left side*) and part of an unmineralized and unstained collagen fibril from turkey tendon observed in vitreous ice in the TEM (*right side*). Level 2: TEM micrograph of a mineralized collagen fibril from turkey tendon. Level 3: TEM micrograph of a thin section of mineralized turkey tendon. Level 4: Four fibril array patterns of organization found in the bone family of materials. Level 5: SEM micrograph of a single osteon from human bone. Level 6: Light micrograph of a fractured section through a fossilized (about 5500 years old) human femur. Level 7: Whole bovine bone (scale: 10 cm).

Many measurements of the bulk elastic, plastic, and ultimate properties of certain members of the bone family of materials have been made. Figure 2 is a plot of the moduli and strengths of several bone types measured parallel and perpendicular to the bone long axes. The range of these values reflects many different variables: the diversity in structures, the orientation of the specimens, the variations in mineralization extent and porosity, the precise locations of the specimens, and not insignificantly, the variations in the procedures used for making the measurements. It is clearly important to sort out the contributions of all these variables to the bulk mechanical properties—the focus of this review.

Understanding structure-function relations in these materials is therefore a challenge. We are easily able to measure bulk mechanical properties of some family members and in some special cases obtain information on certain of the intermediate hierarchical levels, but how can these be related to the structures themselves? An in-depth understanding of this subject ideally requires sorting out the bulk mechanical behavior in terms of the contributions of the sub-structures at each hierarchical level. Unfortunately, even if we were able to comprehensively describe these materials in this way, this still would not constitute an adequate analysis, as many of these materials actually change with time. This in turn affects the mechanical properties. Some of these changes are in part thermodynamically driven, such as the increase in the sizes of the crystals, and some are also biologically mediated, such as the determination of the average proportions of collagen, mineral, and water in a given material. Furthermore, specialized bone cells actively remove older bone and replace it with younger bone, which may even have a slightly different structure such that it is presumably optimized to function in the prevailing stress field at the time of its formation (4). In this sense, these materials are really "smart."

The investigations to date of the structure-function relations of the bone family of materials are for the most part unable to provide anywhere near a complete picture of this subject. Despite this inadequacy, we have chosen to organize this review according to the hierarchical levels of organization. We discuss 5 of the 7 hierarchical levels, which range in scale from nanometers to millimeters, in terms of their structures and mechanical properties. These relations in the more macroscopic structures have been reviewed extensively elsewhere (2, 5).

LEVEL 1: THE MAJOR COMPONENTS

Dahllite (carbonated apatite) crystals, type I collagen fibrils, and water are the major components of the bone family of materials. Whereas dahllite is the only mineral type in mature bone, type I collagen is by no means the only protein present. There are two hundred or more so-called non-collagenous proteins (NCPs) (6), but together they generally comprise less than 10% of the total

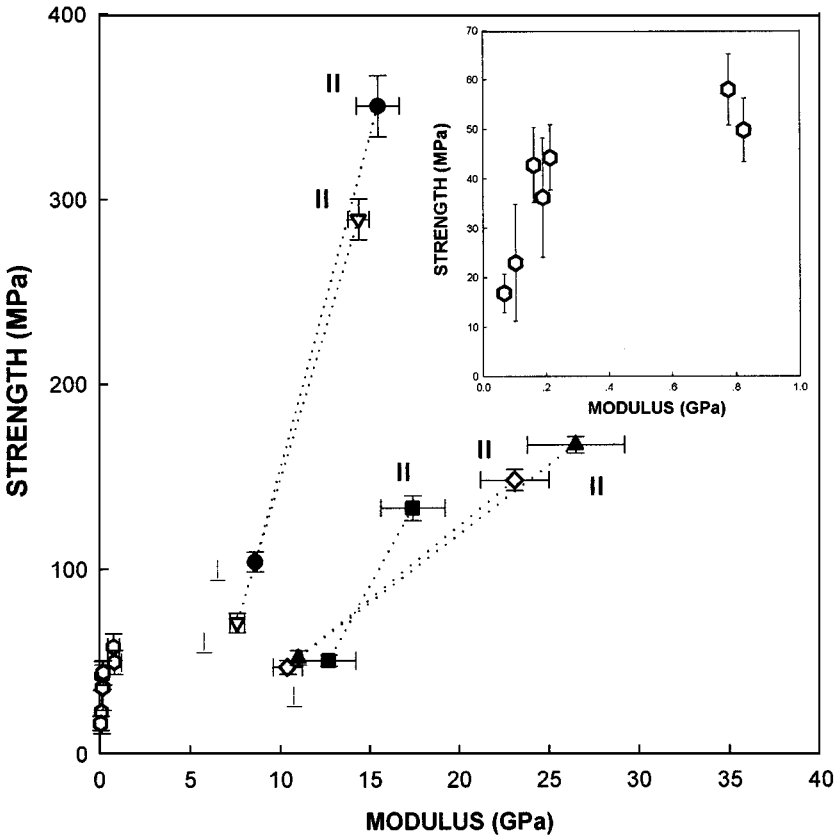


Figure 2 Strength versus modulus of various bone types under different loading modes, in directions parallel and perpendicular to the bone long axes. The *circles* and *inverted triangles* are for baboon tibia planar (circumferential) lamellar bone and osteonal bone, respectively, measured in bending (data from 72); the *squares*, *diamonds*, and *triangles* are for human femur osteonal bone, bovine femur fibrolamellar bone, and bovine femur parallel-fibered bone, respectively, measured in tension (data from 49); *hexagons* are for mineralized turkey tendon (gastrocnemius) measured in tension (data from 47). The insert shows the same values but has an expanded scale. Note that in the latter, proportionality between strength and modulus arises from a progressive change in ash weight (mineral content), rather than an increase in structural isotropy.

protein content. Although some of these proteins may contribute to the mechanical functions, we have no direct evidence for this. Nor do we know whether lipids, which may also be present in fairly large amounts in some bones (7), have any mechanical function.

Dahllite Crystals

The crystals of bone are plate-shaped, with average lengths and widths of 50 × 25 nm (Figure 1; Level 1) (8–10). The crystals are very thin, with thicknesses appearing (in transmission electron microscopy; TEM) to be remarkably uniform. Small angle X-ray scattering (SAXS) is a more reliable means of measuring their smallest dimensions, and the results vary from just 1.5 nm for mineralized tendon up to about 4.0 nm for some mature bone types (11, 12). These crystals are therefore extremely small—in fact they are probably the smallest biologically formed crystals known (10). Unfortunately, we still know very little about their surface atomic structures. An atomic force microscopy (AFM) study of synthetic apatite shows that the surface is highly ordered and matches the bulk structure (13). Nor do we know for sure why these crystals are plate-shaped, even though dahllite has hexagonal crystal symmetry. One proposed explanation is that they grow via an octacalcium phosphate transition phase (14). Octacalcium phosphate crystals are plate-shaped and have a structure very similar to apatite, except for the presence of a hydrated layer.

Reliable measurements of the mechanical properties of biological dahllite are important for understanding bone properties. Measurements on single crystals have not been made to date, presumably because of their small size. Powders under pressure or compact polycrystalline materials have been measured usually by sonic velocity, and the results are compared with measurements made on large geological single crystals (15). Young's modulus of synthetic powdered carbonated apatite is 109 GPa, whereas it is 114 GPa for a large single crystal of hydroxyapatite. We are not aware of a similar measurement made on crystals extracted from bone.

The Type I Collagen Fibril

Type I collagen is characterized by its fibrous nature, with the fibrils in bone generally about 80–100 nm in diameter, when measured in the TEM (Figure 1; Level 1). Their lengths are in effect unknown, because they tend to merge with neighboring fibrils (16, 17). Each fibril is made up of three polypeptide chains about 1000 amino acids long. These are wound together in a triple helix. A triple-helical molecule is thus cylindrically shaped, with an average diameter of about 1.5 nm, and lengths of 300 nm (reviewed in 10, 18, 19). The manner in which they pack to form a fibril is most unusual (19). In an imaginary slice

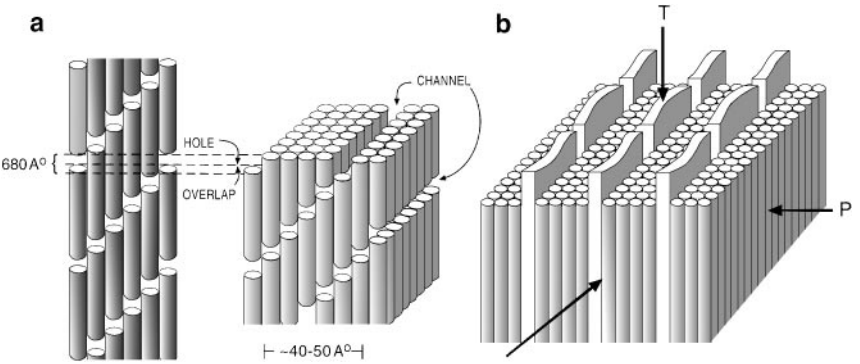


Figure 3 Schematic illustrations (not drawn to scale) of the structure of collagen in terms of the organization of the triple-helical molecules. (*a, left side*) Slice about 1.5 nm thick through a fibril, showing the staggered array pattern first proposed by Hodge & Petruska (20); (*a, right side*) three-dimensional model showing the alignment of the holes to form a channel (21). (*b*) Model showing the proposed location of the platy crystals in the channels (3). The arrows show the three principal symmetry directions. In parallel-fibered bone, T is in the plane transverse to the bone long axis and P is in the plane parallel to the natural outer surface of the bone (48).

through a fibril, 1.5 nm thick (i.e. the thickness of one triple-helical molecule), the triple-helical molecules are all parallel, but their ends are separated by holes of about 35 nm (Figure 3*a*). Furthermore, the neighboring triple-helical molecules are offset or staggered by 68 nm (20). In a second imaginary slice orthogonal to the first, no offset is present and the triple-helical molecules, as well as the holes, are aligned (Figure 3*a*) (21). One key point is that the internal structure of the fibril does not have radial symmetry but is different in all three orthogonal directions.

Fibrils almost never exist alone in biological tissues, including the members of the bone family. They associate with each other to form arrays of aligned fibrils that make up a larger structure called the fiber. It is thus very difficult to measure the mechanical properties of an individual fibril. Furthermore, the packing of fibrils in a fiber may vary from one tissue to another and influence the mechanical properties.

Water

Water is the third major component of the bone family of materials. The importance of water for the mechanical functioning of bone cannot be underestimated. Mechanical measurements of dry bone are different from those of wet bone (22). The water is located within the fibril, in the gaps, and between triple-helical molecules. It is also present between fibrils and between fibers (23).

The Relative Proportions of the Three Major Components

The relative proportions of mineral, collagen, and water vary in a systematic manner between bone types (24). The volume relationships in particular show that the collagen content remains basically the same, whereas the increase in mineral content occurs at the expense of the water content. The average proportions are fixed for any given bone by a complex and only partially understood biological process. The mineral component, in addition, increases with increasing time, as the crystals continue to grow.

Currey (22, 25) has performed a series of studies of the relationships between mineral content and mechanical properties. The approach has been to test a very large sample of bones, even though they have different porosities and structures. The results still clearly indicate that the Young's modulus of compact bone in tension shows a strong positive relationship with mineral content (Figure 4) (25). The ultimate strain and the work under the stress-strain curve decrease

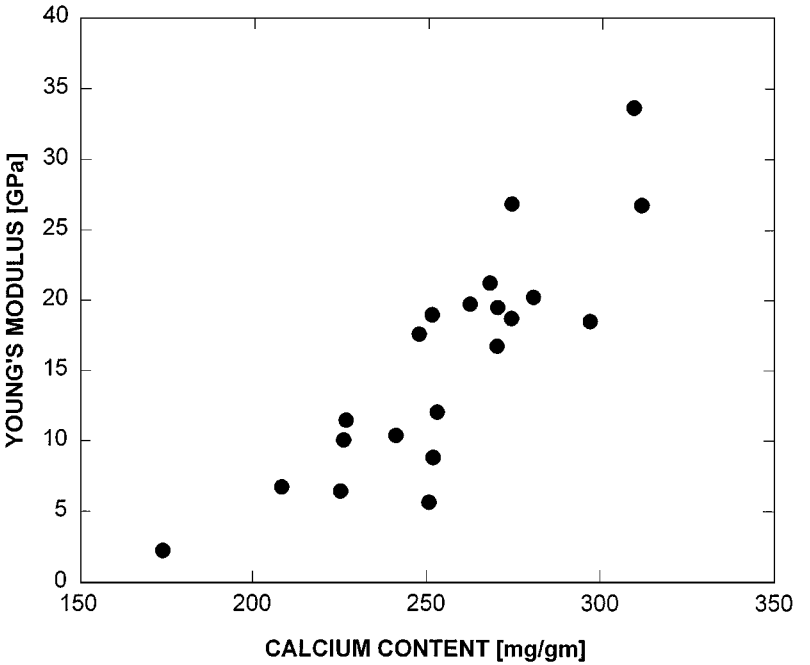


Figure 4 Plot of the calcium content versus Young's modulus for a large variey of bones (data from 25).

with increasing mineral content (22). Interestingly, some of the most unusual bones at both ends of the mineral content spectrum have somewhat anomalous mechanical properties. A fascinating example is the rostrum of the whale, Mesoplodon, which has the highest mineral content (87 wt%) of all the members of the bone family (26). The organization of the crystals is similar to other bones, yet the collagen content and characteristics are quite different (27). Mechanical tests show that it is the stiffest (Young's modulus 49.6 GPa) and hardest bone tested (28). This raises the interesting possibility that collagen was present during the early stages of formation of the rostrum, but was subsequently removed, allowing crystals to continue to grow. Do similar processes, albeit not so severe, occur during the normal ongoing mineralization process?

Mechanical Implications

The three major components of bone have completely different properties. In this sense the material is clearly a composite. The host organic framework has a fibrous structure at the level of individual triple-helical molecules, at the level of fibrils (Level 2), and at the level of assemblages of fibrils (Level 3) that form fibers. In contrast, within a fibril, the structure is crystalline with orthotropic symmetry (Figure 3). This implies the presence of three planes of symmetry orthogonal to each other (Figure 5). The guests in this composite are the plate-shaped crystals. In this respect, bone should be viewed as a platelet-reinforced composite (29). To complicate matters further, the atomic lattice symmetry of the crystals is hexagonal, despite their platy shape. It would be of interest to know if the mechanical properties of individual crystals are really similar to those measured from compressed powders. With the advent of new stiff tips for AFM, measurements of this type may be possible.

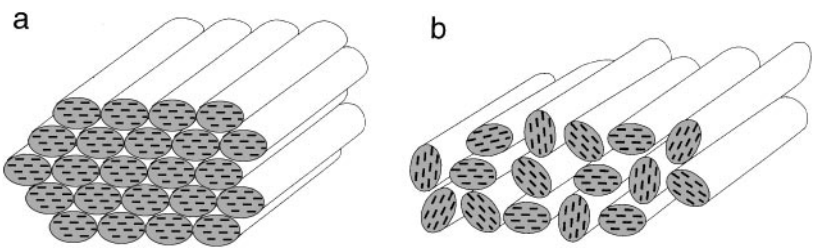


Figure 5 Schematic illustration (not drawn to scale) showing (*a*) an arrangement of mineralized collagen fibrils aligned both with respect to crystal layers and fibril axes. This structure has orthotropic symmetry. (*b*) Arrangement of mineralized collagen fibrils with only the fibril axes aligned. This structure has transversal isotropy.

LEVEL 2: THE MINERALIZED COLLAGEN FIBRIL BUILDING BLOCK

Robinson & Watson's (30) pioneering TEM study reported that the 68-nm banding pattern of stained unmineralized collagen fibrils (Figure 1; Level 1) can also be seen in unstained but mineralized collagen fibrils (Figure 1; Level 2). This implies that the stain and the mineral are at the same location within the fibril, i.e. mainly within the gap regions. TEM observations of unstained individual mineralized collagen fibrils, both after freeze-drying and hydration in vitreous ice (31, 32), showed that the platy crystals are organized in layers that traverse across the fibril (Figure 1; Level 2). Furthermore, electron diffraction patterns show that the crystallographic *c*-axes are well aligned with the fibril long axis; a relation first observed by Schmidt (33) using polarized light microscopy. This layered organizational motif has been confirmed using three-dimensional electron tomography (34), as well as by AFM (35). The layered arrangement of crystals can be easily accommodated inside the collagen fibril (Figure 3*b*). Examinations of individual mineralized fibrils from turkey tendon at different tilt angles, show that they are ellipsoid in cross-section, with the crystal layers aligned with the longer axis of the ellipse (31).

As always in the field of bone structure, the static model is an oversimplification. Studies of crystal growth in collagen fibrils show that the first-formed crystals are indeed in the hole zone (channels) (36, 37) and during the first stages are confined to this zone (32). However, they continue to grow and penetrate into the overlap zone (Figure 3) (38). In so doing, they must push aside the triple-helical molecules. Measurements of the average distances between triple-helical molecules in fibers with mineral and those without show a decrease in distance from about 1.5 to 1.1 nm (39, 40). In addition to direct compression, more space may also be created by partial or (as noted for the whale rostrum) almost complete removal of the collagen.

A key question is whether an appreciable volume of crystals is present outside the fibrils. Katz & Li (23) ingeniously combined structural and volumetric information to infer that in bone an appreciable amount of the mineral is outside the fibrils. Loci of disordered crystals have also been observed in some members of this family (41, 42). The extent to which this occurs may vary considerably between members of the bone family and needs to be better documented.

Mechanical Implications

The organization of the platy crystals in layers across the fibril indicates that at this level the structure is a platelet-reinforced fibril and is fully orthotropic. During the early stages of mineralization, the matrix host limits crystal growth and keeps the crystals separate from each other, at least along the length of the

fibril. The crystals continue to grow, compressing the triple-helical molecules, and eventually join together to form extended sheets. At this stage the crystals can be regarded as having been "straight-jacketed" by the organic framework (43). Clearly the mechanical properties of the collagen fibrils change considerably under such conditions.

An interesting point concerns the nature and role of the interface between the reinforcing mineral phase and its embedding protein matrix. It is well known that the interface plays a critical role in governing specific properties such as compressive and shear behavior, fracture modes and toughness, as well as stress transfer from externally applied loads to the reinforcement. The chemistry of the interface in artificial composites may be tailored for specific functions because in certain cases a weaker interface is desirable, whereas in other cases a stronger interface is preferred. The interface between collagen and apatite crystals is poorly understood. The fact that the collagen framework, directly or indirectly, both orients and controls crystal growth suggests that it could approximate a perfect or ideally strong interface. Thus the primary design of such a composite is probably oriented by nature toward a strong and stiff structure.

LEVEL 3: FIBRIL ARRAYS

In the bone family of materials, mineralized collagen fibrils are almost always present in bundles or arrays aligned along their lengths (44) (Figure 1; Level 3). These bundles are, however, not discrete. Fibers from one bundle may fuse with a neighboring bundle (16). The detailed internal structural organization of the fibril arrays is another poorly documented aspect of these materials. The major gaps in our knowledge arise from the fact that the internal organization of each fibril has an orthotropic structure. Thus understanding fibril array structure, especially in terms of the mechanical implications, should address the question of whether the neighboring fibrils are aligned in all three dimensions, i.e. form an extended crystalline structure or, at the other extreme, are aligned only to their fibril axes. The difference between these two arrangements is important in terms of symmetry; one would be fully orthotropic and the other transversely isotropic (Figure 5). Furthermore, how are the fibril arrays packed to form large ordered structures, and what is the extent of the order/disorder in these structures?

The mineralized tendons of large birds, especially those from the easily obtainable domestic turkey, have been studied in most detail at this level. These tendons are essentially arrays of type I collagen fibrils that reach macroscopic (millimeters) dimensions. They undergo mineralization starting from one end. A TEM study by Nylen et al (37) reported that if the appropriate specimen preparation conditions are used, the fibrils are intimately associated and the

banding patterns in neighboring fibrils are in register with each other (Figure 1; Level 3). Thus the tendon fibril array is clearly highly ordered in two dimensions down to a resolution of tens of nanometers. These observations do not, however, address the question of three-dimensional order.

Traub et al (32) used electron diffraction patterns of the crystals in sections of turkey tendon to demonstrate that locally (over an area of 0.3 μm) the crystal layers in several neighboring fibrils are aligned in all three dimensions. The alignment must be good (better than $\pm 15^\circ$) because the diffraction pattern repeats itself every 30°, as expected from the crystal symmetry. A three-dimensional TEM tomographic study of bone from a chick tibia also showed local alignment of crystal layers in adjacent fibrils (45). Sonic velocity measurements in three orthogonal directions of macroscopic specimens show significant differences (46), implying that orthotropic order at the fibril level may well extend to millimeter distances. This in turn implies that the mechanical properties should also be different in all three directions.

Mechanical measurements of macroscopic-sized mineralized tendons have been made using sonic velocity (46) and in tension (47). The tension measurements show the expected strong dependence on mineral content. The stress-strain curves, however, have an S-shape, except for the most mineralized sample, which behaves like other more mineralized members of this family. The S-shape is attributed to the unfolding of a higher order packing structure (crimps) known to be present in unmineralized collagen (19). Interestingly the presence of more mineral appears to eliminate this structure (47). The unmineralized tendons have low average modulus values of 67 and 103 MPa, whereas the mineralized tendons have average values ranging from 162 to 825 MPa (Figure 2). We caution against extrapolating these measurements to other members of the bone family, as even the most mineralized tendon is poorly mineralized (less than 50 wt%) compared with most bones (46).

Another member of the bone family of materials with a structure similar to tendon is parallel-fibered bone. As its name implies, it is composed of arrays of mineralized fibrils aligned along their fiber axes, but its mineral content (around 65 wt%) is much higher than mineralized tendon and is more representative of most bone types. To date there has been no detailed study of the three-dimensional structure of this bone type, although SEM observations support the notion that it too has long-range orthotropic order (48). The hardness values in three orthogonal directions are quite different (48). In the plane parallel to the macroscopic bone outer surface (periosteum) (P in Figure 3*b*), the hardness values are 480 ± 60 MPa[2], and in the transverse (T in Figure 3*b*) and radial planes, the values are 705 ± 90 and 600 ± 60 MPa[2], respectively (1 kg/mm^2 = 9.81 MPa). The extent of anisotropy is probably much greater because the structure is not perfectly regular to micron-scale dimensions. If these values

are extrapolated down to the nanometer scale of the individual mineralized fibril (Figure 3*b*), the highest hardness values occur when the triple-helical molecules are indented in the plane perpendicular to the fibril axis, and the crystals are indented edge-on. The lowest values occur when the crystals are indented face-on and in the direction in which crystal layers are separated by four layers of triple-helical molecules (P in Figure 3*b*). It is extremely difficult to study this bone type using more informative mechanical tests, because it is generally intimately associated with another bone type (lamellar bone). A single measurement (49) (Figure 2) shows that this bone is highly anisotropic. Clearly, we need to improve our understanding of the elastic and ultimate properties of parallel-fibered bone, as fibril arrays form the basis of all the structural types in this family.

Even though the information is sparse, the mechanical implications are clear. Mineralized fibril arrays are highly anisotropic, with the highest modulus values in tension and, especially, in compression in the direction parallel to the fibril long axes. Thus the basic building module of this family of materials is anisotropic, both in structure and in mechanical properties. This may have advantages for some functions but has clear disadvantages for others. At the next level of organization, the structural diversity might be at least partially rationalized in terms of extents of anisotropy or isotropy of the different sturctures.

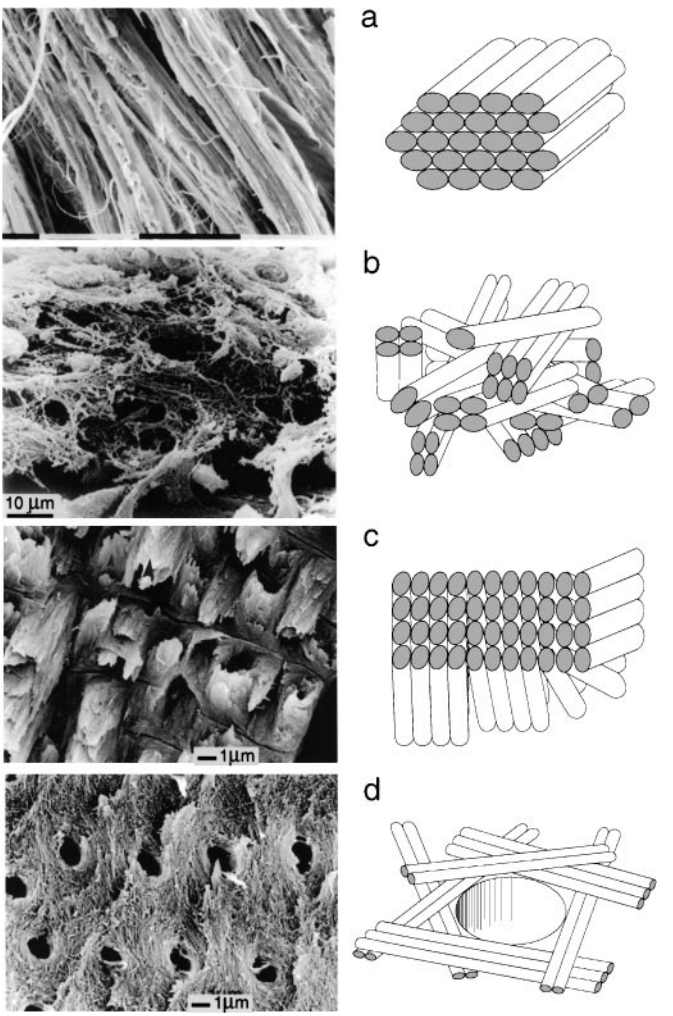
LEVEL 4: DIVERSITY IN FIBRIL ARRAY ORGANIZATIONAL PATTERNS

At Level 4 the conspicuous diversity in structure occurs, with fibril arrays organized in a variety of patterns. Of course, this does not imply that structural diversity does not exist in Levels 1, 2, and 3. The fibril array patterns of four of the most common members of this family are shown in Figure 6. For a more comprehensive description of diversity in fibril array organization see Francillon-Vieillot et al (50) and Pritchard (51).

Pattern 1: Arrays of Parallel Fibrils

This pattern is essentially the extension of the Level 3 structure into the micron and even millimeter scale size range. We mentioned above two occurrences of this structural pattern, namely mineralized tendons and parallel-fibered bone. It is also found in some fish scales and in the skeletons of fish and amphibia (51). The presence of mineralized tendons in the legs of large land-bound birds such as turkeys is somewhat unusual but convenient for study. Far more common is the phenomenon of tendons (called Sharpey's fibers) that are mineralized in the zone of attachment to the bone or tooth surface (16, 50).

Parallel-fibered bone is characteristically found in the bovid family . During early development, bone of this type is formed very rapidly. Parallel-fibered bone is first laid down with the fibrils more or less parallel to the bone long axis. Fairly large sausage-shaped spaces are left between successive layers of newly forming bone. These spaces are subsequently filled with another bone type (lamellar bone) (2). The combination of the two is referred to as fibrolamellar bone (also known as plexiform bone). As was noted in the discussion of Level 3,



we know little about the internal three-dimensional structure of these parallel fibril arrays at the level of crystal layers and fibril organization.

The highly anisotropic structure of parallel fibril arrays has the obvious advantage that they can be aligned in specific directions to optimize their mechanical functions. The attachment of a tendon to a bone surface with the contact zone stiffened by mineralization is a good example of this adaptation. The elastic properties are optimized in a given direction, and the stiffening of the structure essentially fixes the orientation. Parallel-fibered bone has a much higher modulus value (around 26 GPa) in the direction parallel to the axis of long bones, as compared with the orthogonal directions (around 11 GPa) (49) (Figure 2). We noted above (Level 3) that Ziv et al (48) measured Vickers microhardness of parallel-fibered bone on three orthogonal planes, with the highest values also being on the plane perpendicular to the fibril axes (T in Figure 3*b*) and with different but lower values on the other orthogonal planes.

Pattern 2: Woven Fiber Structure

This pattern is in many respects the antithesis of pattern 1. The fibrils are arranged into bundles, some with rather large diameters (up to 30 μm) and many with much smaller diameters (51). The fibril bundles are loosely packed and poorly oriented, particularly in the case of the small-diameter bundles (Figure 6*b*). There is also a large proportion of non-collagenous material in woven bone (16, 51). Treatment of woven bone with NaOCl to remove the accessible organic material reveals a pattern of spherical mineral clusters, with

Figure 6 Four of the most common fibril array patterns of organization. SEM micrographs of fractured surfaces and schematic illustrations (not drawn to scale) of the basic organizational motifs. (*a*) Array of parallel fibrils. SEM: mineralized turkey tendon (scale: 0.1 mm). Schematic illustration showing the localized orthotropic symmetry of a fibril bundle. (*b*) Woven fiber structure. SEM: outer layer of a 19-week old human fetus femur. (Micrograph provided by X Su. Also published in Reference 52.) Schematic illustration showing fibril bundles with varying sized diameters arranged in different orientations. (*c*) Plywood-like structure present in lamellar bone. SEM: fracture surface of a baboon tibia showing the prominent fourth (*large arrowhead*) and fifth (*small arrowhead*) sub-layers (63, 72). Schematic illustration showing the five sub-layer model described in (63) with sub-layers one (*right hand side*), two, and three arbitrarily composed of one fibril layer each, whereas sub-layers four and five are composed of four fibril layers each. Note that the fibrils in each layer are rotated relative to their neighbors (depicted by the change in direction of the ellipsoid cross-section), following the rotated plywood model (67). (*d*) Radial fibril arrays. SEM: human dentin fractured roughly parallel to the pulp cavity surface. The tubules (*holes*) are surrounded by collagen fibrils that are all more or less in one plane. Schematic illustration of the fibril bundles arranged in a plane perpendicular to the tubule long axis. Within the plane they have no obvious preferred orientation.

little evidence of the mineralized collagen fibrils (16). Thus both the matrix and the mineral are disorganized.

Su et al (52) recently revealed a degree of structural complexity, hithertofore unrecognized in human fetal woven bone. The initially formed layers of woven bone are indeed disordered. The extent of order in terms of fibril alignment, however, increases as the spaces between the initially formed layers are filled in. Furthermore, the rate of mineralization is very rapid. Measurements of the microhardness and elastic modulus (4–17 GPa) of these layers using a nanoindenter reflect this mineralization process and in the older specimens show clear anisotropy when comparing cross- and longitudinal-sections. Woven fiber structure may well be disordered as compared with other structural patterns at this level, but this disorder does not result in isotropic properties.

Woven bone is common in the skeletons of amphibia and reptiles, as well as in the skeleton of the mammalian embryo (51). During development, the latter is replaced by other bone types. Embryonic woven bone is probably not weight-bearing (except for gravity). It is also formed relatively rapidly. Woven bone is also the first bone type to be formed after fracture or in other pathologic circumstances, i.e. situations in which rapid formation is a prime concern.

Pattern 3: Plywood-Like Structures

This pattern is characterized by sets of parallel fibrils and/or fiber bundles present in discrete layers, with the fibril orientation in each layer being different (Figure 6*c*). The analogy of this bone structure type to plywood was first made by Weiss & Ferris (53). The analogy to laminated composites is also evident (54).

Plywood-like structural motifs are very common in nature and present a fascinating variety of numerical puzzles, each of which probably has an insightful structure-function relation. Bouligand (55) and Giraud-Guille (56) have not only enumerated many of these structures, but have also pointed out some of the structural misinterpretations that arise when oblique sections through these structures are studied. In certain bone types, for example, this led to the incorrect proposal that collagen fibrils have arc-like curvatures (57).

The simplest plywood-like structure encountered in the bone family of materials has parallel fibril arrays oriented orthogonally to each other in alternate layers. This motif occurs frequently in certain fish scales, many of which (but not all) are fully mineralized (58). It is also the basic structural pattern of cementum, the material that bonds tooth dentin to the jaw bone (59). Orthogonal plywood was first reported to be present in lamellar bone by Gebhardt in 1906 (60). Closer examination of the structure reveals far more complexity. In lamellar bone, the thicknesses of all the layers are not the same. A thin layer or lamella is often followed by a thick one (61). Thus a pair of such thin/thick lamellae

constitutes the basic repeating unit. More careful examination of the plywood structures of such lamellar units in human bone revealed that there is a transition zone between the thin and thick lamellae (62). In many lamellar bones this transition zone is fairly well developed, and it is clear that the structure is not composed of simple orthogonal plywood-like material. Giraud-Guille (57) analyzed the structures of these more complex plywood patterns and showed that the fibril arrays in successive layers progress from one direction through intermediate angles to the orthogonal direction. She referred to these structures as twisted plywood.

Weiner et al (63) made a detailed study of collagen organization in the lamellar bone from rat femurs, using cryomicrotomed and vitrified thin sections (64) of demineralized material cut parallel to the lamellar boundary. This preparation procedure avoids the inevitable tissue distortion due to dehydration and hence the difficulty of interpreting obliquely cut sections. They observed that successive layers of parallel fibrils in a thin/thick lamellar unit processed by an angle of roughly 30° from one layer to the next. They proposed a model in which a lamellar unit is composed of five such sub-layers (Figure 6*c*). The fact that there are five and not six sub-layers oriented progressively every 30°, means that the lamellar unit structure is not symmetrical (65). Comparisons of lamellar bone from different animals showed that in some cases sub-layers one and four were thicker than the others, giving rise to the overall appearance of a thin/thick orthogonal lamellar unit structure. In other cases, however, the five sub-layers are roughly of equal thickness, giving the impression of a continuous structure, more analogous to twisted plywood (63). Although not enough information is currently available on the generality of the five sub-layer lamellar unit model, it conceptually offers a synthesis for all lamellar bone types described. The main variable in terms of collagen fibrils is the different thicknesses of the sub-layers, rather than the angle of procession from one layer to the next. Note, however, that not all investigators of the lamellar structure concur with the Gebhardt plywood model. See Marotti (66) for an alternative model.

The plywood pattern of lamellar bone incorporates another "novel" (for the world of synthetic materials) structural feature. The fibril arrays that make up one sub-layer of the lamellar unit also have an internal crystalline or three-dimensional structure (Level 2). Examination of the orientations of the crystal layers within each of the sub-layers reveals a progressive rotation from the first sub-layer adjacent to the lamellar boundary, where the crystal layers appear to be parallel to the boundary, to the fourth and fifth sub-layers, where they are at a high angle to the boundary. Weiner et al (67) therefore referred to this as rotated plywood (Figure 6*c*). Unfortunately, it has not been possible to accurately measure the rotation angles. Note that as the rotation is always in

one direction, this results in a lamellar unit being asymmetric—an important point when considering its mechanical behavior. Such structures are fascinating for scientists and engineers who usually design composite materials containing single fibers or fiber bundles that have radial symmetry. Layers of platelets within the fibril do not have radial symmetry, and thus an additional degree of freedom is introduced. This could be used for tailoring purposes. Recent model studies deal with such problems in some detail (29, 68, 69).

Lamellar bone may appear to be the ultimate in structural sophistication when it comes to plywood patterns. It is probably not. Consider the pattern of the unmineralized scale of the primitive coelacanth fish (70). A detailed and insightful analysis of the successive orientations of the fibril orientations from layer to layer shows that in every pair of layers the fibrils are orthogonally oriented and that in successive pairs the orientations rotate progressively in a given direction. In fact, if every alternate layer is regarded as a set, then the angular progression is regular. It would be fascinating to understand the mechanical properties of the scale in relation to its structure. The elastic properties could possibly be modeled by means of conventional laminate analysis (54), but other properties such as strength and toughness are more difficult to understand.

Lamellar bone with its rotated plywood structure is very common, especially in mammals, and is the most common bone type in humans. Thus from an anthropomorphic point of view it is very important. However, it is difficult to study the mechanical properties of the structure per se because planar arrays of lamellae are not generally available in the sizes required for standard tests ($\approx$15 mm $\times$ 1 mm $\times$ 1 mm). What is generally available in these sizes are lamellae folded into cylinders (osteons; see Level 5), or sausage-shaped lamellar cylinders in parallel-fibered bone.

Ziv et al (48) measured the microhardness values of planar circumferential lamellar bone from the rat tibia in many different orientations with respect to the rotated plywood structure. The results clearly reveal anisotropy, with the highest values found in the plane perpendicular to the long axis of the bone (around 830 MPa) and lower values in the orthogonal planes (685–735 MPa). However, the extent of anisotropy is much less when compared with parallel-fibered bone of similar mineral content. This implies that the rotated plywood structure results in a material that is more isotropic than the building block from which it is constructed, an important strategy adopted by this material. This in turn may reflect the requirement for lamellar bone to withstand compressive forces in many directions, unlike parallel-fibered bone, which is optimized in one direction. The microhardness study also revealed the asymmetry of the rotated plywood structure, raising the fundamental mechanical question of how this asymmetry does not result in the weakening of the structure by buckling.

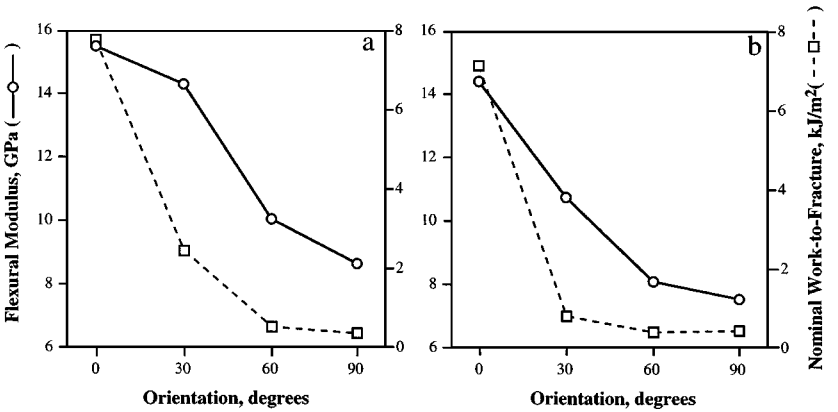


Figure 7 Plots of the flexural modulus and nominal work-to-fracture of baboon tibia (*a*) planar (circumferential) lamellar bone and (*b*) osteonal bone versus orientation of the specimen relative to the whole bone long axis. 0°—the long axis of the specimen is parallel to the long axis of the bone; 90°—the long axis of the specimen is perpendicular to the bone long axis. In all cases, stress was applied perpendicular to the natural bone outer surface and the measurements were made in the three-point bending mode. Data from Liu et al (72).

An invaluable opportunity to study planar arrays of lamellar bone in three-point bending arose when a section of baboon tibia (following treatment with an anti-osteoporotic drug, alendronate) (71) was found to have extensive volumes of circumferential lamellar bone (72). Specimens were examined in four different orientations relative to the bone long axis and were clearly anisotropic with respect to the flexural modulus, bending strength, fracture strain, and nominal work-to-fracture. The highest values were always obtained when the loading axis was perpendicular to the long axis of the bone, namely in the direction in which the greatest compressive forces are normally applied in this bone. The extent of anisotropy for the flexural modulus is around 1.8 (the ratio in the two orthogonal directions), whereas for the nominal work-to-fracture it is as large as 22 (Figure 7*a*). The structure has somehow resulted in the ultimate properties being very anisotropic and distinctly favorable for the direction in which most natural challenges to the bone will occur vis-a-vis fracture, namely with stress being applied perpendicular to the bone long axis. When this occurs, the fracture plane is tortuous, with the crack following either the lamellar boundary planes or the plane perpendicular to the natural surface. The latter fracture surface is almost featureless and resembles a ceramic (Figure 8*b*). In the unnatural situation, when the long axis of the specimen is orthogonal to the bone long axis, the fracture plane reveals the lamellar structure (Figure 8*a*). The challenge now is to relate both the elastic and the ultimate properties of planar

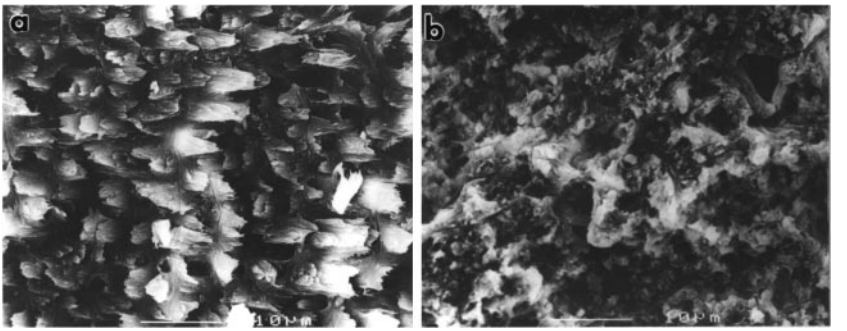


Figure 8 SEM electron micrographs of the surfaces of planar (circumferential) lamellar baboon tibia bone after fracture in (*a*) 90° orientation, and (*b*) 0° orientation (see Figure 7).

arrays of lamellar bone to its structure. The gaps in our current understanding of the structure, especially of the lamellar boundaries themselves, preclude a comprehensive analysis.

Pattern 4: Radial Fibril Arrays

This pattern is characteristic of the bulk of dentin, the material that forms the inner layer of teeth. The collagen fibrils are almost all in the plane parallel to the surface at which dentin formation takes place in the pulp cavity (73). Within this plane, however, the fibril bundles are for the most part randomly to poorly oriented, depending on the specific tooth and the location within the tooth (Figure 6*d*).

The crystal organization follows two quite distinct patterns: (*a*) Within collagen fibrils in dentin, *c*-axes are oriented parallel to the fibril long axes (74), and the crystals are in layers across the fibril (75). (*b*) In loci presumably between fibrils, the crytals have a random orientation and/or radiate out from the center of a locus (41, 76). In human root dentin, there is a preferred *c*-axis orientation in the collagen fibril plane, but there are also many crystals with *c*-axes oriented in all directions. This is presumably the result of both crystal organizational types being more or less equally represented (77). Furthermore, TEM observations of bundles of collagen fibrils show that the crystal layers are not always aligned between neighboring fibrils (75).

Dentin structure is therefore highly anisotropic because the collagen fibrils are essentially confined to one plane. In terms of crystal organization, however, the extent of anisotropy is much less. Other highly anisotropic features of dentin are the tubules, which pass through the structure from the pulp cavity to the region close to the dentin-enamel junction (Figure 6*d*). These holes occupy about 10% or less by volume of the bulk dentin and are oriented roughly

perpendicular to the collagen fibril plane. Therefore, it is surprising that studies of the elastic modulus of dentin have revealed no significant anisotropy in relation to the structure (78, 79). This could, in part, be due to the difficulties of testing such small specimens. Studies of dentin microhardness do reveal significant variations between locations on one plane within the tooth (78). Because of this variation, detection of anisotropy in dentin using microhardness must be made at one specific location. Wang & Weiner (75) performed such tests on human dentin sectioned in different directions and confirmed that dentin is essentially isotropic. In structural terms, we suspect that the essentially isotropic elastic and hardness properties are derived mostly from the crystal organization. We note that there is a large proportion of essentially unoriented crystals and that even in fibril bundles, the crystal layers of neighboring fibrils are not aligned. It is also noteworthy that in contrast to the elastic properties, the fracture properties of dentin are anisotropic, with the crack preferably following the plane in which the mineralized collagen fibrils are present (80).

Mechanical Implications

Level 4 structural organization is in many respects the heart of the subject. It is at this level that structural diversity would appear to be optimized to functional need. We can surmise that mineralized tendons are strong in tension, lamellar bone is built to withstand stresses in many directions, and dentin is structured to withstand compressive forces in one prevailing direction, and so on. Nonetheless, it is evident that serious gaps in our knowledge exist, both with respect to data on the micromechanical properties of these materials (aside from hardness) and in terms of understanding the structure. One reason for the former is the difficulty in acquiring large enough samples of the material comprised only of the particular structural type of interest for mechanical testing. We suspect that the enormous natural diversity in this family of materials has not been thoroughly explored and that suitable materials may be available, albeit from exotic sources. With good data in hand on structure and mechanical properties, the problem of relating structure to function will be less formidable.

LEVEL 5: CYLINDRICAL MOTIFS—OSTEONS

Bones themselves, as opposed to dentin, cementum, scales, and most mineralized tendons, often undergo internal remodeling. This process involves the excavating out of large tunnels by teams of specialized cells called osteoclasts. These tunnels are then refilled by osteoblasts, starting with the deposition of a thin layer of cement on the existing excavated surface, followed by layers of lamellar bone. The process stops when the tunnel is almost completely filled,

leaving a narrow channel at the center that functions as a blood vessel (5) (Figure 1; Level 5). In fact, other even smaller capillary-like features (canaliculi) are also built into the structure. These canaliculi are numerous and house the cells (osteocytes) that remain within the bone material itself. The canaliculi tend to radiate out from the central blood vessel. Thus the structure of an osteon is basically onion-like in cross-section with layers of lamellae surrounding a central hole; in longitudinal section they are cylindrical (Figure 1; Level 5). The osteon also contains many elongated pores.

A brief note on confusing terminology (5): The osteonal structures are also called Haversian systems, erroneously named after John Havers who actually discovered the lamellae in 1691 (81). van Leeuwenhoek discovered the osteons in 1693 (82). These structures are also often referred to as secondary osteons to differentiate them from similarly shaped structures that are laid down de novo, e.g. in the spaces between the parallel-fibered bone in fibrolamellar (plexiform) bone (50).

The widespread phenomenon of remodeling is itself a good indication that it must be important. Despite the hundreds of years that have elapsed since the osteons were identified, we do not have a complete understanding of the advantages, including possible mechanical benefits, that this process affords. Many studies have compared the mechanical properties of secondary osteonal bone with primary bone (83, 84). The primary bone usually used was fibrolamellar (plexiform) bone, which is comprised of both parallel-fibered bone and primary osteons. Secondary osteonal bone composed of cylindrical arrays of lamellae should be compared with primary lamellar bone comprised of planar arrays of lamellae. The latter, as noted above, is not naturally available in large enough quantities for standard mechanical tests. Liu et al (72), however, recently studied such planar lamellar arrays in an unnatural bone produced as a result of treatment by alendronate and have compared it to osteonal bone taken from the same baboon tibia, albeit from the opposing side of the mid-shaft. Figure 7*b* shows the results for the flexural modulus and the nominal work-to-fracture in four different orientations of osteonal bone. The basic trends and absolute values are similar to those of planar lamellar bone (Figure 7*a*), except for the off-axis values, where in the latter, the differences between the curves are a little larger than the on-axis values. It is difficult to definitively ascribe this difference to a particular aspect of the rotated plywood structure, but it may well be related to the presence of a prominent fifth sub-lamella in the lamellar unit of baboon tibia that is oriented 30° from the bone long axis (Figure 6*c*). This asymmetry would be canceled out in a cylindrical structure (Figure 9). In fact, this canceling out effect of the asymmetry due to the folding of the lamellae into cylinders may well be one of the important mechanical advantages of osteonal bone over bone composed of planar lamellar arrays. In general, however, it can

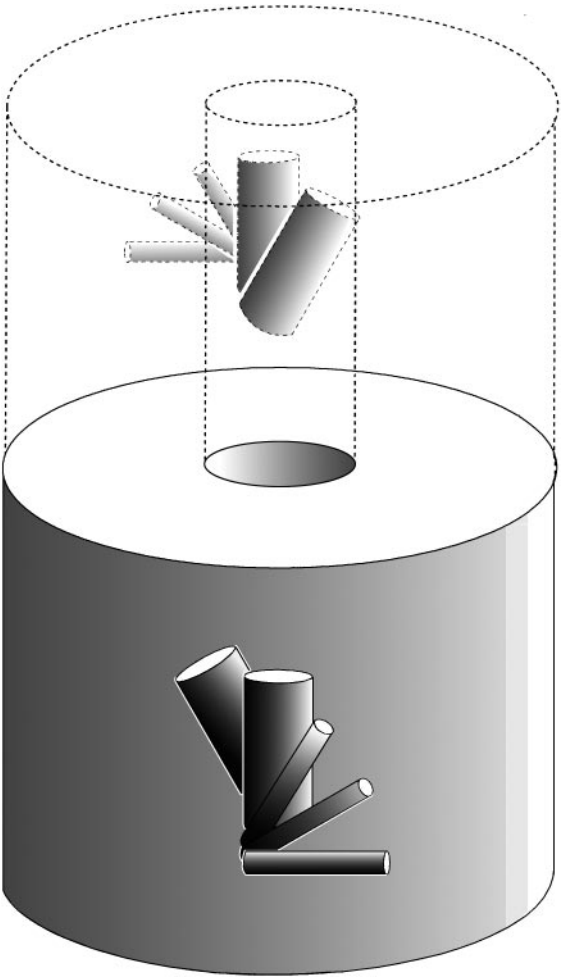


Figure 9 Schematic illustration (not drawn to scale) of a cylindrical osteon-like structure with the five sub-lamellar model superimposed. The orientation of the sub-layers is reversed on opposite sides of the cylinder, thus balancing the asymmetry of the lamellar structure. Figure from Liu et al (72).

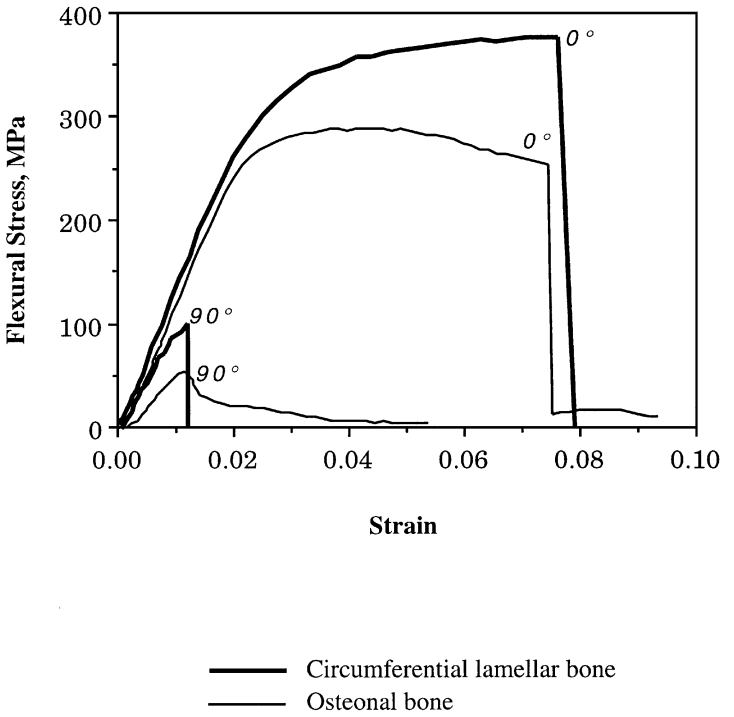


Figure 10 Flexural stress-strain curves of baboon tibia measured in three-point bending at 0° and 90° (see Figure 7). The heavy lines are for planar (circumferential) lamellar bone and the thin lines for osteonal bone. Data from Liu et al (72).

be concluded that the elastic properties are mostly due to the lamellar structure itself and not to the folding of lamellae into cylinders.

This is not the case for the ultimate properties. A comparison of typical sets of stress-strain curves for planar lamellar arrays and cylindrical lamellar structures (Figure 10), shows obvious differences in the fracture behavior. The osteonal bone reveals far more damage-related features and a pronounced tailing effect (72). The latter is related to the observation that following massive fracture, the two pieces of the osteonal bone always remain attached, whereas the planar lamellar bone fractures cleanly into two halves. This may afford important biological benefits. The natural wound healing process of a fractured bone can only take place if the two segments are in close proximity. Otherwise a so-called non-union fracture occurs that will heal only with surgical intervention. There may well be other, yet to be discovered, mechanical benefits of osteonal bone compared with planar arrays of lamellae. In principle, it is possible to predict

the elastic constants of osteons or arrays of osteons, using Lekhnitskii's work on fully orthotropic solids (85). Although algebraically difficult, this should be feasible, providing the data for the basic physical parameters (the relative weight or volume content, the various elastic constants of the components, etc) are available.

LEVEL 6: SOLID VERSUS SPONGY BONE, AND LEVEL 7: WHOLE BONES

Figure 1, Level 6, is a photograph of spongy bone associated with solid or compact bone. Figure 1, Level 7, is a photograph of a whole bone. A vast literature exists on both subjects (e.g. 4), which relates the mechanical properties to the material bone, as well as to their overall structures and shapes. This is beyond the scope of this review. For an overview of the subject, readers are referred to Currey (2), and for a more biological perspective to Martin & Burr (5). For a fascinating as well as historical view, the chapter on bone in Thompson's classic book is recommended (86).

CONCLUDING COMMENTS

Progress is being made on understanding the structure-mechanical function relations in the bone family of materials. Although many gaps in our knowledge exist, especially when the structure is considered in terms of its 7 hierarchical levels, our theoretical understanding of these relations and predictive capabilities are slowly improving. The more we learn, the more we realize that there are striking similarities between bone and man-made polymers and composites and that materials sciences can benefit from the basic work that has been performed in the bone field and vice versa. Simple examples are the high-molecular-weight organic fibers such as aramids (Kevlar) or polyethylene. They are structurally organized like tendons, from the molecular level up. In both the biological and the synthetic materials, the structural and evidently mechanical anisotropies are highest at the molecular scale, but are progressively reduced as the scale increases through sub-fibrillar and fibrillar arrays, up to the micrometer level (the single fiber). At higher levels, both bone and man-made composites have structures and physical properties that show progressively less anisotropy. This occurs, for example, when large arrays of single fiber-based plies or lamellae are arranged into layered or laminated structures at various angles. This is an important characteristic of structural design in nature and synthetically by humans, which in both cases is motivated by the fact that complex macroscopic structures are usually not designed uniquely for a single type of stress, but rather against stresses of various types, applied in various directions. The

design criteria, therefore, are varied rather than single-valued, and structural complexity is unavoidable. This effect also results in the gradual loss of a formal link between the various physical properties at higher and higher organizational levels. For example, by using a scaled-up model it may be possible to predict the stiffness of a fibril based on that of a single molecule, but it is much more difficult to predict the stiffness of a macroscopic composite structure from the stiffness of a molecule. The prediction of strength is even more difficult because of the higher sensitivity of that property to the presence of defects.

Extensive work has been performed in the field of composite materials to predict elastic constants, mostly of complex laminated structures in planar or cylindrical form; e.g. see the laminate theory in Reference 54. In principle, rather complex cases such as the predictions of the elastic constants of osteons or arrays of osteons may also be dealt with using Lekhnitskii's work on fully orthotropic solids (85). We wish to emphasize, however, that especially in the case of biological materials and structures, models can only lead to simplified results, in view of the inherent variability of material and geometrical properties, the aging effects, and our current lack of detailed information of the structures and the properties at the smaller scales. In the case of bone, this structural complexity appears in various often subtle forms. One important example is the interplay of fibrils and laminates at all levels. The power of theoretical models to bridge gaps in our knowledge is possibly the greatest at the level of the single lamella or of the parallel fibrillar arrays, and it is precisely at these levels that experimental results are still lacking, due to the technical difficulties involved in testing very small specimens. Models for bone of various degrees of complexity have indeed been proposed over the years, based mostly on concepts developed in the field of composite materials (29, 49, 87–93). However, only a few of these models incorporate platelet-or ribbon-shaped reinforcement and take into account some of the detailed geometrical and structural features (29, 68, 69). There is clearly a great need for additional careful experimental work with very small specimens with well-defined structures (72, 94).

The family of mineralized collagen-based materials presents a variety of structures that represent solutions to demanding mechanical requirements. The study of the structure-mechanical function relations in such materials is a fascinating subject in its own right and may provide novel ideas that can be applied to the improvement of synthetic materials.

ACKNOWLEDGMENTS

We thank Lia Addadi, Udi Akiva, Talmon Arad, Danmei Liu, Ilana Sabanay, Wolfie Traub, Rizhi Wang, and Vivi Ziv for their invaluable contributions to our knowledge of bone. We also thank Shoshana Neumann for her help in preparing this manuscript. This study was supported by U.S. Public Service

Grant DE06954 from the National Institute of Dental Research. SW holds the IW Abel Professorial Chair of Structural Biology.

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Annual Review of Materials Science
Volume 28, 1998

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