Mechanical properties and the hierarchical structure of bone

shrikant dawankar

Medical engineering & physics

ELSEVIER

Medical Engineering & Physics 20 (1998) 92–102

Medical Engineering & Physics

Mechanical properties and the hierarchical structure of bone

Jae-Young Rho [a,*], Liisa Kuhn-Spearing [b], Peter Zioupos [c]

[a] *Department of Biomedical Engineering, University of Memphis, Memphis, TN 38152, USA*
[b] *Laboratory for the Study of Skeletal Disorders and Rehabilitation, Children's Hospital, Department of Orthopaedic Surgery, Harvard Medical School, Boston, MA 02115, USA*
[c] *Department of Materials and Medical Sciences, Cranfield University, Shrivenham SN6 8LA, UK*

Received 25 June 1997; accepted 22 December 1997

Abstract

Detailed descriptions of the structural features of bone abound in the literature; however, the mechanical properties of bone, in particular those at the micro- and nano-structural level, remain poorly understood. This paper surveys the mechanical data that are available, with an emphasis on the relationship between the complex hierarchical structure of bone and its mechanical properties. Attempts to predict the mechanical properties of bone by applying composite rule of mixtures formulae have been only moderately successful, making it clear that an accurate model should include the molecular interactions or physical mechanisms involved in transfer of load across the bone material subunits. Models of this sort cannot be constructed before more information is available about the interactions between the various organic and inorganic components. Therefore, further investigations of mechanical properties at the 'materials level', in addition to the studies at the 'structural level' are needed to fill the gap in our present knowledge and to achieve a complete understanding of the mechanical properties of bone.

Keywords: Mechanical properties; Cortical bone; Cancellous bone; Structural properties; Material properties; Hierarchical organization

1. Introduction

Bone has a varied arrangement of material structures at many length scales which work in concert to perform diverse mechanical, biological and chemical functions; such as structural support, protection and storage of healing cells, and mineral ion homeostasis. Scale is of importance in discussing bone architecture as the structure is hierarchical and complex. Every technique of assessing bone architecture or the properties of a given structure has its own resolution, and therefore a combination of techniques is required to reveal the material structures and properties at the many different length scales. For example, electron microscopy examines bone ultrastructure at the nanometer level, Fourier transform infrared spectroscopy (FT-IR) and x-rays measure components at the Ångstrom level, light microscopy details features at the level of a few microns, and conventional mechanical testing of small specimens measures the mechanical properties of bone at the hundreds of microns or more level (at best).

In order to understand the mechanical properties of bone material, it is important to understand the mechanical properties of its component phases, and the structural relationship between them at the various levels of hierarchical structural organization [1–3]. These levels and structures are: (1) the macrostructure: cancellous and cortical bone; (2) the microstructure (from 10 to 500 μm): Haversian systems, osteons, single trabeculae; (3) the sub-microstructure (1–10 μm): lamellae; (4) the nanostructure (from a few hundred nanometers to 1 μm): fibrillar collagen and embedded mineral; and (5) the sub-nanostructure (below a few hundred nanometers): molecular structure of constituent elements, such as mineral, collagen, and non-collagenous organic proteins. This hierarchically organized structure has an irregular, yet optimized, arrangement and orientation of the components, making the material of bone heterogeneous and anisotropic (Fig. 1).

It has been shown that the mechanical properties of

* Corresponding author. Tel: 901-678-5485; fax: 901-678-5281; e-mail: jrho@cc.memphis.edu

1350-4533/98/$19.00
PII: S1350-4533(98)00007-1

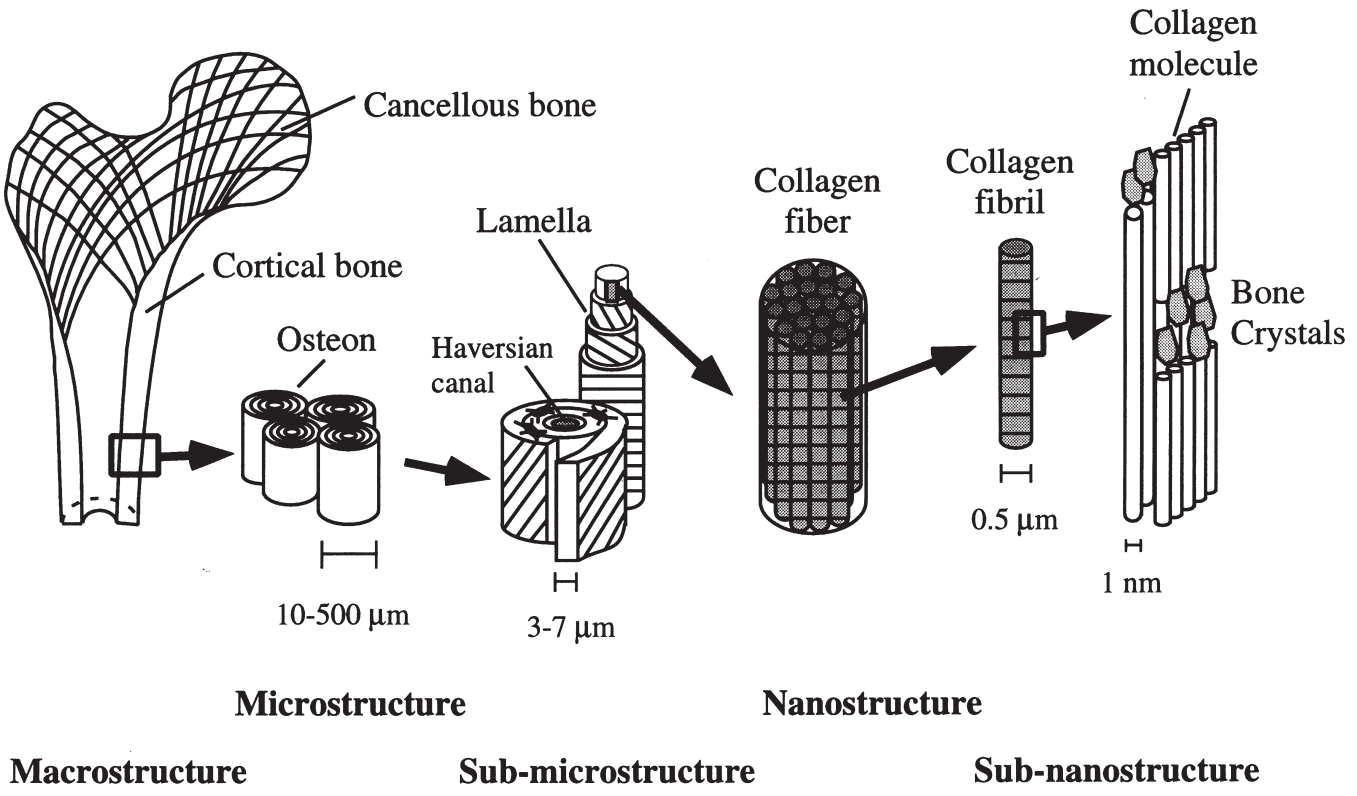

Fig. 1. Hierarchical structural organization of bone: (a) cortical and cancellous bone; (b) osteons with Haversian systems; (c) lamellae; (d) collagen fiber assemblies of collagen fibrils; (e) bone mineral crystals, collagen molecules, and non-collagenous proteins.

bone vary at different structural levels. For example, the Young's modulus of large tensile cortical specimens has been shown to be in the 14–20 GPa range [4] (wet specimen, macrostructural property), while that of microbending cortical specimens was 5.4 GPa (wet specimen, microstructural property [5]). However, it is unclear whether this discrepancy is due to the testing method or the influence of microstructure. Recently, the Young's modulus of osteon lamellar bone measured by nanoindentation was approximately 22 GPa (dry specimen, submicrostructure property [6]) close to that for the macrostructure. There are, at present, no data in terms of Young's modulus for the nanostructure. The basic building blocks (apatite crystals and collagen fibrils) are extremely small, making mechanical testing nearly impossible. The plate-shaped crystals of carbonate apatite are just tens of nanometers long and wide and some 2–3 nm thick [2], the various collagen forms are only a few nanometers wide (fibrils, 1.5–3.5 nm; fibers, 50–70 nm; bundles, 150–250 nm). Unsuccessful attempts have been made to extrapolate the properties of the primary constituents (collagen and mineral) from the macrostructural mechanical properties by working backwards from a mixed composite model [7,8]. Therefore, it seems of primary importance to break down the mechanical testing of bone according to the various levels or architecture within bone material.

2. Macrostructure

2.1. Cortical and cancellous bone

At the macrostructure level, bone is distinguished into the cortical (or compact) and cancellous (or trabecular) types. In cross-section, the end of a long bone such as the femur has a dense cortical shell with a porous, cancellous interior. Flat bones such as the calvaria have a sandwich structure: dense cortical layers on the outer surfaces and a thin, reinforcing cancellous structure within. Although both types of bone (cortical and cancellous) are most easily distinguished by their degree of porosity or density [9,10], true differentiation comes from histological evaluation of the tissue's microstructure. However, in compact coarse-cancellous bone (as described by Enlow [11,12]) the structure is fuzzy and it is difficult to distinguish between the two types of bone with any clarity. This bone is produced by cortical bone wrapping around the struts of cancellous bone, without replacement or remodeling of the old cancellous bone. The microstructure produced by the compaction of cancellous bone is composed of irregular, sinuous convolutions of lamellae. In contrast, the microstructure of cortical bone is composed of regular, cylindrically shaped lamellae. Therefore, reliable differentiation can only be achieved by microscopy methods.

Some workers [9,13,14] consider cortical and cancellous bone to be a single morphological material which can be characterized by a highly variable porosity or apparent density (mass/total volume, including pores). Others [5,15–17] consider these two types to consist of two different materials. In general, cancellous bone material is much more active metabolically, is remodeled more often than cortical bone, and is therefore 'younger' on average than cortical bone. Therefore, even though cancellous and cortical bone may be of the same kind of material, the maturation of the cortical bone material may alter the mechanical properties at the microstructural level.

The nature of cancellous bone, composed of bony trabecular struts and marrow-filled cavities, can be described in terms of structural and material properties [18]. *Structural* properties are defined as the extrinsic properties of both trabeculae and pores, whereas *material* properties are defined as the intrinsic properties of the trabecular struts only. Structural properties are important for global stress analyses, while material properties are important for characterizing various bone pathologies, microlevel stress analyses, and bone adaptation around implants. Microlevel stress analyses may provide valuable information about loosening and bone resorption at the implant–bone interface.

In cortical bone the mechanical properties are influenced greatly by the porosity, the mineralisation level and the organization of the solid matrix. It is, therefore, difficult to predict micro-properties *in vivo* [19,20] by measuring mechanical properties at the macrostructural level. In general, values for mechanical properties of bone at the macrostructural level vary from one bone to another as well as within different regions of the same bone [21,22]. For a number of human bones (including the femur, tibia, humerus, mandible, lumbar vertebrae, and patella), the site-specific orthotropic elastic moduli, shear moduli, Poisson's ratios, and densities have been studied as a function of position [21,23] using ultrasonic techniques. The mechanical properties of human cortical bone from the tibia, femur, and humerus have been found to vary between subjects, although the density was the same. The elastic moduli in the longitudinal direction were not very different between the various types of cortical bone and there was a greater modulus variability along the length of a whole bone than around its circumference (the variation in the mechanical properties around the circumference being less than 10%). The elastic moduli in the radial or circumferential directions correlated to the longitudinal modulus, but the correlation coefficients were relatively low, of the order of 0.001–0.6. However, all three perpendicular moduli correlated even better with the density of the material.

In human cancellous bone, by contrast, there is no difference in the mechanical properties of the humerus, the proximal tibia, and the lumbar spine [21,23]. The stiffness and strength of cancellous bone from these bones were found to be lower than those of the patella, and the distal and proximal femur (patellar cancellous bone had the highest values overall). In human cancellous bone, mechanical properties vary significantly around the periphery and along the length and they also show significant *inter-subject* differences. This could suggest that experiments performed on a large number of samples from one subject are not enough to draw conclusions. The differences in *inter-bone* mechanical properties suggest that predictions of mechanical properties can not be done by consideration of its density alone. When the pronounced anisotropy of cancellous structure in the highly mechanically loaded long bones (the preferential orientation of the struts gave rise to the well known Wolff's law) was taken into account, greater uniformity of measurements resulted, allowing for very accurate predictions [24,25]. However, the results of these compressive tests on cancellous bone specimens are confounded by technical problems like friction and the transfer of load between the platens and the body of the cancellous specimen [26–28].

The lesser regional heterogeneity observed in cortical bone may be the result of the lower turnover rate for this material which engenders changes at the microstructural level. Overall, the differences between the mechanical properties in cancellous bone are much broader than those in cortical bone and indeed may vary by a factor of 2–5 from bone to bone. To a great extent, differences between whole bones may be explained by the variance in their structure and mechanical function. However, the properties of a particular type of bone can not be adequately expressed in single values; our best estimates can only be given in a range of values reflecting both the experimental difficulties and the observed regional heterogeneity.

3. Microstructure

Mineralized collagen fibers form into planar arrangements called lamellae (3–7 μm wide). In some cases these sheets (lamellae) of mineralized collagen fibers wrap in concentric layers (3–8 lamellae) around a central canal to form what is known as an *osteon* or a *Haversian* system. The osteon looks like a cylinder about 200–250 μm in diameter running roughly parallel [29] to the long axis of the bone. Other forms of cortical bone where the mineralized collagen fibers are less well registered and no pattern can be distinguished are called *woven* bone. In some forms of bone, the lamellae are overall tangential to the outer surface of the bone (without forming osteons), and together with woven bone tissue, form a larger plywood-type stacking of thicker layers (150–300 μm) around the complete circumference of the bone in what is called *lamellar* bone. Cancellous bone

is made of an interconnecting framework of trabeculae in a number of combinations, all comprising the following basic cellular structures: rod–rod, rod–plate, or plate–plate. A trabecular rod is about 50–300 μm in diameter.

3.1. Haversian systems

Ascenzi and co-workers examined the mechanical properties of single Haversian systems in tension [30], compression [31], bending [32] and torsion [33]. For tension measurements, samples were excised from bone mostly less than 50 μm wide, smaller than a single whole osteon (which is roughly 200 μm wide). These osteonal segments had elastic moduli of 12 and 5.5 GPa in tension [30] and strengths of 120 and 102 MPa (in this section the first value refers to samples with the majority of lamellar orientation in the longitudinal direction and the second value is for osteons that have adjacent lamella orientations at sharp angles to each other). Later, Ascenzi and Bonucci [31] examined isolated osteons in compression and found that these were half as stiff (6 and 7 GPa) but equally as strong as those in tension (110 and 130 MPa), an intriguing result. Later tests by the same group in bending [32] (the two numbers are for longitudinal and interchanging orientational characterization of lamellae as before) gave values for stiffness of approximately 2 and 3 GPa and bending strength of 390 and 350 MPa. In torsion [33], single osteons had moduli of 20 and 16 GPa and strengths of 200 and 160 MPa. Consideration of all these results suggests that perhaps careful characterization of the lamellar orientation within an osteon can predict its mechanical behavior, which seems to be optimized for its use *in vivo*. Ascenzi et al. [30–33] seem convinced that osteons with longitudinal lamellae are better for tension and torsion and perhaps stronger in bending as well, while osteons with alternating lamellae are more suited for compression. The stiffnesses produced from torsion testing of individual osteons were certainly much higher than those for the whole bone. They did not justify or explain this result. However, this effect may be explained by the use of Cosserat micropolar elasticity, which allows for local rotation at cement sheaths and other internal boundaries [34].

The Ascenzi group also claim to have some microscopic evidence for these results from observations of the lamellae failing during deformation. In compression, cross-hatched fissures at 30–40° appeared and these were not affected by the kind or combination of lamellar architecture. In tension, however, the transverse lamellae failed first and the osteons were kept together only by the longitudinally oriented ones. Other workers that tried to corroborate these results are somewhat doubtful as to whether osteonal architectures can be classified as neatly as by the Ascenzi group. Katz and co-workers [35] confirmed that there is a majority of fibers in the longitudinal direction in osteons and also along the long axis of trabeculae in cancellous bone, but the situation in general is muddled. Marotti [36] claims that fibers in general follow two patterns which constitute thin and thick lamellae; the thin ones are more oriented and compact, the thick ones are more diverse and sparse (somewhat microporous) in their elements.

3.2. Single trabeculae

The trabecular properties are much easier to study in isolation. However, in spite of recent reports [5,15,17,18], there remains some controversy regarding the value of the elastic modulus of single trabeculae (Table 1). Trabecular bone material properties are important for characterizing various bone pathologies, and the remodeled bone adjacent to various joint implants, because they are affected by disease sooner than cortical bone. In the past it was assumed that individual trabeculae, single osteons, and a thin cortical shell possessed the same mechanical properties as those of large cortical bone specimens regardless of their type or size [37]. However, many investigators produced values for the elastic modulus of individual trabeculae, single osteons, and a thin cortical shell that were considerably less than that for whole bone [5,17,18].

Choi et al. [5] found that the elastic modulus of cortical bone obtained from micro-bending specimens (5.4 GPa; dimensions $100 \times 100 \times 1500$ μm) is considerably smaller than that of large tensile specimens tested by others [4] (17.1 GPa). The reason for this discrepancy is not clear, but it could arise from difficulties encountered in making accurate mechanical property measurements by bending small specimens. The possible causes include: (i) the influence of microstructural defects such as cement lines and voids (Haversian and Volkmann canals, lacuna, osteocytes, canaliculi) on the measured displacements; (ii) uncertainties in specimen geometry, which are often exacerbated at small scales; and (iii) problems in properly seating and aligning small bending specimens in small test fixtures [6]. Choi et al. [5] further noted that "the modulus of bone tissue even at a microstructural level may still represent a 'stiffness' rather than an intrinsic property of bone material."

A literature survey of measured and estimated values of the modulus of trabecular bone material [5,17,18,37–45] shows that moduli values range from 1 to 20 GPa (Table 1). Rho et al. [17] showed that the relationship derived from these data, between elastic moduli and density in cancellous bone material, could not be extrapolated from similar data from tests on cortical bone material and its density. This led the authors [17] to conclude that the materials of the two bones were intrinsically different. However, the data are not definitive, as the dependence of the modulus on other parameters such

Table 1
A literature survey of methods for determining the elastic modulus of trabecular bone material and the resulting estimate values. This list is an updated version of the original table published by Rho *et al.* [17]

Source	Test method	Estimate of elastic modulus (GPa)
Wolff (1892) [37]	Hypothesis	17–20 (assumption)
Runkle and Pugh (1975) [38]	Buckling	8.69 ± 3.17 (dry)
Townsend et al. (1975) [39]	Inelastic buckling	11.38 (wet)
Williams and Lewis (1982) [40]	Back-calculating from finite element models	1.30
Ashman and Rho (1988) [18]	Ultrasound test method	12.7 ± 2.0 (wet)
Ryan and Williams (1989) [44]	Tensile testing	0.76 ± 0.39
Hodgskinson et al. (1989) [41]	Microhardness	15 (estimation)
Kuhn et al. (1989) [42]	Three-point bending	3.81 (wet)
Mente and Lewis (1989) [43]	Cantilever bending with finite element analysis	7.8 ± 5.4 (dry)
Choi et al. (1990) [5]	Four-point bending	5.35 ± 1.36 (wet)
Rho et al. (1993) [17]	Tensile testing	10.4 ± 3.5 (dry)
	Ultrasound test method	14.8 ± 1.4 (wet)
Rho et al. (1997) [45]	Nanoindentation	19.6 ± 3.5 (dry): longitudinal direction
		15.0 ± 3.0 (dry): transverse direction

as location, microstructure or density variation were not considered. Use of nanoindentation [45] and finite element analysis (FEA) simulation [24] suggest that in fact the elastic properties of single trabeculae are very similar to the properties of nearby cortical tissue. This matter requires further clarification because imprecise values may lead to misinterpretations of the structural function of each type of bone material or misinterpretation of the role of trabecular bone in the mechanical behavior of normal and implanted joints.

3.3. Sub-microstructure

3.3.1. Lamellae

Bone lamellae are 3–7 μm thick [36], but the arrangement and orientation of the substance of a lamella is not well known. There may be differences in the lamellae encountered in cortical and cancellous bone. The most common perception of the arrangement of the collagen fibers in a lamella of an osteon is that they lie in parallel in each lamella, with a change in the orientation of fibrils from one lamella to the next described figuratively as a twisted plywood or helicoidal structure by Giraud-Guille et al. [46]. According to this representation, adjacent lamellae have different orientations; either longitudinal (with the collagen fibers along the long axis of the lamellar sheet) or transverse (with the collagen fibers perpendicular to the long axis). The osteonal lamellae are wrapped around a central canal, and sequential concentric lamellae have fiber orientations alternating with each other, spiraling around the central canal. Lamellae with alternate orientations are seen as alternately bright, dark, or intermediate in cross-section under a polarized light microscope (PLM) with the intensity of the transmitted light depending on the collagen content, its degree of alignment, the presence of a mineral fraction, and on the orientation of the section [30,47]. The orientations envisaged in this kind of modeling are transverse, longitudinal, or oblique. However, in another model [36] it has been suggested that the arrangement of the fibers in a lamella is highly interwoven with no apparent preferred orientation. Current studies indicate that different types of lamellae are seen under a PLM only because of variations in the density of the collagen fibers in the lamellae and not because of their orientation. Lamellae previously classified (using a PLM) as transverse are made up of dense, interwoven collagen fibers (dense lamellae), while those classified as longitudinal possess fewer fibers in a coarse weave (a less dense or loose lamellae), with a greater amount of mineral. A lamella can no longer be considered to contain individual, highly oriented collagen bundles that do not branch. Overall, the collagen has a basically parallel orientation but the fibers form a continuum both within a single lamellae and between lamellae [48].

Structural information at this level has been obtained using optical microscopy, x-ray diffraction, and electron microscopy. Mechanical properties of individual lamellae in several orientations are needed to comprehend bone anisotropy. Some studies which used selective demineralization and acoustic methods have come up with intriguing theories about the complementary role of the collagen and the mineral [49–52]. These workers have suggested that isolated collagen is more or less isotropic and by the impregnation of mineral it reaches the anisotropic ratios that are known for whole bone (1.7–2.1) in two normal directions. Experimental methods that can resolve the issue of absolute and relative (anisotropy) values for the elastic modulus of microscopic bone tissue would be invaluable. However, mechanical data at the sub-micron level have been unavailable until recently, when nanoindentation tests were used to measure the hardness and elastic modulus of single lamellae [45] and small filler particles in resin com-

posites and other dental restoratives [53]. This technique is able to measure mechanical properties with a resolution better than 1 μm and does not require visual resolution of the indentation. Taking into account the microstructural features of bone, the nanoindentation technique offers a means by which the intrinsic mechanical properties of the individual microstructural components of bone may be measured in a manner which avoids the influences of the inherent defects and inhomogeneities in the microstructure. This technique also allows the mechanical properties to be measured in several different directions at the microstructural level. The mechanical properties of trabecular bone in the transverse direction can also be measured despite the small size of the bone specimen. The nanoindentation method may offer valuable insight into the mechanical properties of bone at the microstructure (or lower) level. These properties can be further used in the development of theoretical micromechanical models and in finite element modeling.

4. Nanostructure

4.1. Collagen fibers from hundreds of nanometers to 1 μm

The most prominent structures seen at this scale are the collagen fibers, surrounded and infiltrated by mineral. The attachment sites of macromolecules onto the collagen framework are not distinctly known, although several immunohistological studies have shown preferential labeling of some macromolecules in a periodic fashion along the collagen molecules and fibers [54].

4.2. Sub-nanostructure

4.2.1. Crystals and collagen fibrils down to tens of nanometers

The sub-nanostructures of the three main materials are crystals, collagens, and non-collagenous organic proteins. The mature crystals are not needle-shaped, but plate-shaped [2]. Plate-like apatite crystals of bone occur within the discrete spaces within the collagen fibrils, thereby limiting the possible primary growth of the mineral crystals, and forcing the crystals to be discrete and discontinuous. The mineral crystals grow with a specific crystalline orientation—the *c* axes of the crystals are roughly parallel to the long axes of the collagen fibrils [55]. The average lengths and widths of the plates are 50×25 nm. Crystal thickness is 2–3 nm [3,56]. The nanocrystalline bone apatite has small but significant amounts of impurities such as HPO_4, Na, Mg, citrate, carbonate, K, and others whose positions and configurations are not completely known [55]. While the x-ray diffraction pattern is that of hydroxyapatite, the near-absence or absence of the hydroxyl group has been proven repeatedly by chemical methods and FTIR and NMR spectroscopy [57]. The primary organic component of the matrix is Type I collagen. Collagen molecules secreted by osteoblasts self-assemble into fibrils with a specific tertiary structure having a 67 nm periodicity and 40 nm gaps or holes between the ends of the molecules (Fig. 2). Non-collagenous organic proteins, including phosphoproteins, such as osteopontin, sialoprotein, osteonectin, and osteocalcin, may function to regulate the size, orientation, and crystal habit of the mineral deposits. Through chelation of calcium or enzymatic release of phosphorus from these proteins, they may serve as a reservoir for calcium or phosphate ions for mineral formation. However, additional studies are needed to conclusively define their actions and mechanisms.

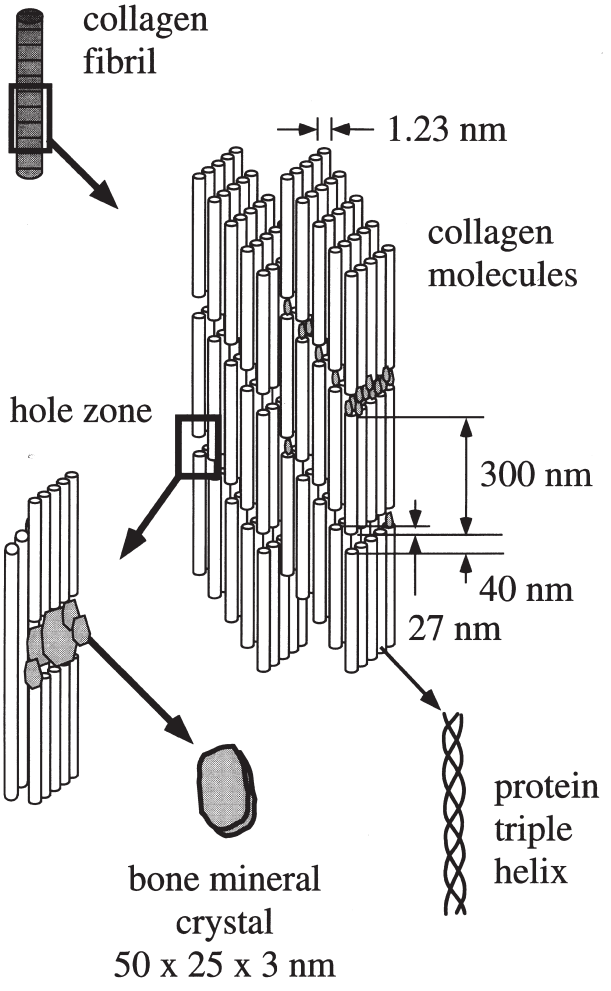

Fig. 2. A schematic diagram illustrating the assembly of collagen fibrils and fibers and bone mineral crystals. The well known 67 nm periodic pattern results from the presence of adjacent hole (40 nm) and overlap (27 nm) regions of the assembled molecules.

5. Composite modeling of bone material

The composition of bone tissue is more complex than most engineering composites. A more fundamental understanding may be achieved by models employing a collagenous matrix and mineral crystals. These organic and inorganic constituents act together to give bone its unique properties. The viscoelastic properties and resistance to fracture cannot yet be explained by explicit molecular mechanisms or commonly measured physical characteristics [58,59], but models of the elastic properties and their anisotropy using composite rules of mixtures for the elements have been suggested [60]. Early attempts viewed bone as a soft collagen matrix with a stiffening filler of mineral crystals added to it and obeying a general rule of mixtures [61]. This rule is useful in estimating the elastic modulus of unidirectional continuous fiber composites, but is probably not suitable for bone. Sweeney et al. [62] mechanically tested bone from which either mineral or collagen was removed. They showed that the individual properties of the mineral and the collagen components were very compromised, and could not add up to the elastic properties of whole bone. They concluded that bone was a two-phase composite in which the mineral and collagen were bound in a complex manner.

Piekarski [63] and Katz [64] adopted a more complex theory of the rule of mixtures; the drawback was that it offered too broad a range of values for the elasticity of bone material [1]. Moreover, none of these early models could satisfactorily explain the anisotropy of bone [1]. Currey's model was based on a microstructural feature [65]: the mineral crystals were assumed to fuse end-to-end and form 'mineral fibers' that reinforce a collagen matrix. A simple superposition of the two components was assumed and although it produced a reasonable estimate for the longitudinal modulus of elasticity, it estimated the transverse modulus of bone to be equal to that of collagen, while experimental evidence shows roughly a 2:1 ratio for the moduli in orthogonal directions [1,66].

The hierarchical model proposed by Katz [35] modeled the properties of an osteon and of cortical Haversian bone by adopting a macrostructural approach. A two-layered hierarchical model, in which the osteons are considered to be the reinforcing fibers, was developed. The 'matrix' in which the fibers are embedded is made up of the organic cement lines and interstitial lamellae. The elastic properties of individual osteons were calculated using a unidirectional fiber-reinforced formulation, based on different percentages of longitudinal collagen fibers in an osteon. Using these calculations for modulus of the osteons, the osteon is considered to be a hollow fiber and the Hashim–Rosen formulation [62] for hexagonally and near-randomly arrayed fiber-composite is directly applied to the analysis of bone. This analysis yielded results which approximated better the observed anisotropy of bone.

In the rule of mixtures formulations the collagen matrix is considered to be a homogeneous continuum and isotropic material, which is a gross simplification. If something is remarkable about bone, it is its *hybrid* design. The collagen matrix is itself in a fibrous form, which may play a crucial role in elasticity and fracture by imparting its own directionality to the structure. However, by some strange turn of fate it has been found that this is not far from the truth. Hasegawa et al. [49] calculated that the anisotropy ratio of demineralized bone material is close to 1.

In a recent model, Braidotti et al. [67] considered an osteon as an *n*-layered cylinder with existing internal stresses and demonstrated the relative importance of the number of lamellae and the lesser importance of orientation of the lamellae on the overall properties of the osteon. Braidotti et al. [68] revised the previous two approaches as follows: (i) the early one by Currey [65], where the mineral directly reinforces the collagen matrix was called an 'ultrastructural' model; (ii) Katz's model [35] (at a higher level of organization), which considers that osteons are the reinforcing phase of whatever surrounds them, was called a 'microstructural' model. Braidotti et al. [68] also came up with their own synthesis, what they called an 'intermediate' model where the mineralized collagen fibers inside a non-collagenous mineralized matrix comprise the reinforcing phase. There are two definite positive elements in this concept of the mineralized fiber model. First, the most generally accepted view of the mineralization process in bone in ontogeny and phylogeny is that early mineralization is initially intrafibrillar (inside the fibers) and then interfibrillar (between the fibers). Intrafibrillarly the mineral occupies up to 30–40% by volume and then starts to fill the space around the mineralized fiber to various degrees [60], ranging from 50–60% for human bone, to something much less for antler bone, to 80–90% for some highly mineralized tissues (i.e. tympanic bulla). Second, the fracture properties of bones with various degrees of mineralization [61] corroborate the dominant role of the mineralized fibers. Less mineralized bones show rough, fibrous fracture surfaces and are in general tougher tissues than more mineralized bones, which show flat, brittle fracture surfaces devoid of any evidence of toughening mechanisms like those observed in composites (fiber pull out, bridging, crack deviation, microfractures and so on).

The models of Sasaki et al. [69] and Wagner and Weiner [70] also modeled bone anisotropy. Both groups assumed some values for the basic components (mineral and collagen), the volume fraction and the geometry of the mineral phase and employed composite reinforcement formulae, such as as those by Halpin and Tsai [71], which are based on the shape of the mineral to determine

the final mechanical properties of the composite material. In this way, both groups derived the properties of a basic lamella, Sasaki et al. [69] assuming the mineral to be needle-like in shape, Wagner and Weiner [70] assuming it to be plate-like. Once the basic lamellar properties had been produced, Sasaki et al. [69] made use of their x-ray analysis data which showed the polar distribution of the lamellar orientation and used a generalized rule of mixtures to derive properties of whole bone. Wagner and Weiner [70] used direct microscopic evidence which showed that the great majority of lamellae are oriented in just two directions; then they also added the contributions of the two subsets of lamellae. Currey et al. [72] contributed to this debate with data they collected on the anisotropic mechanical behavior of dentine material from the tusk of a whale. Dentine has a very regular grain which changes every few hundreds of micrometers and it is possible to isolate small specimens which have a unidirectional orientation (equivalent to the elusive lamellae) and study their properties. These workers [72] expressed the opinion that the previous two models [69,70] are on the right track, but they thought that the plate-like reinforcing phase was a more plausible suggestion.

By contrast, Pidaparti et al. [52] made a conceptual distinction of *inter*- versus *intra*-fibrillar mineralisation and utilized some dentine material in order to obtain some indication for the likely effects of mineralization on the anisotropic elastic properties of collagen fibers. Whole bone was modeled in two phases. In phase I the properties of the mineralized fibers (intrafibrillar mineral) were derived by acoustically measuring the properties of demineralized whole bone and these of mineralized and demineralized dentine. They found [52] that mineralization in uniaxially registered fibers increased the anisotropy threefold and assumed that the same would happen in bone. In phase II, the anisotropic properties of the extrafibrillar mineral were derived on the data provided by Katz and Ukraincik [73] for hydroxyapatite crystals. The two phases were added up in a generalized rule of mixtures and the best fit was obtained for the assumption that 25% of the mineral is inside fibers and the rest lies between the fibers. The authors did not consider particular bone architectures and did not go into the detail of the organization of the various elements. Instead, they produced averaged bone properties at the level of the demineralized bone, mineralized and demineralized dentine and then fitted their model into the directionally varying averaged behavior of whole bone. In this respect they dispensed with the need to produce the properties of mineralized collagen matrix (as did Sasaki et al. [69] and Wagner and Weiner [70]) and also the need to define the orientation of this matrix element. This is very promising, but it depended on two additional assumptions (which may very well be true): (i) that all the interfibrillar mineral is aligned to the long direction of the fiber and (ii) all the extrafibrillar mineral is aligned to the long axis of the bone.

6. Mineral, collagen and their interface

The previous theories of macroscopic modeling are different from the material-level models which explore the relationship between the mineral crystals and the collagen matrix [8], although they did originate from the fiber-composite models of mineral and organic matrix [35]. There are still no reliable data regarding the elastic properties of the mineral crystals and the collagen matrix of bone. Isolated crystals retaining all of the native features of bone mineral can be obtained using low-temperature and non-aqueous methods [55]; however, it is not clear how the mechanical properties of these crystals can be measured at this time. Most likely, the elastic properties of bone mineral crystals are different from those of pure hydroxyapatite [73,74] due to the vacancies and substitutions in the natural crystals. Bone crystallites seem to have the same elastic behavior in all directions as judged by the density of the molecules in each face (Naoki Sasaki, personal communication). If this is true it seems that the anisotropy they impart in bone-collagen matrix is an effect of the elongated shape of the mineral, another result which makes the previous composite reinforcing models even more hopeful. Similar conclusions are reached when one examines the elastic–fracture and toughness properties of some extremely mineralized bone tissues [75]. Examination of the highly mineralized rostrum bone of a Mesoplodon whale, which has only 2–3% collagen w/w, shows that bone fully compacted with mineral retains the same anisotropy ratios (roughly 2:1 in elasticity and toughness) as less mineralized ‘normal’ bone. This seems to underline the importance of the shape, geometry and material properties of the crystals for determining the behavior of whole bone.

The elastic properties of bone-*collagen* have been assumed to be the same as those for tendon or ligament collagen. However, unlike the collagen of soft tissues, it is normal to assume that bone collagen will not show reorientation under stretch and also will not (does not) have the crimp pattern and straightening out effect. Examining the organic remnant after crystal dissolution appears to be the customary method for obtaining bone-collagen properties [76–78]. However, no one can be certain of the constitution and any conditional changes of the phase (either mineral or collagen) that is left behind [49,62,79]. Therefore, it is very difficult to imagine what the bone-collagen properties may be, and certainly the intimate relationship of mineral and collagen and their interplay makes measurement and predictions even more difficult. Further investigations are warranted for a more complete understanding as to how mineral–matrix inter-

actions are related to their effects on mechanical properties at the various hierarchical levels of tissue structure and organization.

7. Research challenges

This review of the mechanical properties of bone has indicated a number of areas in which additional research would advance our understanding of bone as a structure and a material. The following questions remain unanswered:

1. Is there a size effect in bone? If yes, does the critical size depend on the particular property under consideration? What is the hierarchical level at which homogenized material properties can be obtained that are similar to those observed macroscopically in whole bone samples?
2. Bone as a composite material is made of mineral and collagen measured at the level of 1–8 mm^3 volume space. What are the influences of location, orientation, and microstructure on bone properties (both cancellous and cortical)?
3. What are the properties of a single bone lamella? Satisfactory mechanical tests at this level are hampered by technical problems such as: (i) the existence of different types of lamellae that need to be classified and tested; (ii) the arrangement and orientation of the substance of a lamella which are also unknown; (iii) the possibility that lamellae may have internal stresses when in position around the osteon and, therefore, when removed for testing they may yield a different stress–strain response than the actual response in situ; (iv) cortical and cancellous bone have different arrangements of their constituent lamellae.
4. The next level of structure upwards is the osteon for cortical bone and the single trabecula for cancellous bone. Why do individual/isolated trabeculae show on average a lower modulus than that of whole cortical bone samples, while isolated cortical osteonal samples show a similar modulus to that of bulk cortical bone? This issue needs further clarification.
5. Modeling of the material of cortical bone fails to explains all its properties due to the lack of knowledge of the properties of the individual constituents and their associations in the matrix. What is the nature of the interfaces between the constituents of bone at all length scales?

If we are at all successful in answering these questions, various pathophysiological conditions may be easier to understand. Aging, osteoporosis, osteoarthritis, and other degenerative diseases are expected to influence, and be influenced by, the microstructure to different degrees. It may just be possible that certain diseases (or syndromes) affect the constitution and quality of only one level of the hierarchical organization of bone, and therefore they may become easier to understand and treat.

Acknowledgements

The work of Jae-Young Rho was supported in part by the Whitaker Foundation and a Faculty Research Grant from The University of Memphis. Liisa Kuhn-Spearing has been supported by an Institutional NIH Training Grant to the Children's Hospital, Boston. Peter Zioupos has been supported in part by AFOSR, the Wellcome Trust and the NATO scientific exchange program.

References

[1] Mehta SS. Analysis of the mechanical properties of bone material using nondestructive ultrasound reflectometry. PhD Dissertation, The University of Texas Southwestern Medical Center at Dallas, 1995.
[2] Weiner S, Traub W. Bone structure: from angstroms to microns. FASEB 1992;6:879–85.
[3] Landis WJ. The strength of a calcified tissue depends in part on the molecular structure and organization of its constituent mineral crystals in their organic matrix. Bone 1995;16:533–44.
[4] Reilly DT, Burstein AH, Frankel VH. The elastic modulus of bone. J Biomech 1974;7:271–5.
[5] Choi K, Kuhn JL, Ciarelli MJ, Goldstein SA. The elastic moduli of human subchondral trabecular, and cortical bone tissue and the size-dependency of cortical bone modulus. J Biomech 1990;23:1103–13.
[6] Rho JY, Tsui TY, Pharr GM. Elastic properties of human cortical and trabecular lamellar bone measured by nanoindentation. Biomaterials 1997;18:1325–30.
[7] Bundy KJ. Composite material models for bone. In: Cowin SC, editor. Bone mechanics. Boca Raton, Florida: CRC Press, 1989:197–210.
[8] Mammone JF, Hudson SM. Micromechanics of bone strength and fracture. J Biomech 1993;26:439–46.
[9] Carter DR, Hayes W. The compressive behavior of bone as a two-phase porous structure. J Bone Jt Surg 1977;59A:954–62.
[10] Gibson LJ. The mechanical behavior of cancellous bone. J Biomech 1985;18:317–28.
[11] Enlow DH. Principles of bone remodeling. Springfield, IL: Charles C Thomas, 1963.
[12] Enlow DH. The human face: An account of the postnatal growth and development of the craniofacial skeleton. New York: Harper and Row, 1968.
[13] Carter DR, Schwab GH, Spengler DM. Tensile fracture of cancellous bone. Acta Orthop Scand 1980;51:733–41.
[14] Keller TS, Mao Z, Spengler DM. Young's modulus, bending strength, and tissue physical properties of human compact bone. J Orthop Res 1990;8:592–603.
[15] Choi KW, Goldstein SA. The fatigue properties of bone tissues on a microstructural level. Trans. 37th Orthop. Res. Soc. 1991; 16, 485.
[16] Rice JC, Cowin SC, Bowman JA. On the dependence of the elasticity and strength of cancellous bone on apparent density. J Biomech 1988;21:155–68.
[17] Rho JY, Ashman RB, Turner CH. Young's modulus of trabecular and cortical bone material: ultrasonic and microtensile measurements. J Biomech 1993;26:111–9.

[18] Ashman RB, Rho JY. Elastic modulus of trabecular bone material. J Biomech 1988;21:177–81.
[19] Juist J. In vivo determination of the elastic response of bone 2. Ulnar resonant frequency in osteoporotic, diabetic and normal subjects. Phys Med Biol 1970;15:427–34.
[20] Christensen A, Ammitzboll F, Dyrbye C, Cornilessen M, Cornilessen P, Van Der Perre G. Assessment of tibial stiffness by vibration testing in situ: I. Identification of mode shapes in different supporting conditions. J Biomech 1986;19:53–60.
[21] Rho JY, Hobatho MC, Ashman RB. Relations of density and CT numbers to mechanical properties for human cortical and cancellous bone. Med Eng Phys 1995;17:347–55.
[22] Goldstein S. The mechanical properties of trabecular bone: dependence on anatomic location and function. J Biomech 1987;20:1055–61.
[23] Rho JY. Mechanical properties of human cortical and cancellous bone. PhD Dissertation, The University of Texas Southwestern Medical Center at Dallas, Dallas, TX, 1991.
[24] VanRietbergen B, Odgaard A, Kabel J, Huiskes R. Direct mechanics assessment of elastic symmetries and properties of trabecular bone architecture. J Biomech 1996;29:1653–7.
[25] Odgaard A, Kabel J, VanRietbergen B, Dalstra M, Huiskes R. Fabric and elastic principal directions of cancellous bone are closely related. J Biomech 1997;30:487–95.
[26] Linde F, Hvid I. The effect of constraint on the mechanical behaviour of trabecular bone specimens. J Biomech 1989;22:485–90.
[27] Odgaard A, Linde F. The underestimation of Young modulus in compressive testing of cancellous bone specimens. J Biomech 1991;24:691–8.
[28] Keaveny TM, Pinilla TP, Crawford RP, Kopperdahl DL, Lou A. Systematic and random errors in compression testing of trabecular bone. J Orthop Res 1997;15:101–10.
[29] Bullough P. Atlas of orthopaedic pathology. New York: Gower Medical Publishing, 1992.
[30] Ascenzi A, Bonucci E. The tensile properties of single osteons. Anat Rec 1967;158:375–86.
[31] Ascenzi A, Bonucci E. The compressive properties of single osteons. Anat Rec 1968;161:377–92.
[32] Ascenzi A, Baschieri P, Benvenuti A. The bending properties of single osteons. J Biomech 1990;23:763–71.
[33] Ascenzi A, Baschieri P, Benvenuti A. The torsional properties of single selected osteons. J Biomech 1994;27:875–84.
[34] Lakes R. On the torsional properties of single osteons [letter]. J Biomech 1995;28:1409–10.
[35] Katz LJ. Mechanical properties of bone. ASME 1981; AMD vol 45:171–184.
[36] Marotti G. A new theory of bone lamellation. Calcif Tissue Int 1993;53(suppl 1):S47–56.
[37] Wolff J. Das Gesetz der Transformation der Knochen. Berlin: Hirschwald, 1892.
[38] Runkle JC, Pugh J. The micro-mechanics of cancellous bone. Bull Hosp Jt Dis 1975;36:2–10.
[39] Townsend PR, Rose RM, Radin EL. Buckling studies of single human trabeculae. J Biomech 1975;8:199–201.
[40] Williams JL, Lewis JL. Properties and an anisotropic model of cancellous bone from the proximal tibial epiphysis. J Biomech Eng 1982;104:50–6.
[41] Hodgskinson R, Currey JD, Evans GP. Hardness, an indicator of the mechanical competence of cancellous bone. J Orthop Res 1989;7:754–8.
[42] Kuhn JL, Goldstein SA, Choi KW, London M, Feldkamp LA, Matthews LS. Comparison of the trabecular and cortical tissue moduli from human iliac crests. J Orthop Res 1989;7:876–84.
[43] Mente PL, Lewis JL. Experimental method for the measurement of the elastic modulus of trabecular bone tissue. J Orthop Res 1989;7:456–61.
[44] Ryan CB, Williams JL. Tensile testing of rodlike trabeculae excised from bovine femoral bone. J Biomech 1989;22:351–5.
[45] Rho JY, Roy M, Tsui TY, Pharr GM. Young's modulus and hardness of trabecular and cortical bone in various directions determined by nanoindentation. In: Transactions of the 43th Annual Meeting of the Orthopaedic Research Society, San Francisco, CA, 1997:891.
[46] Giraud-Guille MM. Twisted plywood architecture of collagen fibrils in human compact bone osteons. Calcif Tissue Int 1988;42:167–80.
[47] Frasca P, Harper RA, Katz JL. Collagen fiber orientations in human secondary osteons. Acta Anat 1977;98:1–13.
[48] Albright J. The scientific basis of orthopaedics. Connecticut: Appleton and Lange, 1987:289–324.
[49] Hasegawa K, Turner CH, Burr DB. Contribution of collagen and mineral to the elastic anisotropy of bone. Calcif Tissue Int 1994;55:381–6.
[50] Turner CH, Burr DB. Orientation of collagen in osteonal bone [letter]. Calcif Tissue Int 1997;60:90.
[51] Turner CH, Chandran A, Pidaparti RMV. The anisotropy of osteonal bone and its ultrastructural implications. Bone 1995;17:85–9.
[52] Pidaparti RM, Chandran A, Takano Y, Turner CH. Bone mineral lies mainly outside collagen fibrils: predictions of a composite model for osteonal bone. J Biomech 1996;29:909–16.
[53] Willems G, Celis JP, Lambrechts P, Braem M, Vanherle G. Hardness and Young's modulus determined by nanoindentation technique of filler particles of dental restorative materials compared with human enamel. J Biomed Mat Res 1993;27:747–55.
[54] Glimcher M. Mechanisms of calcification: role of collagen fibrils and collagen–phosphoprotein complexes in vitro and in vivo. Anat Rec 1989;224:139–53.
[55] Kuhn-Spearing L, Rey C, Kim HM, Glimcher MJ. Carbonated apatite nanocrystals of bone. In: Bourell DL, editor. Synthesis and processing of nanocrystalline powder. The Minerals, Metals and Materials Society, Warrendale, PA, USA, 1996.
[56] Ziv V, Weiner S. Bone crystal size: a comparison of transmission electron microscopic and x-ray diffraction line-width-broadening techniques. Conn Tissue Res 1994;30:165–75.
[57] Rey C, Miquel JL, Facchine L, Legrand AP, Glimcher MJ. On the question of hydroxyl groups in bone mineral. Bone 1995;16:583–6.
[58] Currey JD. Physical characteristics affecting the tensile failure properties of compact bone. J Biomech 1990;23:837–44.
[59] Lakes R, Katz LJ. Viscoelastic properties of wet cortical bone—2. Relaxation mechanisms. J Biomech 1979;12:679–87.
[60] Lees S, Davidson CL. The role of collagen in the elastic properties of calcified tissues. J Biomech 1977;10:473–86.
[61] Currey JD. Three analogies to explain the mechanical properties of bone. Biorheology 1964;2:1–10.
[62] Sweeney A, Byers R, Kroon R. Mechanical characteristics of bone and its constituents. ASME Human Factors Conference 1965, 65-WA (HUF-7), American Society of Mechanical Engineers, New York, 1–17.
[63] Piekarski K. Analysis of bone as a composite material. Int J Eng Sci 1973;11(6a):557.
[64] Katz LJ. Hard tissue as a composite material—1. Bounds on elastic behavior. J Biomech 1971;4:455–73.
[65] Currey JD. The relationship between the stiffness and the mineral content of bone. J Biomech 1969;2:477–80.
[66] Zioupos P, Currey JD, Mirza MS, Barton DC. Experimentally determined microcracking around a circular hole in a flat plate of bone: comparison with predicted stresses. Philos Trans R Soc London, Ser B 1995;347:383–96.
[67] Braidotti P, Branca FP, Sciubba E, Stagni L. An elastic compound tube model for a single osteon. J Biomech 1995;28:439–44.

[68] Braidotti P, Branca FP, Stagni L. Scanning electron microscopy of human cortical bone failure surfaces. J Biomech 1997;30:155–62.
[69] Sasaki N, Ikawa T, Fukuda A. Orientation of mineral in bovine bone and the anisotropic mechanical properties of plexiform bone. J Biomech 1991;24:57–61.
[70] Wagner HD, Weiner S. On the relationship between the microstructure of bone and its mechanical stiffness. J Biomech 1992;25:1311–20.
[71] Halpin JC, Tsai SW. Environmental factors in composite design. Air Force Materials Laboratory Technical Report, 1967, AFML-TR-67-423.
[72] Currey JD, Brear K, Zioupos P. Dependence of mechanical properties on fibre angle in Narwhal tusk, a highly oriented biological composite. J Biomech 1994;27:885–97.
[73] Katz JL, Ukraincik K. On the anisotropic elastic properties of hydroxyapatite. J Biomech 1971;4:221–7.
[74] Gilmore RS, Katz JL. Elastic properties of apatites. Proceedings of an International Symposium on the Structural Properties of Hydroxyapatite and Related Compounds. Washington, DC: National Bureau of Standards.
[75] Zioupos P, Currey JD, Casinos A, Buffrenil De V. Mechanical properties of the rostrum of the whale Mesoplodon densirostris: a remarkably dense bony tissue. J Zool London 1997;241:725–37.
[76] Jonas J, Burns J, Abel EW, Cresswell MJ, Strain JJ, Paterson CR. A technique for the tensile testing of demineralised bone. J Biomech 1993;26:271–6.
[77] Broz JJ, Simske SJ, Greenberg AR. Material and compositional properties of selectively demineralized cortical bone. J Biomech 1995;28:1357–68.
[78] Bowman SM, Zeind J, Gibson LJ, Hayes WC. The tensile behavior of demineralized bovine cortical bone. J Biomech 1996;29:1497–501.
[79] Aerssens J, Dequeker J, Mbuyi-Muamba J-M. Bone tissue composition: Biochemical anatomy of bone. Clin Rheumat 1994;13(S1):54–62.