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Anisotropy in the compressive mechanical properties of bovine cortical bone and the mineral and protein constituents

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ABSTRACT

The mechanical properties of fully demineralized, fully deproteinized and untreated cortical bovine femur bone were investigated by compression testing in three anatomical directions (longitudinal, radial and transverse). The weighted sum of the stress–strain curves of the treated bones was far lower than that of the untreated bone, indicating a strong molecular and/or mechanical interaction between the collagen matrix and the mineral phase. Demineralization and deproteinization of the bone demonstrated that contiguous, stand-alone structures result, showing that bone can be considered an interpenetrating composite material. Structural features of the samples from all groups were studied by optical and scanning electron microscopy. Anisotropic mechanical properties were observed: the radial direction was found to be the strongest for untreated bone, while the longitudinal one was found to be the strongest for deproteinized and demineralized bones. A possible explanation for this phenomenon is the difference in bone microstructure in the radial and longitudinal directions.

1. Introduction

Bone is a hierarchically structured composite material consisting mainly of a biopolymer (type I collagen), a mineral phase (carbonated hydroxyapatite) and water. There is also an amount of non-collagenous proteins that "glue" the collagen fibers together and attach the mineral to the collagen [1,2]. The structure and mechanical properties of the major bone constituents have been investigated by many research groups for several decades, including seminal works by Currey [3–5], Reilly and Burstein [6], Burstein et al. [7], and Rho et al. [8]. The mechanical properties of cortical bone are highly anisotropic, therefore, significant efforts have been made to examine the properties of bone in different anatomical directions [9–12]. Fig. 1 shows the orientation of the longitudinal, radial and transverse bone directions. One should keep in mind that measured strengths and stiffness values for bone are highly dependent on the test method, hydration condition, age, gender, histology, porosity and mineral content.

Reilly and Burstein [9] investigated the anisotropic compressive and tensile properties of cortical bone and found that the Young's modulus and maximum strength in the longitudinal direction are more than twice those in the transverse and radial directions. Bonfield and Grynpas [10] studied the mechanical anisotropy of cortical bone at varying angles to the bone growth direction (the longitudinal direction corresponded to 0°, the transverse direction to 90°) by ultrasonic measurement. They found that the Young's modulus gradually decreased with increasing angle (from 0° to 90°), and there was a plateau between 20° and 70°. Information on the mechanical properties in the radial direction was not reported. The bulk mechanical properties of bone are greatly affected by its microstructural features. Two types of bone are found in cortical bone, namely osteonal bone and periosteal bone, as shown in Fig. 1. Osteonal bone consists of osteons made up of thin (2–6 μm) lamellar sheets oriented in a concentric cylindrical structure. These osteons are 150–250 μm in diameter and align parallel along the long axis of bone. Interstitial lamellae (remnants after bone remodelling) occupy the space around the osteons. Periosteal bone consists of a circumferential lamellae structure which is parallel to the bone surface and is made of fibrolamellar bone. The periosteal bone is reported to be stronger and more highly anisotropic than osteonal bone [4]. The elastic properties of microstructural components in human and bovine osteonal bone have been investigated by several groups using nanoindentation. Rho et al. [13] showed that the Young's modulus of the interstitial lamellae (~26 GPa) was higher than that in the osteons (~22 GPa) in the longitudinal

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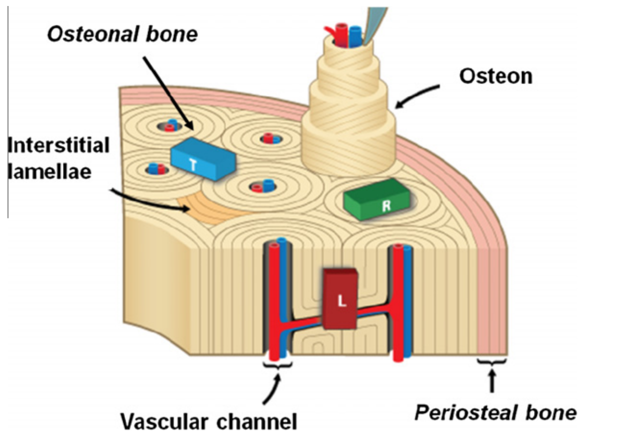

Fig. 1. Schematic diagram of bone microstructure and sample orientations for the three anatomical directions in cortical bone. Sample orientations: L, longitudinal; R, radial; T, transverse. The samples are not shown to scale.

direction for human cortical bone. The average Young's modulus (including both osteons and interstitial lamellae) in the transverse direction was found to be ~17 GPa. Swadener et al. [14] and Fan et al. [15] proposed and verified methods to predict the nanoindentation moduli for different bone directions based on the previous ultrasound studies by Rho [16]. A possible mechanism for bone anisotropy at the 10–100 μm scale was suggested by Seto et al. [17]. They performed tensile experiments on relatively small samples (a fibrolamellar unit) obtained from the periosteal region (see Fig. 1). An extremely high mechanical anisotropy in the Young's modulus (of the order of 1:20) and tensile strength (of the order of 1:15) between the transverse and longitudinal directions in wet bovine femur bone was reported. Furthermore, they proposed that the periodic presence of mechanically weak heterogeneous layers filled with soft organic constituents inside the fibrolamellar bone accounted for this high anisotropy. These weak interfaces act as damping elements and suppress crack propagation on the 10–100 μm scale.

One of the main reasons for bone anisotropy is the preferential orientation of collagen fibers and mineral crystals along the bone growth direction. This topic has been investigated by several groups [18–20]. Landis et al. [18] investigated the ultrasound interaction between collagen and mineral crystals in chicken bone by high voltage electron microscopic tomography and found that individual platelet-shaped mineral crystals were periodically arranged along collagen fibrils preferentially aligned along the main bone axis. Martin et al. [19,20] found that the longitudinal fiber orientation in cortical bone contributed greatly to the increased elastic modulus and strength in four point bending.

The mineral/protein interaction is important to understand how bone constituents affect the mechanical properties. The mechanical properties of the protein and mineral constituents can be investigated separately by demineralization and deproteinization, respectively. Mechanical testing results in compression and tension on deproteinized bone were summarized by Piekarski [21] and Mack [22], but information on the orientation of the bone was not provided. Burstein et al. [7] investigated the tensile mechanical properties of partially demineralized bone using HCl solution at varying concentration. They found that bone in tension demonstrated plastic behavior: the yield point and maximum strength progressively decreased as demineralization proceeded, while the slope of the plastic region was the same for all demineralization stages. These findings demonstrated that bone stiffness in the plastic region is a function of collagen properties only. The contribution of the two main bone constituents to elastic anisotropy was investigated by Hasegawa et al. [11] and Iyo et al. [23]. Hasegawa et al. [11] performed acoustic velocity measurements on demineralized and deproteinized dog femur in the longitudinal and transverse directions. They found that the collagen matrix is highly isotropic and proposed that the minerals play the major role in the anisotropic behavior of whole bone. Iyo et al. [23] investigated the effect of mechanical anisotropy on Young's modulus relaxation. Their model consisted of a combination of two processes: a fast one, attributed to relaxation of the collagen matrix, and a slow one, attributed to the mixture of collagen and mineral phases. Moreover, they suggested that the latter process, corresponding to both collagen and mineral constituents, was responsible for the anisotropic behavior of bone, in contrast to what had been suggested by Hasegawa et al. [11]. A detailed examination of the mechanical properties of the major bone constituents (mineral and collagen parts) in different anatomical directions is important to better understand the mechanical behavior of bone. Skedros et al. [24] used acoustic microscopy to evaluate the elastic modulus of untreated, demineralized and deproteinized cortical bone of deer calcanei for different bone cortices. It was found that the anisotropy ratio, defined as the ratio between the acoustic velocity squared for the longitudinal and transverse bone directions, was significantly different from that for both demineralized and deproteinized bone, demonstrating that not only untreated bone, but also the main bone constituents (the mineral and collagen phases) were anisotropic. The anisotropy ratio was higher for cortices that were adapted for tension and compression, and were less for cortices that were adapted for a combination of compression/shear or tension/shear. These results clearly indicate that the degree of anisotropy of bone greatly depends on its functions and adaptations. Macione et al. [12] investigated the properties of partially demineralized bone using an ultrasound technique. They showed that the elastic modulus in the longitudinal direction could be predicted using ultrasound measurements on the transverse and radial directions.

The mechanical properties of demineralized and deproteinized cancellous bone were recently studied by several groups. Chen et al. [25] developed and verified methods to fully demineralize and fully deproteinize cancellous bovine femur bone without altering the microstructure. It was found that minerals form a continuous, stand-alone structure after removing all the protein, and mature cancellous bone was indeed an interpenetrating composite, in agreement with Rosen et al. [26], who found a well-organized mineral structure in deproteinized bovine cortical bone. The compressive mechanical properties of demineralized and deproteinized cancellous bone were further investigated by Chen and McKittrick [27]. It was shown that both the relative elastic modulus and compressive strength increased with relative density. Moreover, a strong synergistic effect between the mineral and protein phases was found and rule of mixture did not apply, proving strong chemical bonding and interactions between the two phases. Lubarda et al. [28] derived the elastic modulus of untreated cancellous bone based on the measured properties of the mineral and protein phases in order to understand osteoporotic degradation. The demineralization kinetics for cancellous and cortical bone were thoroughly studied by Castro-Ceseña et al. [29]. It was shown that the mineral and protein phases of cortical bone are independent structures that can be mechanically tested, corroborated the findings of Chen et al. [25], but mechanical testing was not performed.

To the best of our knowledge there has been no study of the mechanical properties of demineralized and deproteinized cortical bone as a function of anatomical direction, which is the goal of this study.