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Contributions of Trabecular Rods of Various Orientations in Determining the Elastic Properties of Human Vertebral Trabecular Bone

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Abstract

Trabecular bone networks consist of two basic microstructural types: plates and rods. Although trabecular rods represent only a small fraction of total bone volume, their existence has important roles in failure initiation and progression. The goal of this study was to quantitatively examine the contributions of trabecular rods in various orientations to the anisotropic elastic moduli of human vertebral trabecular bone. Twenty-one human vertebral trabecular bone specimens were scanned by micro computed tomography (μ CT). A coordinate system of orthotropic axes representing the best elastic orthotropic symmetry was determined for each sample. Individual trabeculae segmentation (ITS), a 3D image analysis technique, was performed to identify each individual trabecular rod and determine its orientation in the orthotropic coordinate system. Next, three rod-removed images were created where longitudinal, oblique, or transverse trabecular rods were removed, respectively, from the original μ CT images. The original and three categories of rod-removed images were then converted to finite element (FE) models for evaluation of their elastic moduli and anisotropy. Both the transverse and oblique rod-removal caused significant decreases in all six elastic moduli. However, the removal of longitudinal rods only caused significant changes in E_{33} , G_{23} , and G_{31} but not in any transverse/in-plane elastic properties (E_{11} , E_{22} , and G_{12}). The analysis of covariance (ANCOVA) with repeated measures was applied to detect the moduli change in the different models caused by the effects beyond just bone volume loss. The results suggested that the loss of transverse rods induced a significant decrease in in-plane mechanical competence, which was greater than what could be explained only by the associated bone volume loss. In contrast, the reduction in the axial Young's modulus caused by the loss of transverse rods was proportional to the bone volume decrease. Furthermore, the loss of longitudinal rods affected the axial Young's modulus through both bone volume loss and architectural change. With aging, the reduction in in-plane mechanical competence would be magnified by the preferential loss of transverse rods. The predictive ability of bone mineral density, a surrogate of BV/TV in clinical measurements, may reduce more quickly for transverse mechanical properties than for the axial mechanical properties.

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Conflict of Interest:

Drs. Guo and Liu are inventors of software derived from this work. All other authors have no relevant conflicts of interest.

Keywords

bone microstructure; individual trabeculae segmentation; micro computed tomography; trabecular orientation; trabecular rod

Introduction

Advanced imaging techniques have extended traditional X-ray computed tomography (CT) and magnetic resonance imaging (MRI) techniques to be able to depict trabecular bone microstructure at a resolution of 10 μm , which is equivalent to $\sim 1/10$ of the thickness of an individual trabecula. On the basis of *in vivo* μMRI of trabecular structure, Wehrli *et al.* first provided *in vivo* evidence that osteoporosis is associated with a conversion of trabecular plates to rods and eventual disconnections of trabeculae [1]. Using μCT imaging and a finite element (FE) modeling technique, recent studies have quantitatively evaluated the roles of trabecular plates and rods on the mechanical properties of trabecular bone, and they suggested that trabecular plates play an important role in determining apparent Young's moduli and yield strength of human trabecular bone [2–5]. Nevertheless, the existence of a certain number of trabecular rods may be able to optimize the balance of mechanical competence and the energy to maintain the physiological environment of trabecular bone at various anatomic locations. This view is supported by a quantitative study of trabecular bone failure initiation and progression which demonstrated that yielding initiates in trabecular rods [2]. Hence, trabecular rods could be one of the important factors that determine the ability of trabecular bone to resist damage initiation and accumulation.

The arrangement and orientation of individual trabeculae are highly adapted to their mechanical environment. A recent study based on the analysis of femoral, tibial, and vertebral trabecular bone microstructure showed that the majority of the trabecular plates align with the longitudinal axis, whereas more than half of the trabecular rods aligned with the transverse plane [3]. Moreover, a quantitative study of vertebral trabecular bone under compressive loading demonstrated that longitudinal trabecular plates withstand most axial loading while transverse trabecular rods serve as essential links to stabilize the whole structure [2]. It has been found that changes in trabecular orientations are often associated with aging [6–8]. Some studies have suggested that in vertebral trabecular bone there is a greater relative loss and thinning of transverse trabeculae, and maintenance or even increase in thickness of vertical trabeculae with aging [7,9]. As the percentage of trabecular rods in a trabecular bone network increases with aging, evaluating the contributions of trabecular rods in different orientations to the mechanical properties of trabecular bone will improve our understanding of trabecular bone micromechanics and provide quantitative knowledge in the etiology of osteoporosis.

The key technique involved in this study was the explicit classifications of trabecular type and orientation assessment at an individual trabecula level. The individual trabeculae segmentation (ITS) technique [3], which spatially decomposes the trabecular bone microstructure into individual trabecular plates and rods, has been developed recently and used to study moduli-morphology relationships [3,4], the failure process of individual trabeculae [2], and the efficacy of osteoporosis treatment [10]. In this study, μCT -based finite element (FE) models of human vertebral trabecular bone were used to quantitatively examine the contributions of variously oriented trabecular rods to elastic moduli and anisotropy. With the aid of the ITS-based orientation analysis, three rod-removed models were created where either longitudinal, oblique, or transverse trabecular rods were removed from the original μCT images of trabecular bone, respectively. The original and three categories of rod-removed images were then converted to FE models for evaluation of their apparent mechanical properties. Subsequently, elastic moduli and anisotropy of rod-removed models were statistically compared to those of

the original model. In this manner, the contributions of trabecular rods in various orientations to apparent mechanical properties of trabecular bone were quantitatively examined.

Materials and Methods

Trabecular Bone Sample Preparation and Imaging

Twenty-one cylindrical on-axis human trabecular bone cores (~8 mm in diameter and ~20 mm in length) oriented in the superior-inferior direction were harvested from L3 (n=2), L4 (n=14), and L5 (n=1) vertebral bodies of seventeen donors (8 males/9 females; age 74 ± 14) [4,11]. The subjects were screened to exclude metabolic bone diseases or bone cancer, and X-ray radiographs were taken to ensure that there was no evidence of damage or other bone pathologies. Samples were scanned at $21 \mu\text{m}$ resolution using a μCT system (μCT 20, Scanco Medical AG, Switzerland). The central $\sim 4 \times 4 \times 4 \text{ mm}^3$ cubical sub-volume of each specimen was extracted from the reconstructed image and thresholded using a global thresholding technique where the minimum between the bone and bone marrow peaks in the voxel gray value histogram was chosen as the threshold value. Isolated voxels or disconnected voxel-clusters were removed from the largest bone component [12,13] and the resulting images were used for the further analyses.

Orthotropic Coordinate System and Stiffness Tensor of Original μCT Images by FE Analysis

A FE model was generated from each image by converting voxels to 8-node brick elements. The FE model based on the original μCT image was referred to as “original model” in this paper. The trabecular bone tissue was modeled as an isotropic, linear elastic material with a Young’s modulus (E_s) of 15 GPa and a Poisson’s ratio of 0.3 for all the models [14]. Using an element-by-element pre-conditioned conjugate gradient solver [15], six FE analyses were performed for each model, representing three uniaxial compression tests along three image axes (x, y, and z) and three uniaxial shear tests. The general anisotropic stiffness matrix was first determined based on the results from the above analyses, and then a new coordinate system of orthotropic axes (X_1 , X_2 , and X_3) representing the best orthotropic symmetry was calculated using an optimization procedure [16]. Axis X_3 was along the longitudinal direction of vertebral body. On average, the angle between axis X_3 of the orthotropic coordinate and the principal orientation of trabeculae calculated by the fabric tensor [17] was within ± 10 degrees.

The tensor transformation of the anisotropic stiffness matrix to a new coordinate system yielded the full orthotropic stiffness matrix [18]. The elastic moduli (three Young’s moduli $E_{11} < E_{22} < E_{33}$ and three shear moduli G_{23} , G_{31} , G_{12}) were then derived from the orthotropic stiffness matrix. Elastic anisotropy, defined as the ratio between the maximum and minimum Young’s modulus (E_{33}/E_{11}), was calculated for each original model.

ITS-Based Morphological Analyses

ITS, a 3D image analysis technique, was performed to fully decompose the trabecular bone microarchitecture into individual trabecular plates and rods according to their topology and geometry [3]. The detailed description of the algorithms of ITS and the illustrations on trabecular bone images can be found elsewhere [3,4].

The orientation of each trabecular rod was obtained by employing 3D principal component analysis on its corresponding curve skeleton. The angle Φ_3 was defined as the angle between the orientation of a trabecular rod and the X_3 axis in the orthotropic coordinate of the trabecular bone image; according to its angle Φ_3 , the orientation of each trabecular rod was determined (longitudinal ($0^\circ \leq \Phi_3 \leq 30^\circ$), oblique ($30^\circ < \Phi_3 \leq 60^\circ$) or transverse ($60^\circ < \Phi_3 \leq 90^\circ$)) (Figure 1). The local diameter along the long axis of each trabecular rod was evaluated and the average was defined as the diameter of the rod. By adopting the American Society of Bone and Mineral

Research bone histomorphometric nomenclature [19], the following morphological parameters were reported: bone volume fraction (BV/TV), plate bone volume fraction (pBV/TV), rod bone volume fraction (rBV/TV), and trabecular rod number density (rTb.N, 1/mm) [3].

Longitudinal/Oblique/Transverse Rod-Removed Models

Based on the segmented trabecular bone image, longitudinal, oblique, or transverse trabeculae were artificially removed from the original 3D μ CT image of trabecular bone to form three rod-removed models, respectively (Figure 2). Three sets of specimen-specific FE models based on rod-removed μ CT images were constructed for each specimen: (1) longitudinal rod-removed; (2) oblique rod-removed; and (3) transverse rod-removed models. In all rod-removed models, trabecular plates were untouched. The FE analyses (three uniaxial compression and three shear tests) as described before were performed on all three rod-removed models of each specimen. For each rod-removed model, three Young's moduli, three shear moduli, and the elastic anisotropy under the orthotropic coordinates (X_1 , X_2 , and X_3) of the corresponding original model were calculated.

Statistical Analyses

Analysis of variance (ANOVA) with repeated measures and the Tukey Honestly Significance Difference (HSD) *post-hoc* test were used to investigate any significant difference ($p < 0.05$) of BV/TV, elastic moduli, and anisotropy between the original model and the rod-removed models. The moduli-density relationships between the original model and the three rod-removed models were compared by the analysis of covariance (ANCOVA) test with repeated measures. The repeated measures ANCOVA was able to determine whether architectural changes in rod-removed models caused the different levels of reductions in elastic moduli after accounting for the effect of bone volume loss. The ANCOVA test was performed using SPSS software (Version 10.0; SPSS, Chicago, IL, US). All the other statistical analyses were performed using KaleidaGraph 3.6 software (Synergy Software, Reading, PA).

Results

For each category of rod-removed models, there were significant changes in BV/TV and rBV/TV compared to the original model; pBV/TV remained constant for each sample as trabecular plates were unchanged (Table 1). Transverse rods were found to represent $60.3 \pm 8.1\%$ of all rods in terms of bone tissue volume, and $58.2 \pm 6.0\%$ of all rods in terms of trabecular number. Longitudinal rods represented $12.5 \pm 6.2\%$ of all rods in terms of bone volume, and $12.2 \pm 4.7\%$ of all rods in terms of rod number. Oblique rods represented $27.2 \pm 8.3\%$ and $29.7 \pm 6.8\%$ of all rods in terms of bone tissue volume and trabecular number, respectively. On average, the reduction in BV/TV caused by the removal of rods in different orientations was significantly different (transverse > oblique > longitudinal rod-removal, $p < 0.05$) (Figure 3).

A significant percentage reduction in all elastic constants was observed when transverse or oblique rods were removed; in contrast, removal of longitudinal rods did not affect the in-plane/transverse elastic constants E_{11} , E_{22} , and G_{12} (Table 2 and Figure 3). The greatest reduction occurred in the transverse elastic properties E_{11} , E_{22} , and G_{12} followed by the removal of transverse rods. Except for the elastic modulus E_{33} , the removal of transverse rods resulted in the largest reductions in elastic moduli whereas the removal of longitudinal rods resulted in the least reductions. The difference between the reductions in moduli caused by rod removal (transverse > oblique > longitudinal rod-removal) was statistically significant as shown by an ANOVA test. For the elastic modulus E_{33} , the reduction caused by the removal of longitudinal rods was statistically equal to that caused by the removal of oblique and transverse rods. Furthermore, the elastic anisotropy increased significantly after the removal of transverse rods but remained constant ($p > 0.05$) after the removal of oblique or longitudinal rods (Table 2).

Results from the ANCOVA test indicated that BV/TV-moduli relationships were significantly changed in E_{11} , E_{22} , G_{12} , and G_{31} ($p \leq 0.01$) and remained the same in E_{33} , and G_{23} ($p > 0.05$) after transverse rod-removal. These results suggest that reduction in E_{33} and G_{23} following transverse rod-removal could be explained by the changes in BV/TV. However, the cause of significant reduction in E_{11} , E_{22} , G_{12} and G_{31} was greater than what could be explained only by the loss in bone volume. In contrast, BV/TV- E_{33} relationship changed after longitudinal rod-removal, suggesting that the significant change in elastic modulus E_{33} was caused by factors beyond BV/TV ($p = 0.02$). The oblique ($p = 0.034$) rod-removal had a significant influence on shear modulus G_{31} independent of bone volume change. However, other than the direct effect caused by bone volume loss, removal of any category of rods did not have an impact on shear modulus G_{23} (Figure 4 and Table 3).

Discussion

Trabecular bone networks consist of two basic microstructural types: trabecular plates and rods. Earlier studies have demonstrated the importance of plate-like structure in determining the Young's moduli and strength of trabecular bone [2–5]. Although trabecular rods represent only ~15% of total bone volume, their existence has important roles in damage initiation and progression while trabecular bone undergoes daily loading or abnormal loading. As orientations of trabeculae are influenced by its mechanical environment due to bone adaptation, the differently oriented rods may have various biomechanical functions. In this study, with the aid of the ITS-based orientation analysis, each individual trabecular rod was classified as longitudinal, oblique, or transverse in the 3D trabecular bone microstructure. Transverse rods were found to be dominant in the total rod bone volume and total rod number while longitudinal rods had the least bone tissue volume and the lowest trabecular number. Both the transverse and oblique rod-removed models had significant decreases in all six elastic moduli. However, longitudinal rod-removal only caused significant changes in E_{33} , G_{23} , and G_{31} , but not in any transverse/in-plane elastic properties (E_{11} , E_{22} , and G_{12}). It is intriguing that the removal of the transverse rods caused the same reduction as the longitudinal rods in E_{33} , the Young's modulus in the longitudinal axis. A previous study has reported that transverse trabecular rods are essential links stabilizing the whole structure under compressive loading [2]. The findings of this study confirmed the importance of transverse trabecular rods in axial elastic modulus as well as in the transverse elastic properties of trabecular bone.

Since the elastic moduli are dependent on bone volume fraction, without comparing the moduli-BV/TV relationships between different models, it is not clear whether the significant reduction in moduli of transverse rod-removed model was caused by a fundamental architectural change or merely by bone volume change. The ANCOVA test was able to explore the differences in moduli changes of various model types with statistical blockage of the bone volume effect. As expected, the removal of transverse rod was indicated to have a significant influence on in-plane elastic properties E_{11} , E_{22} , and E_{12} independently of bone volume change. However, the removal of oblique and longitudinal rods no longer retained influence on any of the in-plane elastic moduli E_{11} , E_{22} , and E_{12} after the bone volume effect was taken into consideration. In contrast, for Young's modulus in the longitudinal direction, even though longitudinal rods only represented a small portion of total rod bone volume, their removal was the only statistically significant factor that associated with the reduction in E_{33} by architectural change. Hence, we conclude that in addition to the associated bone volume effect, loss of transverse rods significantly impairs in-plane elastic properties of trabecular bone, whereas longitudinal-axial Young's modulus is influenced only by the loss of longitudinal rods. The results for the out of plane shear moduli G_{23} and G_{31} are more complicated. Indeed, G_{23} and G_{31} involve complex interactions of trabeculae in various orientations. The underlying mechanisms cannot be quantified in the current study as transverse directions (anterior-posterior or medial-lateral) were not monitored. This could be explored in the future study.

Certain limitations were associated with this study. First, the cubic bone images were extracted from cylindrical bone cores, and therefore the relationship between anatomic directions and X_1 and X_2 axes in the elastic orthotropic coordinates was unknown. The anatomic orientations, including anterior-posterior and medial-lateral, of each sample should be more carefully monitored in the future study. Second, bone tissue was modeled as an isotropic and elastic material and assumed to be homogeneous within each specimen. However, variations in mineralization and mechanical properties exist in trabecular bone tissue. More studies are required to obtain data on trabecular tissue properties of various trabecular types/orientations. It will be of interest to incorporate these details in future analyses.

In conclusion, we utilized the ITS-based 3D orientation analysis and μ CT-based FE analysis to quantify the roles of longitudinal, oblique, and transverse trabecular rods to anisotropic elastic moduli. It has been shown for the first time that the removal of transverse rods could induce a significant decrease in in-plane mechanical competence, which was greater than what could be explained by the associated bone volume loss. In contrast, the reduction in the axial Young's modulus caused by the loss of transverse rods was proportional to the bone volume decrease. Furthermore, the bone volume and architectural change caused by the loss of longitudinal rods only affected the axial Young's modulus. Previous studies have shown a preferential loss of transverse trabeculae over longitudinal trabeculae with aging [20,21]. If that is also true for trabecular rods, the adverse effects of these processes on in-plane mechanical competence would be magnified with the preferential loss of transverse rods given the critical role of transverse rods implied by the current study. Bone mineral density (BMD), a surrogate of BV/TV, is still widely used as a clinical measurement of bone quality to predict bone strength. With aging, its predictive ability for the transverse mechanical properties may reduce more quickly than that for the axial properties.

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Figure 1.

Illustration of the ITS-based segmentation of trabecular rods on an image of a vertebral trabecular bone sample. According to the angle between the longitudinal axis and trabecular rod orientation, each individual rod was classified as transverse, oblique or longitudinal.

Figure 2.
(A) Original, (B) longitudinal rod-removed model, (C) oblique rod-removed model, and (D) transverse rod-removed model of an image of a vertebral trabecular bone sample.

Figure 3.

The normalized BV/TV and elastic moduli of longitudinal, oblique, and transverse rod-removed model. * indicates significant difference compared to the original model (repeated measures ANOVA tests, $p < 0.05$).

Figure 4.

Correlations between BV/TV and elastic moduli (A) E_{11} , (B) E_{22} , (C) E_{33} , (D) G_{23} , (E) G_{31} , and (F) G_{12} of original, longitudinal rod-removed, oblique rod-removed and transverse rod-removed model. * Indicates significant difference of elastic moduli between rod-removed model and original model independent of bone volume fraction (repeated measures ANCOVA test with BV/TV as covariate, $p < 0.05$).

Table 1

Bone volume fraction, rod bone volume fraction and trabecular rod number (Mean $\pm$ SD) of original and rod-removed models. SD: standard deviation.

	Original	Longitudinal Rod Removed	Oblique Rod Removed	Transverse Rod Removed
BV/TV	0.105 $\pm$ 0.032	0.103 $\pm$ 0.032	0.101 $\pm$ 0.032	0.94 $\pm$ 0.031
rBV/TV	0.017 $\pm$ 0.007	0.015 $\pm$ 0.006	0.013 $\pm$ 0.006	0.007 $\pm$ 0.003
rTb.N	1.61 $\pm$ 0.23	1.54 $\pm$ 0.20	1.44 $\pm$ 0.23	1.20 $\pm$ 0.17

Table 2

Elastic moduli (Mean $\pm$ SD) of original and rod-removed models. SD: standard deviation.

	Original Model	Longitudinal Rod-Removed Model	Oblique Rod-Removed Model	Transverse Rod-Removed Model
E ₁₁ (MPa)	247.0 $\pm$ 132.5	234.6 $\pm$ 126.8	205.4 $\pm$ 116.5	138.9 $\pm$ 85.5
E ₂₂ (MPa)	416.1 $\pm$ 209.6	396.9 $\pm$ 209.1	351.8 $\pm$ 184.9	261.8 $\pm$ 176.1
E ₃₃ (MPa)	673.0 $\pm$ 265.5	569.5 $\pm$ 265.1	577.4 $\pm$ 244.7	532.1 $\pm$ 247.0
G ₂₃ (MPa)	172.5 $\pm$ 99.0	160.1 $\pm$ 98.6	146.5 $\pm$ 88.5	132.6 $\pm$ 93.7
G ₃₁ (MPa)	114.0 $\pm$ 54.1	104.8 $\pm$ 53.7	94.6 $\pm$ 49.0	81.8 $\pm$ 44.7
G ₁₂ (MPa)	101.6 $\pm$ 53.5	96.8 $\pm$ 52.6	86.9 $\pm$ 49.1	64.2 $\pm$ 43.2
Anisotropy	3.14 $\pm$ 1.42	2.74 $\pm$ 1.25	3.27 $\pm$ 1.47	4.57 $\pm$ 2.12

Regression coefficients (SE) between elastic moduli and bone volume fraction of original, longitudinal rod-removed, oblique rod-removed, and transverse rod-removed models. SE: standard error.

	$E = \frac{a+b*(BV/TV)}{}$	Original Model	Longitudinal Rod-Removed Model	Oblique Rod-Removed Model	Transverse Rod-Removed Model
E ₁₁ (MPa)	a	-139.4 (46.3)	-129.6 (42.1)	-127.5 (38.4)	-86.1 (31.9)
	b	3677.1 (422.0)	3546.2 (391.5)	3307.4 (364.3)	2384.8 (321.8)
E ₂₂ (MPa)	a	-209.4 (65.9)	-214.6 (63.5)	-177.4 (60.4)	-193.6 (68.8)
	b	5952.2 (600.6)	5954.0 (591.7)	5257.3 (573.1)	4828.1 (694.9)
E ₃₃ (MPa)	a	-109.1 (88.7)	-178.5 (94.2)	-112.4 (85.0)	-131.3 (86.4)
	b	7443.4 (809.1)	7284.0 (876.8)	6853.0 (806.8)	7032.3 (872.8)
G ₂₃ (MPa)	a	-77.5 (48.9)	-82.7 (47.8)	-74.1 (41.6)	-81.8 (45.6)
	b	2379.0 (445.9)	2364.2 (445.3)	2191.2 (395.1)	2272.0 (460.5)
G ₃₁ (MPa)	a	-50.7 (15.1)	-52.9 (15.9)	-49.9 (13.7)	-43.1 (13.3)
	b	1567.7 (137.57)	1535.4 (147.6)	1435.3 (130.1)	1323.7 (134.0)
G ₁₂ (MPa)	a	-59.6 (15.9)	-58.5 (15.1)	-56.3 (14.5)	-50.7 (15.6)
	b	1534.4 (145.1)	1512.8 (140.4)	1422.2 (138.1)	1218.3 (157.7)