Tissue Mineral Density Dependent Mechanical Properties of Individual Trabecular Plates and Rods Do Not Differ in Anatomic Directions but Individual Trabecular Directions

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Introduction: Human trabecular bone, a crucial bone compartment susceptible to osteoporosis, consists of individual trabecular plates and rods. These trabecular plates and rods are distributed distinctly along the longitudinal, transverse, or oblique anatomic directions of the skeleton. In addition, individual trabecular plates and rods are formed in preferred lamellar layers, parallel to their trabecular directions (Fig 1.A). Therefore, it is expected that mechanical properties of bone tissue are different in alignment with (axial) or against (lateral) the direction of individual trabeculae, i.e., having anisotropic mechanical properties. The tissue properties of individual trabeculae and their dependence on tissue mineral density (TMD) are important bone quality measures. However, anisotropic mechanical properties of individual trabeculae along various anatomic directions are currently not available. The objectives of this study are as follows: to measure anisotropic tissue modulus and TMD of individual trabeculae; to examine their dependence on trabecular type and anatomic direction; and to determine the relationship between anisotropic tissue modulus and TMD of individual trabeculae.

Methods: Twelve cylindrical trabecular bone cores were harvested from human proximal femurs. Trabecular cores were embedded in a weak exothermal plastic (Vishay MicroMeasurements). After curing, the samples were rehydrated and imaged at 25µm resolution by µCT (Scanco Medical) and then analyzed by individual trabecula segmentation (ITS) technique to provide the individual trabecular types and their anatomic directional information [1] (Fig 1.D). Subsequently, the samples were cut and polished with progressive grades of silicon carbide paper (800, 1200, 2400, 4000 grit) and finished with a 0.3µm alumina suspension under wet condition. The polished samples were scanned with five density calibration phantoms (0, 100, 500, 750, 1000 mg HA/cm³) at 12.5µm resolution and 500ms integration time to reduce the noise. Individual trabeculae on the polished surface were subjected to microindentation. The slope at the initiation of unloading on the force-displacement curve was used to calculate the tissue modulus [2]. Three indentation tests were performed under wet condition on both axial and lateral cross-sections (Fig 1.B) of each selected plates and rods in longitudinal (L), transverse (T), and oblique (O) directions, respectively (Fig 1.A, C). The microscope images of selected trabeculae were registered with the corresponding 12.5µm resolution µCT images using a landmark initialized mutual information-based registration toolkit (national library of medicine insight segmentation and registration toolkit, Fig 1.D). The point-by-point registered grayscale values of the µCT image at the indentation sites (average of 4 voxels) were converted to TMD using calibration phantoms. In total, 432 indentations were made and linear regressions were used to correlate anisotropic tissue modulus with TMD, according to trabecular types or anatomic directions.

Results: The tissue modulus and the co-localized TMD of trabecular plates were significantly higher than those of trabecular rods (p<0.001, Fig. 2A, B). The axial tissue modulus of individual trabeculae was significantly higher than the lateral tissue modulus for both trabecular plates and rods in all the three
anatomic directions (p≤0.05, Fig 2.C). In general, the tissue modulus correlated strongly with TMD ($R^2=0.52$). These correlations did not differ significantly between plates and rods or different anatomic directions. However, the axial modulus-TMD correlation was significantly different from that in lateral direction (Fig 2.D).

**Discussion:** The study reported novel findings in mechanical properties of individual trabecular plates and rods. Despite trabecular plates possessing distinct morphology from rods; the differences in their microscopic mechanical properties are mainly a result of the mineralization. For both plates and rods, we confirmed anisotropic elastic moduli dependent on different anatomic directions. Surprisingly, the heterogeneous tissue modulus correlated with TMD regardless of trabecular type and anatomic direction, suggesting that distinctly oriented trabecular plates and rods may have similar mineral/collagen ultrastructure. However, the correlation significantly differs in the axial and lateral trabecular directions, which further indicates that the underlying ultrastructure may play a critical role in the tissue mechanical properties of the individual trabecula. It remains to be seen how these heterogeneous anisotropic tissue properties play a role in whole bone mechanical competence.

**Significance:** This study reported new bone quality measures of individual trabeculae. The heterogeneous and anisotropic tissue properties of the individual trabecula are likely the results of the mineral/collagen ultrastructure. It is important to include heterogeneous anisotropic tissue properties in assessing bone fragility.
Fig 1. Illustration of the anatomic directions, the axial and lateral trabecular directions of individual trabecular plates and rods in proximal femur and TMD measurements. A. Classification of anatomic directions of plates and rods. B. Axial and lateral directions of trabecular plates and rods. C. Selection of individual trabeculae of different types and anatomic directions. D. Image registration of microscope photo (front) and μCT image (back) to locate the indentation sites in μCT image.
Fig 2. Tissue modulus and TMD vary in different trabecular types, anatomic directions and individual trabecular directions. (Plate: n=216; rod: n=216)
A. Plates have higher tissue modulus than rods. *p<0.05;
B. Plates have higher TMD than rods. *p<0.05;
C. Tissue modulus in axial direction is higher than in lateral direction. *p<0.05. P and R denote plate and rod; L, T, O denote longitudinal, transverse and oblique.
D. Tissue modulus closely correlates with TMD. However, the correlations differ between axial and lateral indentation directions, *p<0.05

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