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Prediction of trabecular bone principal structural orientation using quantitative ultrasound scanning

Liangjun Lin, Jiqi Cheng, Wei Lin, and Yi-Xian Qin*

Orthopaedic Bioengineering Research Laboratory, Department of Biomedical Engineering, Stony Brook University, Stony Brook, NY, USA

Abstract

Bone has the ability to adapt its structure in response to the mechanical environment as defined as Wolff's Law. The alignment of trabecular structure is intended to adapt to the particular mechanical milieu applied to it. Due to the absence of normal mechanical loading, it will be extremely important to assess the anisotropic deterioration of bone during the extreme conditions, i.e., long term space mission and disease orientated disuse, to predict risk of fractures. The propagation of ultrasound wave in trabecular bone is substantially influenced by the anisotropy of the trabecular structure. Previous studies have shown that both ultrasound velocity and amplitude is dependent on the incident angle of the ultrasound signal into the bone sample. In this work, seven bovine trabecular bone balls were used for rotational ultrasound measurement around three anatomical axes to elucidate the ability of ultrasound to identify trabecular orientation. Both ultrasound attenuation (ATT) and fast wave velocity (UV) were used to calculate the principal orientation of the trabecular bone. By comparing to the mean intercept length (MIL) tensor obtained from μ CT, the angle difference of the prediction by UV was 4.45° , while it resulted in 11.67° angle difference between direction predicted by μ CT and the prediction by ATT. This result demonstrates the ability of ultrasound as a non-invasive measurement tool for the principal structural orientation of the trabecular bone.

Keywords

Quantitative ultrasound; Non-invasive measurement; Anisotropic structure; Trabecular bone; Ultrasound attenuation; Fast wave velocity

1. Introduction

According to Wolff's law, bone has the ability to adapt its structure in response to the mechanical environment, and the alignment of trabecula is heavily influenced by the stress applied to it (Martin et al., 1998). This internal structural anisotropy of trabecular bone substantially affects its mechanical properties in different orientations, and the anisotropic deterioration of the trabecular structure increases the risk of fracture. Therefore, simply

*Correspondence to: Department of Biomedical Engineering, Stony Brook University, Bioengineering Building, Room 215, Stony Brook, NY 11794-5281, USA. Tel.: +1 631 632 1481; fax: +1 631 632 8577. Yi-Xian.Qin@sunysb.edu (Y.-X. Qin).

Conflict of interest statement

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measuring the “quantity” of bone such as bone mineral density (BMD) by using dual-energy x-ray absorptiometry (DXA) is not sufficient to fully characterize the “quality” of bone. Since being introduced by Langton et al., quantitative ultrasound (QUS) has been widely used for bone health status assessment (Ashman et al., 1987; Cardoso et al., 2003; Gluer, 1997; Kaufman et al., 2007; Lin et al., 2006; Nicholson et al., 1994; Njeh et al., 1997; Qin et al., 2007). Compared to the current gold standard, DXA, QUS has the advantages such as safe, low cost, portable, radiation-free and easy to operate. As ultrasound wave propagates through bone, both the velocity and the amplitude of the wave are modified. This modification can provide useful information of the mechanical properties of bone, i.e., the elastic modulus (Njeh et al., 1999).

For the past few years, many researchers have been investigating extensively on the interaction between ultrasound wave propagation and the anisotropic structure of trabecular bone. Cardoso and Cowin (Cardoso and Cowin, 2011, 2012; Cowin and Cardoso, 2011) studied the relation between fabric tensor—a measurement of the degree of anisotropy of the trabecular bone—and the wave propagation equations in anisotropic porous media. An angle-dependent model to the classic Biot’s theory (Biot, 1956a, b) to predict the ultrasound velocity in an anisotropic trabecular bone model was also introduced (Lee et al., 2007). Mizuno et al. reported in vitro studies on the different effects of the structural anisotropy of trabecular bone on the velocity of the fast wave and slow wave components of the broadband ultrasound signal (Mizuno et al., 2009; Mizuno et al., 2008; Mizuno et al., 2010). Hosokawa and Otani (1998) investigated the same subject, and later on computational simulation work was also reported by Hosokawa (2011). Han and Rho (1998) reported that a combination of BMD and elastic anisotropy of the bone resulted in an enhanced correlation with broadband ultrasound attenuation, a spectrum of ultrasound attenuation in frequency domain.

Although those investigations mentioned above have shown how ultrasound wave propagation in trabecular bone is influenced by the anisotropic structural property of trabecular bone in both computation simulations and in vitro experiments, some basic and fundamental questions remained unanswered: if ultrasound wave can be used to characterize the anisotropy of the porous structure of trabecular bone, can we use quantitative ultrasound to predict such principal alignment of trabecula, and to what degree? Which quantitative ultrasound parameter has the better ability to provide information to characterize the anisotropy of the trabecular structure? The objective of this study is to employ quantitative ultrasound to non-invasively predict the principal structural orientation of trabecular bones, and use the longest vector of mean intercept length (MIL) tensor, the current gold standard, to validate the accuracy of such prediction.

2. Materials and methods

2.1. 3-D volumetric trabecular sample preparation

Seven trabecular bone cylinders (Ø 38.1 mm) were cut from distal bovine femurs using cylindrical cutting bit. Then seven trabecular bone balls (Ø25.4 mm) were machined from the bone cylinders using lathe machine. Anatomical orientations were first marked on the surface of the cylinder. Then half of the bone ball was machined, and the orientation markers were extended to the newly machined semi-spherical surface before the other half of the

bone ball was machined. Anatomical poles were marked on the surfaces of the bone balls to indicate the three principal anatomical orientations—anterior–posterior (AP), medial–lateral (ML) and proximal–distal (PD). The marrow inside the trabeculae was flushed out using dental water-pick. For preservation, the bone balls were wrapped in gauze soaked with saline and 70% ethanol “half-and-half” solution and stored in 4 °C refrigerator. Before quantitative ultrasound measurement, the bone balls were put into a vacuum chamber for 3 h to remove the air bubbles trapped among the trabeculae.

2.2. Quantitative ultrasound measurement

Quantitative ultrasound measurement was performed by using a scanning confocal acoustic diagnostic navigation (SCAN) system (Xia et al., 2007), consisted of a computer-controlled 2-dimensional scanner unit and a pair of focused transducers (V302-SU-F2.00IN, Olympus NDT Inc., Waltham, MA) with a center frequency of 1 MHz. The diameter of the transducers is 25.4 mm, and the confocal length of the transducers is 50.8 mm. Therefore, the transducers were coaxially installed 101.6 mm away from each other, aligned with the center of the bone ball which was placed in a rotation stage at the midpoint of the two transducers. The rotation stage consisted of a holder for the bone ball and a base with angular calibration (Fig. 1). For ultrasound measurement, ultrasonic pulses transmitted through the center of the bone ball, and the bone ball was rotated 10° around the long axis of the rotation stage between every two scans. For every bone ball, ultrasound measurement was performed around all three anatomical axes, resulting 36 scans around each axis and 108 scans in total for each trabecular bone ball. Because of the shape of the bone ball sample, for each measurement in different scanning angles of a bone ball, the distance that ultrasound wave traveled in the bone sample should be the same. Therefore, the difference of amplitude and signal arrival time among the received signals in different scanning angles of a bone ball was induced by the anisotropic material property. The ultrasound results were then processed and visualized in a custom written MATLAB program. For each measurement, ultrasound attenuation and velocity were calculated using the classic substitution method (Langton et al., 1984). Based on the previous work showing that the speed of fast wave is more related with the change of the incident angle than the speed of slow wave, and that the amplitude of both the fast wave and slow wave are influenced by the incident angle (Hosokawa and Otani, 1998; Mizuno et al., 2010), the fast wave of the ultrasound pulse was chosen for the velocity calculation via the following equation:

$$c_b = \frac{c_w d}{d - c_w \Delta t} \quad (1)$$

here c_b is the ultrasound velocity in the bone sample, c_w is the ultrasound velocity in the water, d is the diameter of the bone ball, and Δt is the phase difference of the same signal landmark of the reference signal and the sample signal. In this study, 1480 m/s was used for the velocity of ultrasound in water (Langton et al., 1990).

2.3. μ CT scanning and imaging

μ CT with a resolution of 18 μm was performed on each trabecular bone ball by using a μCT 40 system (SCANCO Medical AG, Brüttisellen, Switzerland). In order to eliminate the influence from the surface condition induced by the machining of the bone ball, a cubic volume ($15 \times 15 \times 15 \text{ mm}^3$) of trabecular bone in the center of the bone ball was used for evaluation. The longest vector of the MIL tensor $\mathbf{H}$ was obtained from the μCT system. Currently, MIL is the gold standard of quantifying the structural anisotropy (Whitehouse, 1974). When straight lines in an arbitrary direction intercept with a medium, the average value of the intercept lengths is defined as MIL, and the degree of anisotropy is defined as the ratio of the longest MIL in the principle direction of the medium to the shortest MIL in the orthogonal direction. By the definition of MIL tensor, the longest vector indicates the direction in which smallest number of intercepts occurs. According the equation:

$$\text{MIL}(\theta, \varphi) = \frac{L_{TOT}}{N(\theta, \varphi)} \quad (2)$$

where $\text{MIL}(\theta, \varphi)$ is the length of a MIL vector in a certain direction, L_{TOT} is the total length sectors of lines in that certain direction and $N(\theta, \varphi)$ is the number of intersections, the number of intersections and MIL vector length is inversely proportional to each other; a long MIL vector indicates small number of intercepts (Tabor, 2011). Therefore, the longest MIL vector indicates the smallest number of intersections, which should be the main direction of trabeculae.

A piece of rubber foam was used to fix the sample in certain orientation during the CT scanning, and a screw was inserted in the foam and included in the CT scanning as the reference of the sample's orientation. The MIL tensor was computed in a cube volume within the sample. Based on the μCT data, the average trabecular thickness for all the samples was 189 μm , about 10 times of the scanning resolution 18 μm . This resolution should be fine enough to characterize the geometrical information of the sample. To binarize the images, a threshold value of 138 was used and it was based on triangularization of surfaces.

2.4. Calculation of the principal structural orientation of bone ball

A Cartesian coordinates system (x -, y - and z -axis) with the origin set at the center of the bone ball was defined to calculate the principal structural orientation. The quantitative ultrasound parameters were plotted against the scanning incident angle on the corresponding orthogonal plane in the Cartesian coordinates system. Based on the ultrasound parameter around three different axes (AP, ML and PD), a specific angle in which the ultrasound with the peak measurement was chosen for calculation for the specific orthogonal plane. In the 3-dimensional Cartesian coordinates system, a plane normal to the orthogonal plane was determined based on that angle, and the three normal planes of all three corresponding measuring anatomical planes can be denoted in the following three equations:

$$\begin{aligned}
 a_1x + b_1y + c_1z &= 0 \\
 a_2x + b_2y + c_2z &= 0 \\
 a_3x + b_3y + c_3z &= 0 \quad (3)
 \end{aligned}$$

The normal vectors of these planes, (a_1, b_1, c_1) , (a_2, b_2, c_2) and (a_3, b_3, c_3) , were used to calculate the three intersecting lines between every two planes using the following equations:

$$\begin{aligned}
 \mathbf{I}_{12} &= (a_1, b_1, c_1) \times (a_2, b_2, c_2) \\
 \mathbf{I}_{13} &= (a_1, b_1, c_1) \times (a_3, b_3, c_3) \\
 \mathbf{I}_{23} &= (a_2, b_2, c_2) \times (a_3, b_3, c_3) \quad (4)
 \end{aligned}$$

Theoretically, these three vectors should represent the same direction because all three planes intersect at the same line. This is based on the assumption that there is one principle orientation in the bone ball sample, and the ultrasound measurement in the direction along this orientation should have the highest values. The peak values recorded in the rotational measurements around three anatomical axes were in the direction which is the projection of the principal orientation on the orthogonal planes. For example, the peak value in the measurements rotating around x -axis was in the direction of the projected vector of the principal orientation on the y - z plane. Therefore, when using the intersections of every two normal planes to determine the principal orientation, the results should be the same line, because there can be only one intersecting line between every two planes.

Due to the inevitable measurement error, intersection vectors of every two planes differed from each other. Therefore, a center vector $\mathbf{I}(x, y, z)$ is calculated from the intersection vectors ($\mathbf{I}_{12}$, $\mathbf{I}_{13}$ and $\mathbf{I}_{23}$). The three intersection vectors were converted from Cartesian coordinates system (x, y, z) to Spherical coordinates system (r, θ, ϕ) . Then the angular components of the vectors were averaged as the angular components of the center vector. The magnitude of the center vector was arbitrarily decided as 1. The center vector was then converted back to Cartesian coordinates system to compare with the longest vector of the MIL tensor, $\mathbf{H}(x_H, y_H, z_H)$ by using the following equation:

$$\varphi = \cos^{-1} \left(\frac{\mathbf{H} \cdot \mathbf{I}}{|\mathbf{H}| |\mathbf{I}|} \right) \quad (5)$$

An illustration of the mathematic approach of the calculation for the principal orientation was shown in Fig. 2.

3. Results

The received ultrasound wave from all incident angles for the same bone ball showed angle-dependent pattern in both amplitude and signal arrival time (Fig. 3). To further analyze this pattern, the x -axis value (arrival time) and the y -axis value (amplitude) of the first high

peaks of all the 36 received ultrasound signals in different incident angles around one anatomical axis of a trabecular bone ball are plotted in Fig. 4. The high peaks and low valleys in these curves indicate that both amplitude and arrival time are affected by the anisotropy of the trabecular structure. These peaks and valleys also showed geometrical symmetry: the two peaks/valleys are located approximately 180° away from each other, indicating that they come from the same ultrasound propagating pathway, only in opposite directions. The peak values of the 180° apart measurements were averaged. Four consecutive scans are plotted in Fig. 5, in which both fast and slow wave components of the ultrasound pulses can be observed. It is demonstrated that, as the scan angle changes, the signal arrival time of the fast wave components changes gradually. On the other hand, little change in arrival time can be observed for the slow wave components.

According to the μ CT data, the average porosity of the samples was 0.67. Of all 21 rotational measurements (3×7), the phenomenon that the fast wave amplitude is larger than the slow wave amplitude was observed in 14 measurements. And in the other seven measurements, the slow wave amplitude was larger than the fast wave amplitude. Due to the certain existence of overlapping between the fast wave and slow wave components, the first high peak of the whole ultrasound pulse signal was chosen as the landmark to calculate the ultrasound velocity in Eq. (1), and the amplitude of the whole ultrasound pulse signal was used to calculate the attenuation of the signal.

Similar to the amplitude and arrival time curves in Fig. 4, the ultrasound attenuation and velocity curves from 36 scans around each anatomical axis of a bone ball showed similar trends with adjacent peak and valley positions (Fig. 6). The ultrasound velocity measured from all seven bone balls ranged from 1700 m/s to 2300 m/s, and this range is in accordance to the velocity of the fast wave in bovine trabecular bones reported by other investigators' work (Cardoso et al., 2003; Hosokawa and Otani, 1997; Lee et al., 2007; Mizuno et al., 2008; Mizuno et al., 2010). Despite that these attenuation and velocity results showed high correlations for all seven bone balls with an overall average R^2 value of 0.785 (Table 1), certain phase shift can be observed. In Table 2, the angle difference between the principal structural orientations predicted by quantitative ultrasound and μ CT is listed. Both ultrasound attenuation (ATT) and the velocity of the fast wave (UV) were used for the prediction. Here, $\mathbf{I}_{12}$, $\mathbf{I}_{13}$ and $\mathbf{I}_{23}$ are the vectors of the interception lines calculated from Eq. (4) and $\mathbf{I}$ is the center vector calculated from $\mathbf{I}_{12}$, $\mathbf{I}_{13}$ and $\mathbf{I}_{23}$. For the prediction, the principal structural orientation predicted using ATT has an average angle difference of 11.67° from the $\mathbf{H}$ vector predicted by μ CT, while the orientation predicted using UV has a difference of only 4.45° . Statistically, a paired t-Test showed that the prediction from UV has a significantly smaller error than ATT ($P < 0.02$).

4. Discussion

This work presented a non-invasive quantitative ultrasound method to predict the principal structural orientation of trabecular bone. Both attenuation and fast wave velocity of the ultrasound signal were used for independent predictions, and these predictions were evaluated by comparing to the current gold standard—the longest vector of the MIL tensor. For the ultrasound velocity calculation, the velocity of the fast wave component was chosen

based on the previously reported phenomenon that fast waves are more related to the material properties of the solid phase in a porous medium, whereas the slow waves are more related to the liquid phase (Hosokawa, 2006; Hosokawa and Otani, 1997, 1998; Mizuno et al., 2009; Mizuno et al., 2008; Mizuno et al., 2010; Yamamoto et al., 2009). These investigators all agreed that when the ultrasound wave propagates along the direction of the trabecular bone alignment, the phase velocity of the fast wave is the fastest. The explanation for this phenomenon is that the ratio of trabecular structure along its alignment is the highest and the compression velocity of ultrasound in bone material is much higher than in water.

By definition, the longest vector of the MIL tensor is the direction in which a straight line intercepts with the most bone content. Therefore, a pathway along this direction, in which the “solid/liquid” or “bone/water” ratio is the highest, is expected to give rise to the greatest fast wave velocity—as presented in this study. Although certain bone–water or water–bone interfaces still exist along the trabecular structural orientation, their effect on the velocity of ultrasound wave is much smaller than that on the attenuation. Besides the energy absorption, the scattering and refraction caused by these interfaces substantially contribute to the measurement of ultrasound attenuation. This difference represents itself as the phase shift we observed in the ultrasound velocity and attenuation curves around one anatomical axis of a bone ball (Fig. 6), and eventually resulted in the angle difference between the predictions for the principal orientations (Table 3). The overall average angle difference is 8.96° with the maximum of 21.48° and the minimum of 1.06° .

The prediction using fast wave velocity has a smaller angle difference (4.45°) with the MIL vector than the prediction using the attenuation (11.67°). This finding is in agreement with the work of Mizuno et al. (2010), in which the velocity of fast wave was shown to have a higher correlation with the MIL tensor than the ultrasound attenuation. The prediction of fast wave ultrasound velocity is not only better than attenuation by the average angle difference, but also by the angle variance of the interception vectors $\mathbf{I}_{12}$, $\mathbf{I}_{13}$ and $\mathbf{I}_{23}$. As shown in Table 2, the average angle difference for these individual interception vectors from the ATT prediction was 14.13° , with a standard deviation of 9.03° . On the other hand, the average angle difference for the individual interception vectors from the UV prediction was 11.59° , with a standard deviation of 7.02° . The variance of individual interception vectors were reduced by the introduction of the center vector $\mathbf{I}$. The calculation of $\mathbf{I}$ was based on assumption that, theoretically the magnitudes of $\mathbf{I}_{12}$, $\mathbf{I}_{13}$ and $\mathbf{I}_{23}$ are the same, and the angle variance came from the error of measurement and interpretation of the ultrasound attenuation and velocity curves. Therefore, these individual vectors were normalized and converted into a spherical coordinates system to calculate the center vector by averaging the angular components of the coordinates of the individual vectors.

This center vector approach lowered the error in calculating the predicted orientations from both ultrasound attenuation and velocity by reducing the prediction error from 14.13° to 11.67° for ATT and from 11.59° to 4.45° for UV. Considering that the increment of the rotational ultrasound scan was 10° , an angle error of 4.45° for the prediction is promising and satisfactory. Such small error demonstrated the feasibility of using quantitative ultrasound to non-invasively predict the principal structural orientation. Although, as shown in this study, ultrasound velocity has a better prediction for the MIL tensor than attenuation,

future study should take combining the predictions from ATT and UV as a comprehensive method into consideration. Ultrasound velocity can provide reliable information of the structural alignment of the trabecular bone, but the mechanical properties in such alignment direction is yet to be tested. It is very crucial to verify the mechanism behind the difference between the predictions for the structural orientation by different ultrasound parameters. By integrating the potential angle-dependent mechanical information carried by ultrasound attenuation with the existing prediction protocol, a more precise and extensive method to provide information of both structural and mechanical anisotropy of the trabecular bone tissue is expected to be established.

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Fig. 1.

Schematic representation of the ultrasound measurement experiment set up. A trabecular bone ball is placed onto a holder with a rotation stage. The stage was placed in a water tank filled with degassed water. Two ultrasound transducers used for the measurement were placed on both sides of the bone ball, with the bone ball at the confocal point. The ultrasound signal was transmitted from one of the transducers and received by the other one after propagating through the bone ball sample and the water between the sample and transducers.

Fig. 2.

The trabecular bone ball (dark sphere) was placed in a Cartesian coordinates system defined by the orthogonal anatomical planes (frontal, sagittal and transverse planes) with the center of the ball placed at the origin of the coordinates. Based on the rotational ultrasound measurement around three anatomical axes, the angles α , β and γ were determined. These are the angles in which the ultrasound measurements obtained peak values. According to these angles, three vectors (dash arrows) on the corresponding orthogonal anatomical planes were determined, i.e., the vector on the transverse plane is determined by the ultrasound measurements around proximal–distal axis. Along the direction of each of these three vectors, a plane was defined normal to the corresponding anatomical plane. These normal planes (gray planes) intersect at one line (dash line). The normal vectors of these normal planes were used to define the intersection line using Eqs. (3) and (4). A vector (dark arrow) along the direction of that intersection line is defined as the principal orientation predicted by ultrasound, and the angle difference ϕ compared to the longest vector of MIL tensor (light arrow) was calculated using Eq. (5).

Fig. 3.

Typical ultrasound waves transmitted through the trabecular bone ball in 36 different incident angles around one anatomical axis. Both amplitude (value on y -axis) and arrival time (value on x -axis) of the ultrasound waves showed angle-dependent pattern. As shown in the figure, the arrival time difference between the first high peaks of scanning in 80° and 170° (90° apart) is $2.5 \mu\text{s}$.

Fig. 4.

By tracing the first high peaks of the 36 received ultrasound signals around one anatomical axis of a trabecular bone ball (a), similar angle-dependent patterns between the incident angle and the amplitude of the signal (b) and signal arrival time (c) can be observed. The high peaks and low valleys in these curves indicate that both amplitude and arrival time are affected by the anisotropy of the trabecular structure. These peaks and valleys also showed geometrical symmetry: the two peaks/valleys are located approximately 180° away from each other, indicating that they come from the same ultrasound propagating pathway, only in opposite directions.

Fig. 5.

The fast wave and slow wave components of the received ultrasound pulses in four consecutive incident angles. The arrival time of the fast wave component gradually altered as the scanning angle changed, while the arrival time of the slow wave component remained relatively unaffected.

Fig. 6.

Typical ultrasound attenuation and fast wave velocity curves of all the ultrasound scans around one anatomical axis of the same bone ball. Similar trends with adjacent peak/valley positions can be observed, while certain phase shift also exists between two curves.

Table 1

Average Pearson correlation coefficient values ($R^2 \pm$ standard deviation) between ultrasound velocity and attenuation for all seven bone balls around three different anatomical axes.

	AP	ML	PD	Average
R^2	0.768 $\pm$ 0.109	0.752 $\pm$ 0.087	0.826 $\pm$ 0.079	0.785 $\pm$ 0.094

Table 2

Angle difference (°) between the principal structural orientations predicted by μ CT and quantitative ultrasound calculated from Eq. (5). In this table, I_{12} , I_{13} and I_{23} are the vectors of the intersection lines calculated from Eq. (4), and I is the center vector of I_{12} , I_{13} and I_{23} . Both of ultrasound attenuation (ATT) and velocity of the fast wave (UV) results were used for the prediction. Overall, the orientation predicted by UV has less average angle difference, 4.45° , with the μ CT orientation than the prediction of the ATT, whose average angle difference is 11.67° .

	Bone 1	Bone 2	Bone 3	Bone 4	Bone 5	Bone 6	Bone 7	Average $\pm$ standard deviation
ATT								
I_{12}	5.45	9.48	21.34	9.86	13.01	7.59	1.77	14.13 ± 9.03
I_{13}	33.34	24.75	10.56	8.00	18.32	6.74	20.18	
I_{23}	29.44	16.73	9.88	2.72	25.46	17.76	4.33	
I	20.52	16.06	10.74	3.13	18.61	5.03	7.65	11.67 ± 6.83
UV								
I_{12}	6.98	2.72	22.14	10.91	28.36	12.12	6.43	11.59 ± 7.02
I_{13}	8.26	1.24	9.43	18.53	5.35	15.54	22.98	
I_{23}	6.22	4.93	11.49	12.41	11.73	16.96	8.73	
I	6.30	2.41	5.45	0.82	7.16	4.34	4.67	4.45 ± 2.20

Table 3
Angle difference between the predicted trabecular structural orientation by UV and ATT.

	Bone 1	Bone 2	Bone 3	Bone 4	Bone 5	Bone 6	Bone 7	Average ± standard deviation
Angle difference between UV and ATT (°)	21.48	14.19	5.57	3.22	13.35	1.06	3.89	8.96±7.48