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STEREOSCOPIC ANALYSIS OF TRABECULAR BONE ORIENTATION IN PROXIMAL HUMAN TIBIAS

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Abstract

The three-dimensional orientation of trabeculae is a key factor in determining the load carrying capabilities of cancellous bone. Previous biomechanical studies have shown that proximal tibias resected parallel to the articulating surface are stronger and stiffer than the contralateral tibias resected perpendicular to the long axis of the bone. However, morphologic evidence was not provided to help explain the mechanical differences.

To determine the orientation of the trabeculae in the medial condyle for both parallel cut and perpendicular cut specimens, a scanning electron microscope and stereoscopic techniques were used. Data showed that tibias cut parallel to the articular surface had trabeculae oriented nearly vertical with a mean angle of $4.5^\circ \pm 14.7^\circ$ (range, $0^\circ$ to $56.3^\circ$). The contralateral tibias cut perpendicular to the long axis of the tibia had trabeculae oriented at a mean angle of $36.0^\circ \pm 12.2^\circ$ (range, $16.1^\circ$ to $67.4^\circ$) from vertical.

The differences between the two resection techniques were shown to be significant ($p < 0.01$) using an Analysis of Variance.

This study provided morphologic evidence to explain why previous specimens cut parallel to the articular surface had stronger and stiffer cancellous bone than the contralateral specimens cut perpendicular to the long axis of the tibia. This information is important in understanding the load carrying capabilities of cancellous bone and how it may be applied to improving the clinical results of primary total knee arthroplasty.

Key Words: 3-D stereoscopy, trabecular orientation, cancellous bone, total knee arthroplasty, joint replacement, tibia, bone morphology, parallax, resection technique, scanning electron microscope

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Introduction

It has been suggested that the trabeculae in weight bearing regions of cancellous bone develop along the axes of principal stress [9, 12, 26, 28, 29, 39]. This contributes to the structural anisotropic (direction dependent) properties of human cancellous bone [11, 34, 38]. Studies have shown that the stiffness and strength of cancellous bone is dependent upon the orientation of the trabeculae [5, 11, 29, 34, 38]. Therefore, trabecular orientation is a key factor in determining the load carrying capabilities of cancellous bone.

A recent investigation by Hofmann, et al. [19], determined that the orientation of the tibial resection during total knee arthroplasty significantly affects the load carrying capabilities of the underlying subchondral bone. Clinical evidence revealed that the tibias resected parallel to the anatomic posterior slope of the articular surface (surface-parallel) during primary total knee arthroplasty experienced a lower incidence of tibial component subsidence when compared to tibias resected perpendicular to the long axis of the bone (axis-perpendicular) (Figure 1). Results of mechanical testing indicated that the surface-parallel resected bone exhibited 40% greater load carrying capacity and 70% greater stiffness than axis-perpendicular resected bone. It was speculated by the investigators [19] that the trabecular orientation may be a factor in the mechanical differences. However, morphological data was not provided to explain the observed differences in the mechanical properties of the cancellous bone.

To understand the mechanical properties of cancellous bone, various attempts have been made to analyze the trabecular structure [5, 7, 11, 29, 32, 36, 38]. However, three dimensional structural analysis is difficult due to the anisotropy of cancellous bone [5, 10, 35, 37]. Pugh, et al. [29] recognized that trabeculae are not all oriented in ideal columns. From studies using longitudinal thin sections through human knees, they [29] observed that some trabeculae had an angled orientation, but most trabeculae were oriented normal to the articular surface of the bone. Takechi [32] used
microradiographs of the proximal tibia to relate trabecular arrangement to mechanical function. While quantitative results were not given, it was reported that the trabeculae oriented perpendicular to the articular surface of the tibial condyles help support the compressive loads from the condyles of the femur. [32] A crossing trabecular pattern near the tibial tuberosity was also observed, serving to resist the tension forces from the hamstrings and the quadriceps muscles attaching to the proximal tibia [32].

Mechanical testing by other investigators [1, 2, 13, 24] has shown that the anteromedial condylar region of the bone found in the proximal tibia is stronger than the posteromedial and lateral condylar regions when tested in compression. However, the morphologic analysis related to these studies was either not reported, or it was restricted to qualitative descriptions from two-dimensional images of the bone. Williams, et al. [38] reported a qualitative description of trabeculae located beneath the articular surface of the proximal human tibia. They [38] found that the trabeculae were oriented in vertical columns perpendicular to the surface, but when viewed in the transverse plane, the plate and rod formation of the trabeculae appeared quite curved. This curvature in the horizontal plane minimizes the Euler bending [8, 33] of the trabeculae under axial compression, contributing to an increased mechanical strength [38]. The difficulties of accurately measuring three dimensional structures from a single test plane, and the lack of quantitative morphologic analyses reported with mechanical testing support the need for an accurate and efficient three-dimensional analysis technique.

Stereoscopy

Two methods have been cited in the literature for generating stereomicrographs using the scanning electron microscope (SEM). These methods allow for visualization of complex three dimensional structures, and ultimately the measurement of trabecular orientation. The Shift Method involves photographing the specimen at two different positions in the field after laterally displacing the specimen [20, 27]. While this method uses simple calculations for analysis, it is limited by the amount of overlap available for each micrograph [20, 27]. Consequently, at magnifications greater than 50x, sufficient overlap of the image is not obtainable [20, 27]. The Tilt Method involves tilting the specimen surface between exposures, providing complete overlap of the stereomicrographs at any magnification [3, 4, 16, 20, 21, 22, 27]. This proven method was chosen to generate the stereomicrographs for this study.

The purpose of this study was to utilize three dimensional stereoscopic techniques to measure the angle of trabecular orientation in the proximal human tibia resected axis-perpendicular and surface-parallel. This information will help explain the load carrying capabilities of cancellous bone and how the choice of resection angle can improve the clinical results of primary total knee arthroplasty.

Materials and Methods

Specimen Preparation

Five matched pair of fresh frozen proximal human tibia were obtained. All specimens were from individuals who died from either traumatic deaths or from cardiovascular disease. As shown in Table 1, the tibias were from 3 male donors and 2 female donors. The age of the donors ranged from 53 to 64 years (58.4 ± 4.7). The posterior slope of the tibias ranged from 8.5° to 12.0° (10.2° ± 1.8°). None of the bone specimens had any evidence of gross deformities or advanced disease states. Following the osteometric methods published by Ruff et al. [30], the long-axis of the tibia was determined for each specimen. This reference axis was used to control the tibias for reproducible alignment and sectioning.

To prepare the tibias for sectioning, a transverse cut was made through the midshaft region of each tibia using a 0.625 cm (0.25 inch) wide, 0.0625 cm (0.025 inch) thick band saw blade with 6 teeth per cm (15 teeth per inch). The distal half of each tibia was discarded from this study. The distal portion of the midshaft of each proximal specimen was inserted into a 5 cm diameter cylinder fabricated of aluminum. Set screws in the aluminum device were advanced to the cortical surface of the tibia, and hand-tightened with an Allen wrench so that no movement of the bone was evident.

<table>
<thead>
<tr>
<th>Specimen Number</th>
<th>Gender</th>
<th>Donor Age (years)</th>
<th>Posterior Tilt (degrees)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>60</td>
<td>8.5</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>53</td>
<td>12.0</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>64</td>
<td>8.5</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>54</td>
<td>12.0</td>
</tr>
<tr>
<td>5</td>
<td>Female</td>
<td>61</td>
<td>10.0</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>58</td>
<td>10.2</td>
</tr>
<tr>
<td>Std. Dev.</td>
<td></td>
<td>5</td>
<td>1.8</td>
</tr>
</tbody>
</table>
The proximal tibia was then oriented according to the previously marked reference axis such that either the articular surface of the medial condyle was parallel to the saw blade, or the long axis of the tibia was perpendicular to the saw blade, then clamped in a steel vise. The vise was firmly held to the table of the saw using an electromagnetic chuck (O.S. Walker Company, Inc., Worcester, Massachusetts). Once the specimen was aligned, a cut in the transverse plane was made through the lowest point of the ellipses defined by the articulating surfaces of the tibial condyles of each tibia using a water-cooled bone saw with a 20.3 cm (8 inch) diameter circular saw blade with 4 teeth per cm (10 teeth per inch) (Cleveland Twist Drill Co., Cleveland, OH). The proximal tibia was further resected to a level 6 mm below the initial cut, producing 5 mm thick wafers of cancellous bone.

The angles of these cuts were determined from two main surgical resection techniques currently used by orthopaedic surgeons in total joint replacement [18, 23]. One specimen from each pair was cut parallel to the natural articulating surface of the tibia [18], while the contralateral tibia was cut perpendicular to the long axis of the tibial shaft [23]. To avoid data bias, a coin toss was used to determine which specimen of each matched pair was cut surface-parallel and which was cut axis-perpendicular. This entire process resulted in five paired wafers of bone.

After cutting, the cancellous surface of each specimen was Water Picked (Teledyne, Ft. Collins, CO) to remove marrow and fatty tissue. The specimens were dehydrated using ascending grades of ethyl alcohol from 70% to 100%, and then placed in a vacuum desiccating chamber to dry for over 48 hours. Since the full wafers could not be imaged in the SEM chamber, the 5 mm wafers were sagittally sectioned into thirds and the medial third was used for this analysis (Figure 2). The medial third was chosen due to its large surface area, making it an important region for supporting the loads of the tibial component [24, 25]. Each specimen was then placed into a vacuum sputter coater (Hummer, Model VI-A, Chestnut Hill, MA), where the pressure was reduced to 40 mTorr and a thin layer of gold was sputtered onto the cancellous surface for two minutes. The specimen was removed from the coater and placed onto the specimen stage of the JEOL JSM-330A for imaging.

**Generating Stereomicrograph Pairs**

A tilt goniometer stage on the SEM allowed the specimen to easily be tilted about the X-axis. While there are many angles of tilt used by other investigators [17, 22, 27], the 10° used in the current study is recommended by Boyd [3, 4], and Howell [20]. This angle provided the necessary cues to perceive relative depth, and allowed for comfortable viewing with the stereoscope based on the interocular distance of the general population [3, 4, 20].

To generate stereomicrographs using the JEOL secondary electron detector, the specimen was first tilted to -5° with respect to the horizontal. The image was then photographed using Type 55 Polaroid film (Polaroid Corp., Santa Ana, CA), at a magnification of 50x, an accelerating voltage of 25kV, a load current of 100 µA, a working distance of 15 mm, and an aperture diameter of 240 µm. After three distinct features were marked on the viewing screen using a wax pencil, the specimen was tilted to +5° with respect to the horizontal. Unless the area of interest happened to lie in the tilt axis of the specimen stage, translation of the specimen occurred in both the x and y planes. Using the appropriate SEM stage controls, the specimen was moved so that the image was realigned with the marked position on the viewing screen. As the specimen was tilted, the brightness and contrast of the image changed due to the slope of the specimen with respect to the electron beam and collection system. After these features were readjusted, the image was photographed a second time. To insure that the second image had the same magnification and rotation as the first image, and to reduce the tilt error, the z height stage control was used to adjust the focus [3, 4, 16, 21].

**Viewing and Measuring in the Third Dimension**

A four mirrored Topcon stereoviewer (Ted Pella, Redding, CA) was used to view the three dimensional image created from the stereomicrographs. This instrument has two lenses which are positioned at their focal length above the stereomicrographs and separated by the interocular distance of the observer [4]. It was placed over a light box so that the negatives of the photomicrographs could be viewed via transmitted light. This provided better detail and decreased potential errors associated with viewing positive prints [4, 27].

Care was taken to properly orient the stereomicrographs beneath the stereoviewer to avoid incorrect perception by the observer. Following the convention indicated by Howell, et al. [21], the photomicrograph with the lower goniometer angle (-5°) was placed on the left beneath the stereoviewer, and that with the higher angle (+5°) placed on the right. Failure to follow this convention resulted in an inverted image where elevations appeared as depressions.

To measure the parallax, the difference in vertical displacement of two points, a floating dot parallax bar
stereomicroscope (Gordon Enterprises: Ladd Research Industries, Inc., Burlington, VT) was used. This instrument consists of two glass disks, each engraved with a small dot, mounted onto a bar whose length can be varied by means of a screw micrometer. The micrometer is accurate to 0.01 mm, which corresponds to an actual distance of 0.2 µm on the magnified scale of the photomicrographs. To obtain a correct measurement of the parallax, it must be measured perpendicular to the axis of tilt \([17, 27]\). For this reason, the stereomicrographs were rotated 90 degrees counterclockwise beneath the stereoviewer and the parallax bar rested along the horizontal. The parallax bar was aligned so that each disk was positioned over the stereomicrographs being viewed under the stereoviewer. For each of the five paired specimens, six to nine trabecular tilt angles were calculated from the measured parallaxes and lateral displacements. As seen on the photomicrographs (Figure 3 and 4), the orientation of the points chosen to be measured were arranged in a right triangle. Points \(P_0\) and \(P_1\) were located on the cut surface of the trabecular bone, while point \(P_2\) was located perpendicular to \(P_1\) on the sloped surface of an individual trabeculae. By matching the altitude of the floating dot with various points on the stereomicrographs, their parallax separation could be read from the micrometer. If the two dots were positioned on exactly the same surface as the corresponding points on the stereomicrographs, a point of light was seen lying in the plane of the surface \([4]\).

For two points whose parallax readings are \(P_1\) and \(P_2\), their relative displacement is given by \([4, 15, 16, 21, 22, 27]\):

\[
h = \frac{(P_1 - P_2)}{2} M \sin(\theta/2) \tag{1}
\]

where \(M\) = the magnification of the stereomicrographs and \(\theta\) = the difference in tilt angles between stereomicrographs. The height displacement and the corresponding angle of tilt is determined by the location of the image points chosen to be measured. An investigation by Heidenreich, et al. \([15]\) showed that the parallax of the two image points needs to be 0.4 mm or greater to produce accurate results. Due to limitations with the optical depth of field of the SEM and the resulting photomicrographs, there was difficulty in distinguishing individual features of the trabeculae at large height displacements. This limited the depth at which the parallax could be measured. Also, the trabeculae were not oriented into ideal columns. Their bases were continuous with the connecting rod and plate formation of cancellous bone \([12, 28, 34, 38]\) creating trabeculae with wide, flared bases. For these reasons, point \(P_2\) (Figure 4) was chosen to lie on the sloped surface of the trabeculae and within the focused depth of field, not on the flared bases of the trabeculae. By meeting these conditions with all of the parallax measurements made in this study, the angle of tilt could be accurately calculated.

Using a digital micrometer (Mitutoyo, Model 500-215, Japan), the lateral displacement between the two points was measured from the surface of the photomicrograph with the lower tilt angle. It was determined that the error associated with measuring from the tilted photomicrograph was small when compared to measurements made from a \(0^\circ\) tilted photomicrograph. The micron bar scale on the photomicrographs allowed this lateral displacement value to be converted into real dimensions. Through the use of simple trigonometry, the angle of the slope \(\Theta\) between the two points could be calculated with the following equation:

\[
\Theta = \tan^{-1} \left( \frac{l}{h} \right) \tag{2}
\]

where \(h\) = the calculated height displacement and \(l\) = the measured lateral displacement in real dimensions. This corresponds to a two dimensional projection of the two points. (Figure 5)

The previous equations assume there is a parallel projection geometry in the SEM, which is nearly the case for pictures taken with magnifications above 500x \([4, 20, 21]\). While the magnification used in this study was only 50x, work completed by Kristensen, et al. \([27]\) indicated that these equations are accurate at any magnification.

**Results**

Qualitative stereoscopic analysis showed the trabeculae of the surface-parallel cut specimens oriented nearly vertical (Figure 3), while the trabeculae of the axis-perpendicular cut specimens displayed an obvious tilt (Figure 4). The calculated angles data are shown in Table 2. A mean angle of \(4.5^\circ \pm 14.7^\circ\) (range, \(0^\circ\) to \(56.3^\circ\)) was measured for the surface-parallel cut specimens. For the axis-perpendicular cut specimens, a mean angle of \(36.0^\circ \pm 12.2^\circ\) (range, \(16.1^\circ\) to \(76.4^\circ\)) was measured. Using an Analysis of Variance \([31]\), it was shown that the measured trabecular orientation from the surface-parallel cutting technique was significantly different from the axis-perpendicular cutting technique (\(p \leq 0.01\)).

**Discussion**

This analysis provided preliminary morphologic evidence that can help explain why the cancellous bone of the proximal tibia cut parallel to the anatomic posterior slope of the tibial articular surface was stronger and stiffer than the cancellous bone cut perpendicular to the long axis of the tibia \([19]\). This stereoscopic analysis demonstrated that the specimens cut parallel to the articular surface had trabeculae predominantly oriented normal to the resected surface. All tibias cut perpendicular to the long axis of the tibia had trabeculae predominantly oriented at an angle to
Measurement of Trabecular Bone Orientation

Figure 3. SEM stereomicrograph of trabecular bone cut using the surface-parallel technique.

Figure 4. SEM stereomicrograph of trabecular bone cut using the axis-perpendicular technique.

Figures 3 and 4. The lettered points show the orientation of the points used to measure parallax and angle of trabecular tilt. Points P0 and P1 are located on the cut surface of the bone, while point P2 is located on the surface of an individual trabecula.

From basic mechanics theory, Euler buckling will occur on vertically oriented trabeculae loaded in compression [8, 33]. As the trabeculae are angled further from vertical, the mode of failure changes from Euler buckling to bending, and the load carrying capability decreases. A surface-parallel resection technique would result in trabeculae being oriented more normal to the tibial component in total knee replacement. This would improve the load carrying capabilities of the cancellous bone in the resected proximal tibia.

Methods for determining the angles of trabecular orientation are necessary to understand the role the trabeculae play in the mechanical strength and stiffness of bone. Investigators need to be aware of the morphology of the test specimens since it has been shown that the trabecular orientation significantly influences the stiffness and strength of cancellous bone [2, 5, 11, 28, 29, 34, 38].

The wide range of trabecular orientations measured in this study indicate that the loading pattern [9, 12, 26, 28, 29, 39] in the proximal tibia is complex. Due to the anisotropic structure of cancellous bone, the trabecular orientation may change with anatomic location. Further investigations are needed to quantify the trabecular orientation as a function of anatomic location across the resected tibial plateau. When revision surgery is being considered, a better understanding of the trabecular orientation distal to the articular surface of the tibia is necessary if trabecular orientation is to help improve implant stability. The application of the knowledge gained in this study can help improve the clinical results of primary total knee
arthroplasty. The 3-dimensional techniques applied in this study can further the understanding of the load carrying capabilities of cancellous bone.

Acknowledgments

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References


TABLE 2. Results of 38 measured angles from surface-parallel and axis-perpendicular cuts.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Parallel Cut</th>
<th>Perpendicular Cut</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0 0.0</td>
<td>24.5 21.7</td>
</tr>
<tr>
<td>2</td>
<td>0.0 0.0 54.8</td>
<td>33.7 25.0</td>
</tr>
<tr>
<td>3</td>
<td>0.0 0.0 56.3</td>
<td>43.9 67.4</td>
</tr>
<tr>
<td>4</td>
<td>0.0 0.0 34.0</td>
<td>21.1 25.2</td>
</tr>
<tr>
<td>5</td>
<td>0.0 0.0 28.3</td>
<td>21.9 50.7</td>
</tr>
</tbody>
</table>

Mean 4.5
Std. Dev. 14.7
Range 0-56.3
n 38 38

Measurement of Trabecular Bone Orientation


Discussion With Reviewers

Anonymous Reviewer 5: Scanning electron microscopy was not required for this investigation and macrophotography would have sufficed. The data from this study could have been obtained from longitudinal cuts only with the two different slopes interpolated from the same specimen.

Authors: Experts [10, 14, 35, 37] in the field of stereology have published that it is impossible to measure precisely three dimensional structures from a single plane -- especially with an anisotropic material such as cancellous bone. The best that one could hope for, is a good estimate. To obtain accurate, 3-dimensional data, extensive sectioning of the structure in three planes is required if proper stereological principles are to be followed from single plane images. While the structure could appear to be fairly uniform in one plane of sectioning, it could not be proven that the structure does not alter along other planes. We feel that the SEM was the best imaging technique for this study due to its superb image resolution, the ease of specimen preparation, and the accuracy of the 3D stereoscopic principles that are well documented in the literature.

S. B. Goodman: The authors imply that resection of the tibia parallel to the articular surface would be the optimal surgical technique during total knee replacement. However, this assumes that trabecular orientation for different types of arthritis (e.g. osteoarthritis, rheumatoid arthritis, etc.) is the same as that fund for "normal" cadaveric specimens. Do the authors have any stereoscopic analyses to support this assumption?

Authors: It is well established in the world literature that osteoarthritis is a focal disease within articular cartilage and subchondral bone. The surgeon commonly resects away these regions of pathology during total joint arthroplasty. We are not aware of any data which might suggest there are correlated changes in the trabecular orientation with osteoarthritis. This requires future investigations.

Rheumatoid arthritis is a systemic autoimmune disease in which the disease and pharmaceutical therapies are both known to affect the load carrying capabilities of bone. To our knowledge, there are no reported studies which demonstrate changes in trabecular orientation with this disease. This also would be worth investigating in the future using accurate quantitative techniques as described in this study.

S. B. Goodman: The authors examined the most medial portion of the medial plateau of the tibia for this study. Do the authors believe that their conclusions are applicable to other portions of the proximal tibia?

Authors: We have anticipated this question and are currently completing the analysis of a 3-dimensional mapping study of the resected proximal tibia. We will
be reporting the results of our studies of the entire tibial wafer as well as "maps" at incremental depths throughout the proximal tibia. We are interested in determining how the "parallel-cut versus perpendicular-cut" comparison affect trabecular orientation distal to the tibial articular surface. This would be especially important when the surgeon is dealing with revision arthroplasty.

K. Draenert: There is a hypothesis which claims that bone is a shell around an inner pressure system -- like a shell of an egg. What are the authors trying to say with respect to the slope of the shell?
Authors: Assuming the trabecular bone is like a "shell" around an inner pressure system, the orientation of the resected "shells" would be angled as a unit if an axis-perpendicular resection technique is used. This places the entire "shell" in a bending moment and decreases its load carrying capability. The "inner hydraulic pressure system" would not aid in the load carrying capabilities since mechanical testing has shown that trabecular bone is not hydraulically strengthened by the presence of marrow under physiological loading conditions [6]. The goal of this study, was to develop a technique which would allow measurement of trabecular orientation or outer "shell" in three dimensions. This was accomplished.

K. Draenert: It is important to map the measurements and show the topography of the select area.
Authors: We agree. We are currently completing the mapping phase of our investigations and will be reporting this data within a few months.

K. Hodde: Does the difference in measured and calculated mean tilt angles of the trabeculae to the cut surface agree with the differences in resection angles between the two main surgical techniques?
Authors: The results of this study represents the trabecular orientation of the complete medial side of the resected specimens. When the data is averaged on final analysis, it represents a broad range of trabecular orientations. This is clinically pertinent since the tibial component is supported by the entire plateau represented by the average trabecular orientation.

T. A. W. Gruen: It is not known if the observer knew the nature of the specimen, as this may have led to the high percentage of zero readings in Table 1.
Authors: This is true. Observer bias can skew the data. However, at the onset of this study, we developed measurement criteria which we hoped would minimize this problem. Using the parallax technique described, it is difficult to bias the data collection process. However, future mapping studies will use a randomized sampling method to help guarantee that observer bias does not occur.

T. A. W. Gruen: The precise region of interest within the medial third of the 5 mm thick wafer of cancellous bone was not specified in this study. This is critical as there are substantial variations within the medial condyle region.
Authors: We recognize these variations across the tibial plateau. For this reason, a second study is being completed which maps the angular variations throughout the proximal tibia at specific, anatomic regions. However, the purpose of this present study, was mainly to develop and demonstrate the application of the technique.

T. A. W. Gruen: What is known about the inherent variation between the left and right sides from the same pair of normal specimens?
Authors: There are numerous factors which could influence the orientation of the trabeculae. From the biomechanical literature, left/right comparisons are common. The literature base for trabecular orientation is, at this time, sparse. To try and avoid any bias in this study, we used a coin toss to randomly select which tibia of the pair would be cut perpendicular and which would be cut parallel.