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The Elastic Moduli and Fatigue Properties of Canine Trabecular Bone Tissue

Kuiwon Choi*
Biomedical Research Center, Korea Institute of Science & Technology, Seoul 136-791, Korea
Gon Khang
School of Electronics & Information, Kyung Hee Univesity, Kyunggi-do 449-701, Korea
Steven A. Goldstein
Orthopaedic Research Laboratories, The University of Michigan, MI 48109, U.S.A.

The elastic modulus and fatigue properties of canine and human trabecular bone tissues (single trabeculae) were experimentally determined on a microstructural level using four-point bending cyclic test, and they were compared based on microstructural characteristics and mineral density. The results showed that canine trabecular bone tissue had significantly lower modulus and lower fatigue strength than human tissue. The observed microstructural differences between the two tissues may be more responsible for the differences, although the lower mineral density in canine tissue might also have contributed to the lower modulus and fatigue strength.

Key Words : Trabecular Bone, Fatigue, Elastic Modulus, Four-Point Bending

1. Introduction

Canine bone has been extensively used as a model for human bone due to many similarities in physiological (Faugere et al., 1985; Martin et al., 1981; Snow et al., 1984; Snow and Anderson, 1986) and mechanical behavior (Vahey et al., 1987; Kuhn et al., 1989b) between the two species. Through both in vitro and in vivo canine experiments, valuable information has been obtained, which has contributed greatly to the understanding of bone mechanics, remodeling mechanisms as well as many orthopaedic problems (Bos et al., 1983;Enneking et al, 1975; Harris et al., 1972;Bobyn et al., 1980;Ducheyne et al., 1977. Burr et al., 1985; Vahey et al., 1987: Kuhn et al., 1989b; Goldstein et al., 1990).

On the other hand, it has also been reported that some differences in mechanical behavior exist between the two species on a continuum level (Kuhn et al., 1989b). They reported that canine trabecular bone cubes displayed a lower modulus than human bone for the same density and speculated that differences in microstructure between the two species may be responsible. This suggested that the limitations of the use of canine trabecular bone tssue as a model for human tissue needed to be established on a microstructural level, so that results from canine models could be properly interpreted.

Recently, more research has been directed towards understanding trabecular bone mechanics on a microstructural level in order to investigate bone-implant interface conditions (Pedersen et al., 1990) and remodeling mechanisms (Hollister et al, 1991). As such, several microstructural models for canine trabecular bone have been proposed which require micro mechanical properties of the structural elements (trabecular tissue or individual trabeculae) being modeled. Although Kuhn's study (Kuhn et al., 1989b) suggests that canine trabecular bone tissue may have a lower modulus than human

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* Corresponding Author,
E-mail : choi@kist.re.kr
TEL : +82-2-958-5921 ; **FAX** : +82-2-958-5909
Biomedical Research Center, Korea Institute of Science & Technology, 39-1 Hawolgok-dong, Seongbuk-gu, Seoul 136-791, Korea.

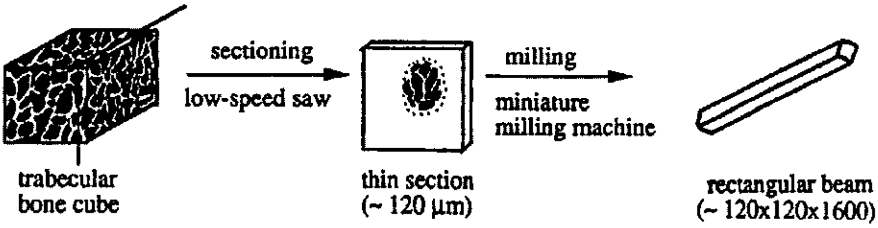


Fig. 1 Schematic diagram of specimen preparation procedures (sectioning-milling)

trabecular tissue, the micro mechanical behavior of canine trabecular bone tissue is virtually uncharacterized.

The purpose of this study was to determine the elastic modulus and fatigue properties of canine trabecular bone tissue on a microstructural level and to compare the properties to those of human trabecular bone tissue previously reported by Choi and Goldstein (1992).

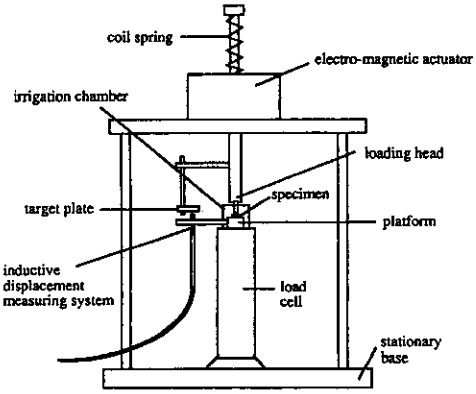


Fig. 2 Schematic diagram of the four-point bending test apparatus

2. Materials and Methods

All trabecular specimens were obtained from one pair of canine tibiae from a skeletally mature mongrel dog. The tibiae were obtained within two hours of death, cleaned of soft tissue, and stored at −30℃ until specimen preparation. The identical protocols for specimen preparation and testing methods described in the previous study (Choi and Goldstein, 1992) were utilized to compare the micro properties between the two species.

The specimen preparation procedures are illustrated in Fig. 1. Thin parallel trabecular bone sections were cut from the proximal portion of the tibia using a low-speed diamond saw (Model 11-1180 Isomet, Buehler, Lake Bluff, IL 60044). Relatively continuous and long trabeculae were selected and milled into rectangular beam specimens using a specially designed milling machine. The specimen dimensions were measured using a digital image processing system (Recognition Technology Inc., Westborough, MA 02582) at high magnification (0.95 μm/pixel). The specimen height and base ranged from 70 to 140 μm. The length of each specimen was approximately 1600 μm. Only specimens with base-to-height ratios between 0.5 and 2.0 were included in this study. Specimens were kept moist at all times during the procedures and stored at −30℃ until testing; Twenty four trabecular specimens were prepared for mechanical testing.

The mechanical testing (static and fatigue) apparatus is schematically illustrated in Fig. 2. Loading was provided by an electro-magnetic actuator with a servovalve control system (Model ECM 2-LI-D, Continental Hydraulics, Savage, MN 55387). A high resolution load cell (Model FTD-G-100, Schaevitz Engineering, Camden, NJ 08101) and an inductive displacement measuring system (KD-2300-. SSU, Kaman Instrumentation Co., Colorado Springs, CO 80933) were used to measure load and deflection. Prior to fatigue testing, static four-point bending tests were conducted. Each specimen was loaded to approximately 0.04 N at a constant loading rate of 0.02 N s^{-1}. The bending modulus was calculated from the load-deflection data using linear elastic beam theory. Each specimen was then tested in cyclic four-point bending with a sinusoidal loading waveform at a frequency of 2 Hz in water and at room temperature until complete failure. The run-out number of cycles was set at 200,000. The minimum bending stress was fixed at approximately 20 MPa and the maximum stress values varied from 80 to 110 MPa.

After mechanical testing, each specimen was scanned on a micro computed tomography system (Kuhn et al., 1989a; Choi and Goldstein, 1992) to determine relative mineral density (unit=grey scale mm^{-2}, U). Relative mineral density was calculated by integrating each X-ray attenuation profile, assumed to be proportional to mineral density, and normalizing by the specimen cross-sectional area. Since this density varies with scanning parameters, such as magnification and X-ray current, the canine specimens and human trabecular specimens were scanned simultaneously to obtain a valid comparison of relative mineral densities.

In order to characterize the microstructure of canine trabecular tissue, microstructural characteristics and fracture patterns along with microdamage patterns were observed using scanning electron microscopy (SEM) and backscattered image (BSI) analyses (Autoscan Model U-2, ETEC Co., Hayward, CA 94545) at a high magnification.

3. Results

A summary of static test results and mineral density measurements are presented in Table 1. The canine trabecular tissue modulus (n=24) ranged from 1.92 GPa to 5.47 GPa, with a mean value of 4.00 GPa (s.d.=0.88). The mineral density values for canine trabecular tissue ranged from 0.197 U to 0.297 U, with a mean value of 0.251 U (s.d.=0.024). No significant correlation was found between modulus and mineral density.

Table 1 The modulus and mineral density measurement results for canine and human trabecular bone tissues. Modulus values are reported in units of GPa, and mineral density measures in units of U (grey scale mm^{-2}). Standard deviations are shown in parentheses. P-values from two-tailed student t test indicate significance of the differences between two species

	Canine	Human	Significance
Modulus	4.00(0.88)	5.72(1.27)	P<0.01
Mineral density	0.251(0.024)	0.294(0.030)	P<0.01
	N=24	N=23	

Fatigue results are summarized in Fig. 3 in the form of a median S-N curve. The numbers on the arrow indicate the number of run-out specimens which did not fail after 200,000 cycles. This S-N

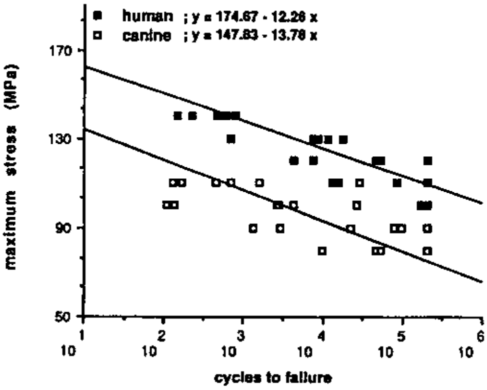


Fig. 3 Median S-N curves for both canine and human trabecular tissues

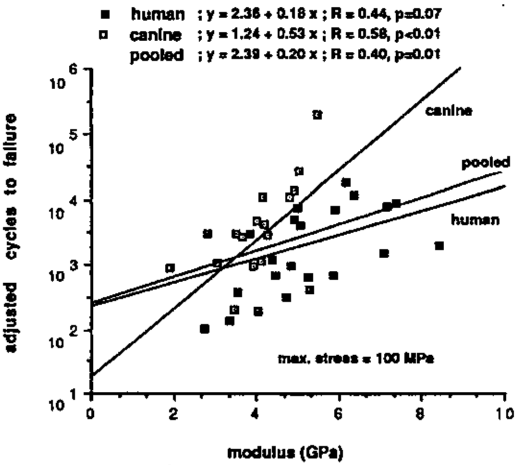


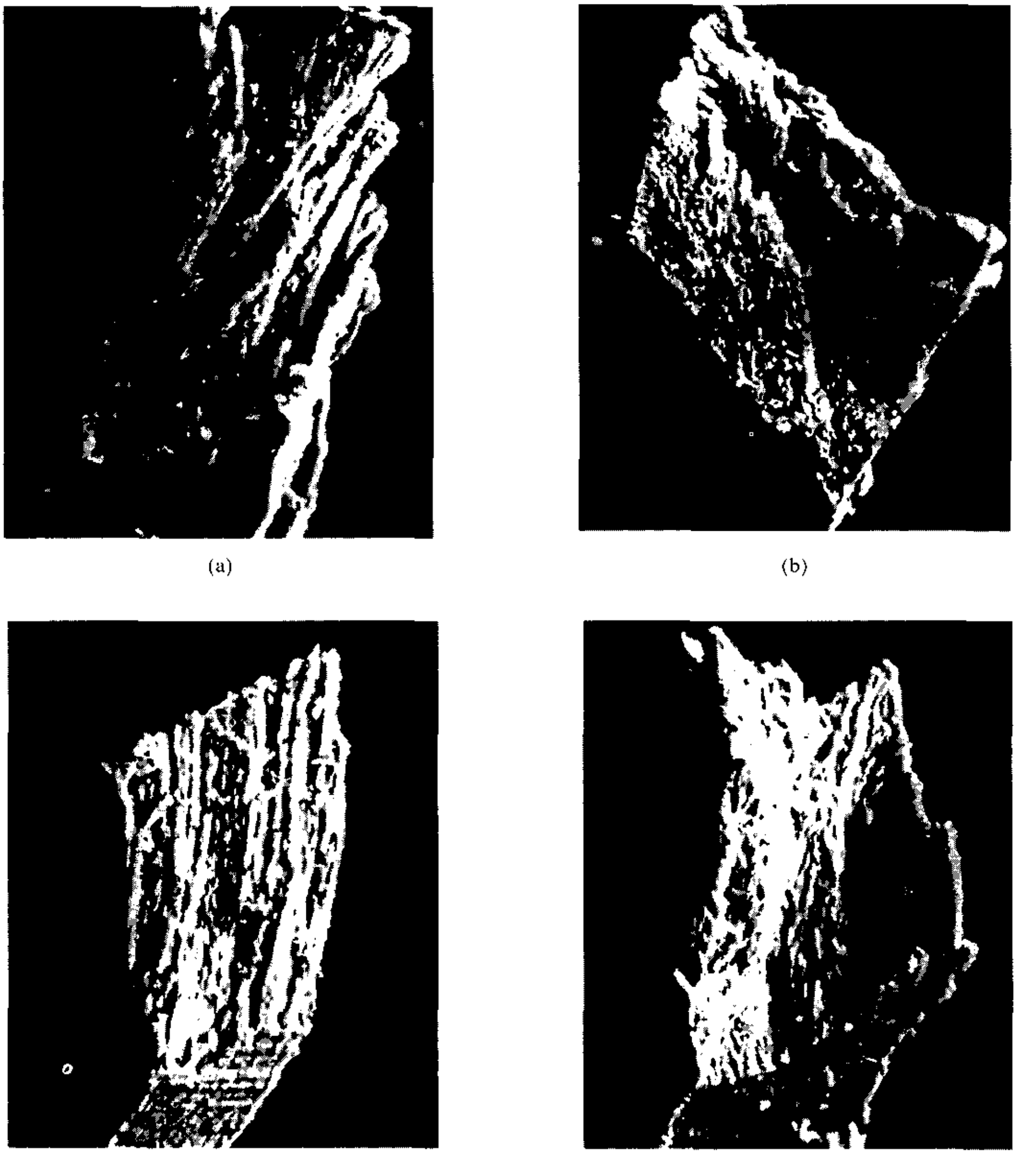
Fig. 4 A significant correlation between modulus and fatigue life was found in cannine trabecular bone tissue. No correlation was found in human tissue. However, a significant relationship was found when both canine and human data were pooled together

relationship is expressed as the following linearized equation

$$\log N = 11.779 - 0.082\ (S)$$

where; N and S refer to number of cycles to failure and maximum stress value respectively. Figure 4 illustrates the relationships between human and canine modulus and adjusted fatigue life for a maximum stress value of 100 MPa. A significant relationship was found in the canine trabecular tissue ($r = 0.58$, $p = 0.008$) indicating the specimens with higher moduli showed higher fatigue strength. However, no significant relationship was found between mineral density and adjusted fatigue life.

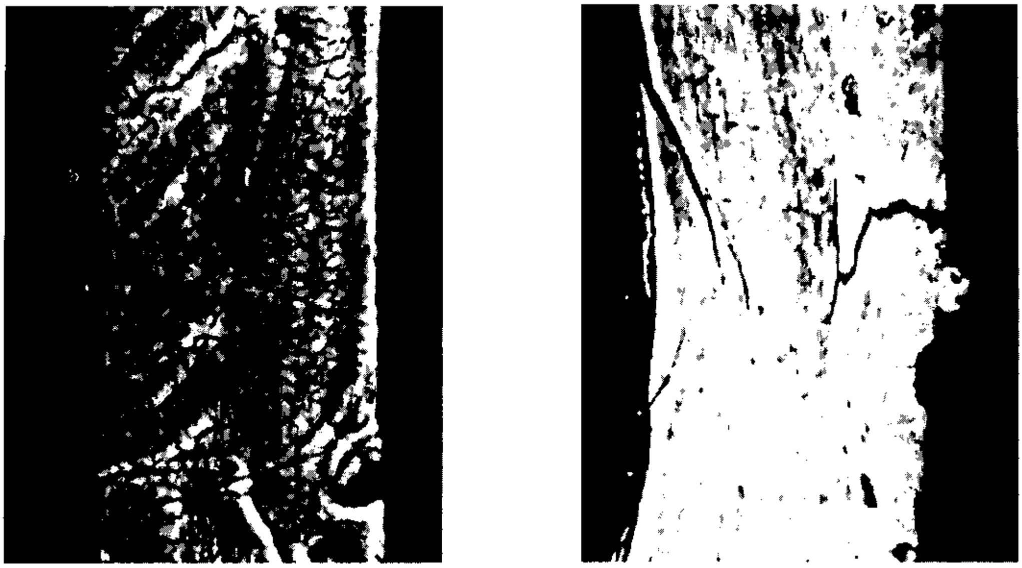
Most of the specimens illustrated large defor-



(a) (b)

(c) (d)

Fig. 5 SEM analyses (x400) on fracture surfaces of canine (a, b) and human (c, d) trabecular bone tissues. Both tissues show oblique (a, c) and haphazard (b, d) fracture patterns. Note that canine tissue exhibits significantly deformed surfaces compared to relatively brittle surfaces of human tissue



(a) (b)

Fig. 6 BSI analyses (x400) on canine (a) and human (b) trabecular bone tissues. Microcracks were observed in both tissues, however, the number and patterns of the microcracks differed between the two species. More microcracks in canine tissue may reflect the weaker nature of its bone matrix

mation before complete failure and 25% of the specimens (6 of 24) were significantly deformed without complete fractures. Examples of fracture surfaces of the canine trabecular specimens are illustrated in Fig. 5(a) and (b). An oblique fracture with significantly deformed (compliant or ductile) surfaces was a typical pattern (Fig. 5 (a)). More haphazard fracture patterns were also observed (Fig. 5(b)), perhaps reflecting the complicated nature of the trabecular tissue microstructure. An example of the fatigue damage pattern for one of the canine specimens is illustrated in Fig. 6(a) from BSI analyses. The trabecular tissue demonstrated a 'mosaic-like' structure, composed of multiple trabecular packets. Lamellar orientation was not clear in some of the trabecular packets. Uneven mineral distribution is indicated by the non-uniform grey levels seen in the BSI photograph. Numerous microcracks were observed throughout the specimens and were associated with lacunae or cement lines.

4. Discussion

A study of the fatigue properties of canine trabecular bone tissue has been performed using identical specimen preparation and testing methods as the author's previous study of human trabecular bone tissue (Choi and Goldstein, 1992). In the previous study, the effects of specimen preparation technique and testing methodology on the modulus and fatigue properties were extensively examined. No artifactual effects due to machining had a significant effect on the property measurements, thus the preparation technique proved to be a valid method for producing regularly-shaped bone microspecimens for mechanical tests. However, inevitable error sources involved in bending tests, such as the effects of yielding, anisotropy, and heterogeneity on stress estimation, remain to be evaluated. One of the objectives of the present study was to compare the properties of canine and human trabecular bone tissue to determine the limitations of canine trabecular bone tissue as a model for human trabecular bone. Although the above error sources may limit the accuracy of the results, the comparison of the results between the two species would be valid.

A two-tailed student t test showed that canine trabecular tissue had a significantly lower modulus (about 30%) and lower mineral density (about 15%) as compared to human tissue (Table 1). The lower mineral density of canine tissue does not agree well with previous findings (Gong et al, 1904; Kuhn et al., 1989b) where no significant differences in average ash weight density and apparent density were found between human and canine trabecular bone cubes. While this discrepancy could be explained by the different measuring methods, it should be noted that the specimens in the current study were obtained from only one donor per species. Biological age difference between the two specimen groups, a 59 year old human versus a 3 year old dog (=approximately 25 human years), might have also contributed to the discrepancy.

Another source for the discrepancy would be a scaling difference along with the differences in specimen preparation methods. The ash weight and apparent densities in the other studies mainly represent 'bone mass' such as number of trabeculae within a trabecular bone volume (Goulet et al., 1991), while the density measure in the current study reflects degree of mineralization within a localized area (individual trabeculae). As a true measure of the fraction of mineral in bone tissue, the ratio of ash weight density to apparent density was reported for both canine and human trabecular tissue (Gong et al., 1964 and Kuhn et al., 1989b), where no significant difference was found between the two species. While this density ratio takes unmineralized osteoid and less mineralized surfaces of trabeculae into account, the mineral density measure in the current study does not include them since all the surfaces of trabeculae have been removed during specimen preparation procedures. Thus, it would be difficult to make density comparisons between the continuum and microstructural level studies due to the 'scaling effect' and different specimen preparation techniques.

It has been generally accepted that there exists positive correlations between mineral density and modulus for both cortical and trabecular bone on a continuum level (Currey. 1969; Mather, 1968; Schaffler and Burr, 1988; Carter and Hayes, 1977; Ciarelli et al, 1991). However, the relationships for trabecular tissue on a microstructural level are virtually uncharacterized. No significant correlations between modulus and mineral density were found in either canine or human trabecular tissue, when the specimens were stratified by species. A significant but weak correlation, however, was found between modulus and mineral density when all the specimens were pooled together (Fig. 7). The mineral density explained only 26% of variance in the modulus, which suggests that the remaining large variance may be explained by microstructure. Kuhn et al. (1989a) reported significant but weak correlations between modulus and mineral density for human iliac crest trabecular tissue. In contrast, a significant positive correlation was reported by Choi et al. (1990) for human tibial trabecular tissue, where mineral density explained 58% of the variance in the modulus. These inconsistent results indicate perhaps that the range of mineral density was low and alterations were much more sensitive to small ultrastructural variations. This aspect is demonstrated in Fig. 7 where a correlation between modulus and mineral density was found only when all the human and canine specimens were pooled together. At this point, it is very difficult to draw any conclusions about the role of mineral density in determining trabecular tissue modulus on a microstructural level, although the data of

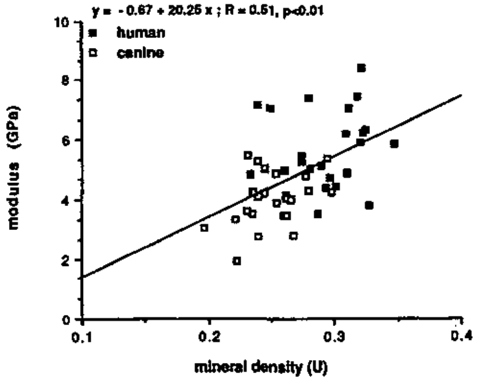


Fig. 7 A linear regression analysis demonstrates a significant but weak correlation between modulus and mineral density when both human and canine trabecular tissue were pooled together

the current study suggest that the lower canine tissue modulus may be related in part to lower mineral density.

Perhaps the combined differences in microstructure, ultrastructure and the mineral distribution pattern between the two species could be responsible for the observed modulus difference. This aspect will be discussed later. While no direct evidence was found in previous investigations to support or dispute the lower modulus of canine trabecular tissue, the study by Kuhn et al. (1989b) suggested that canine trabecular tissue might have different mechanical properties due to its 'unique microstructural characteristics. They reported that canine trabecular bone cubes displayed a lower modulus than human bone for the same density and speculated that differences in microstructure between the two species may be responsible. The lower modulus of canine tissue might have partly contributed to this finding.

A comparison of the fatigue properties between human and canine trabecular bone tissue was illustrated in Fig. 3. An analysis of covariance showed that canine trabecular tissue had significantly lower fatigue strength than human trabecular tissue ($p<0.01$). No significant correlation was found between mineral density and fatigue life for both human and canine bone tissue. This finding does not agree with the results previously reported in other studies where positive correlations were found between apparent bone density and fatigue life in cortical bone (Carter et al., 1976 and 1981; Swanson et al., 1971). Perhaps the scaling effects on mineral density measures and greater variation in microstructure of trabecular specimens used in this investigation, compared with larger cortical specimens, might have contributed to the disagreement.

A significant correlation between modulus and fatigue life was found in canine trabecular tissue, while no relationship was observed in human tissue. However, a significant positive correlation was found, when both canine and human data are pooled together (Fig. 4). This finding is consistent with many previous investigations on cortical bone specimens (Caner et al., 1981, Keller et al., 1985). Since modulus is a mechanical property which accounts for both mineral density and structure, it may serve as a valid estimator for fatigue properties regardless of bone type or specimen size.

The effects of microstructure on fatigue strength have been documented both on a continuum level (Caner et al., 1976) and a microstructural level (Choi and Goldstein, 1992). These results indicated that microstructure affects fatigue strength more than mineral density when structurally different bones were compared. Although both human and canine trabecular tissue demonstrated similar fracture patterns, the texture of fracture surfaces looked different between the two species. This could be explained by the differences in mineral density and microstructure. While both human and canine trabecular tissue consist of trabecular packets, the number of the packets per unit area or average packet size may be different between the two species.

Canine trabecular tissue may contain more packets (or cement lines), as a result, more internal interfaces than human tissue since it has a relatively higher turnover rate (Dannucci et al., 1987; Frost, 1969; Darby and Meunier, 1981). BSI analyses seem to suggest that canine trabecular tissue had more cement lines than human tissue. However, this requires a further quantitative study. The possible contribution of the cement lines to trabecular tissue micro properties has been previously discussed by the authors (Choi and Goldstein, 1992). It was hypothesized that more cement lines would result in lower fatigue strength by providing more crack initiation sites and propagation pathways. It should be noted, however, that the effects of microstructure cannot be completely separated from those of mineral density since the two variables might be interrelated. As stated by Carter et al. (1976), "…the microstructure may correlate (either strongly or weakly) with bone density which may in turn dramatically affect the mechanical property of interest…". Thus, the lower fatigue strength of canine tissue could be a consequence of a lower mineral density associated with its microstructural characteristics.

The different mechanical behavior between two species could be due to 'possible' differences in ultrastructural characteristics, such as mineral crystal size, mineral-collagen fiber interactions, lamellar structure, and arrangements of each lamellae within each packet. To the best of our knowledge, no evidence has been found previously which supports these possible differences. BSI results in this investigation (Fig. 6) seem to suggest that significant ultrastructural differences exist between canine and human trabecular tissue. Human trabecular tissue (Fig. 6(b)) appears to have a better organized lamellar structure with a preferred orientation within each packet. Microcracks in human tissue were relatively larger and fewer in number compared to those in canine tissue (Fig. 6(a)) and were associated with cement lines. This suggests that relatively stronger bone matrix, compared to cement lines, might have prevented crack formation and propagation (Schaffler and Burr, 1987; Burr et al., 1988).

By contrast, canine trabecular tissue (Fig. 6 (b)) shows some packets with no distinct lamellar structure and orientation. Numerous tiny microcracks were observed throughout the tissue while relatively larger cracks were found along cement lines. This might have resulted from the weak nature of canine trabecular tissue, due perhaps to its microstructural and ultrastructural characteristics along with its lower mineral density. Figure 5 illustrated different fracture surface textures between the two species which may also suggest a difference in ultrastructure. While canine tissue showed significantly compliant (or ductile) surfaces (Fig. 5(a)), human tissue demonstrated relatively brittle fracture surfaces (Fig. 5(c)).

5. Conclusions

A great deal of investigations in the area of orthopaedic biomechanics have been conducted through the use of canine models. While many similarities in physiological and mechanical behavior have been reported between canine and human bone, it has also been reported that some differences in mechanical behavior exist between the two species on a continuum level. Therefore, it is very important to establish the limitations of the use of canine models so that results from canine models can be properly interpreted for a specific investigation.

In this study, the use of canine trabecular tissue as a model for human tissue on a microstructural level was examined by comparing their modulus and fatigue properties along with mineral densities and microstructures. Canine trabecular tissue showed a lower modulus and lower fatigue strength than human tissue. The observed microstructural differences between the canine tissue and human tissue may be more responsible for this difference, although the lower mineral density in canine tissue might also have contributed to the lower modulus and fatigue strength.

The role of mineral density in determining the micro mechanical properties of trabecular tissue is not conclusive. Further studies including bone specimens from various species with significantly different mineral densities and microstructure would provide more insights into understanding structure-function relationships on a microstructural level. Based on the observations in this study, possible differences in ultrastructure between the two species were suggested and require further investigations.

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