The Use of Dynamic Mechanical Analysis to Assess the Viscoelastic Properties of Human Cortical Bone

Junro Yamashita,[1] Benjamin R. Furman,[2] H. Ralph Rawls,[2] Xiaodu Wang,[1,3] C. Mauli Agrawal[1]

[1] Center for Clinical Bioengineering, The University of Texas Health Science Center at San Antonio, San Antonio, Texas

[2] Division of Biomaterials, Department of Restorative Dentistry, The University of Texas Health Science Center at San Antonio, San Antonio, Texas

[3] College of Engineering, The University of Texas at San Antonio, San Antonio, Texas

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Abstract: The purpose of this study was to examine the use of a dynamic mechanical analyzer (DMA) system to study the viscoelastic nature of bone. Cortical bone specimens from human femora were tested isothermally for 150 min at 37°C and the loss factor (tanδ) and storage modulus (E') were measured. To explore the effects of test conditions on tanδ and E', different levels of applied stress, two specimen sizes, and two hydration conditions (wet and vacuum-dried) were evaluated. Finally, nonisothermal tests were performed, wherein specimens were heated up to 70°C at different heating rates: 1°C/min, 3°C/min, and 5°C/min. The results indicated that a threshold level of minimum applied stress was required to obtain repeatable and relatively constant values of tanδ. Specimen size did not significantly affect tanδ although it influenced E'. Moisture content had a significant effect on tanδ; vacuum-dried specimens exhibited a lower tanδ compared to wet specimens. Lastly, heating rates influenced tanδ values with lower rates producing more consistent results. The study demonstrated that DMA can be used as an effective tool to test bone. © 2000 John Wiley & Sons, Inc. J Biomed Mater Res (Appl Biomater) 58: 47–53, 2001

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INTRODUCTION

Bone is a natural composite material consisting primarily of three phases: mineral, organic, and water. These three phases are not independent of each other, but rather work in harmony to determine the biomechanical properties of bone.[1] The mineral phase is essentially composed of hydroxyapatite crystals and is relatively brittle in nature. It is well accepted that this phase is the primary component responsible for the elastic properties of bone.[2] Type I collagen constitutes over 90% of the organic phase; collagen molecules cross-link with neighboring collagen molecules to make a three-dimensional lattice-like network. Additionally, collagen fibrils are strengthened by mineral deposits, and vice versa.[3] Although the movement of collagen fibrils is constrained by mineral,[4,5] from the composite material perspective, Type I collagen may play an important role in the viscoelastic properties of bone due to its polymeric molecular structure.

The other main phase is water, but the exact function and location of this water in bone is not fully understood. Water may exist in various forms and locations in bone; the so-called "unfrozen water" contributes to stabilizing the collagen triple helix by means of hydrogen bonding.[6-8] Water is also bonded to crystal surfaces for ion exchange,[9] and some is bonded to extrafibrilar noncollagenous proteins.[10] Other water exists as bulk water in pores, both within and on the surface of bone.

Water may play a notable role in determining the mechanical properties of bone; for instance, the degree of hydration of bone greatly influences its biomechanical properties in *in vitro* tests.[11,12] Moreover, the viscoelastic creep behavior of bone during mechanical tests is considered to be determined by both water and collagen.[13,14]

The viscoelastic properties of bone can play a significant role in high-energy impact type fractures. Parkkari et al.[15] described that the incidence of hip fractures in the elderly population depends on the magnitude of impact energy created by the fall. In another study, Robinovitch et al.[16] reported that the impact produced by a fall to the side has the potential to cause hip fracture in elderly individuals. In fact, most fractures occur under dynamic loading conditions. A better understanding of the viscoelastic behavior of bone would help to further elucidate the mechanism of such fractures. However, the majority of efforts reported to date have focused largely on the elastic behavior of bone in connection

Correspondence to: Dr. C. Mauli Agrawal, Center for Clinical Bioengineering, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive — MC 7774, San Antonio, TX 78229-3900 (e-mail: agrawal@uthscsa.edu)

with changes in the constituent phases, such as those in bone mineral density. Less attention has been paid to the viscoelastic aspect of bone. Black et al.[17] measured the dynamic mechanical properties of human bone under tensile conditions using different loading frequencies at 37.5°C and reported that frequency affected the storage modulus. Lakes et al.[18] described the viscoelastic response of bone under torsional loading as a function of temperature. These studies suggest that the viscoelastic behavior of bone may have a pronounced effect on its fracture behavior under dynamic loading. Beyond these studies, there are few published documents available that focus on the viscoelasticity of bone, especially cortical bone.[19]

The purpose of the present study was to explore the use of a dynamic mechanical analyzer (DMA) as a tool for investigating the viscoelastic properties of bone, and furthermore, to study the effects of various test parameters and establish a reliable technique for using the DMA for bone research. Although the DMA is widely used for characterization of nonbiologic materials such as plastics and metals, its use for bone has not been described yet. In this study, we mainly performed isothermal tests of human cortical bone at body temperature using the DMA and measured the loss tangent and storage modulus as representative measures of bone viscoelastic properties.

MATERIALS AND METHODS

To determine an appropriate test technique for studying bone using a dynamic mechanical analyzer, the effects of various tests parameters on the loss tangent of human cortical bone were examined. These included the magnitude of applied stress, specimen size, and specimen moisture. Additionally, the effects of test duration and temperature scan rates were also studied. Lastly, to gather more information about bone, its storage modulus was also measured.

Specimen Preparation

Human femora ranging in age from 21 to 64 years were obtained from the Musculoskeletal Transplant Foundation (Edison, NJ). None of the donors died of apparent bone-related diseases. Cortical bone specimens were extracted from the diaphysis of the femora using a band saw and an engineering milling machine. During the entire specimen preparation procedure, bone was kept cool and hydrated via copious irrigation with phosphate buffered saline solution (PBS).

Effects of Specimen Size. To examine the specimen size effect on the viscoelastic properties, two different sizes (Small and Large) of rectangular prismatic bone specimens were evaluated. The Small specimens ($n = 8$) were 6.0 × 1.3 × 0.8 mm (length × width × thickness) in dimensions, while the Large specimens ($n = 24$) measured 12.0 × 2.5 × 1.5 mm. The long axis of the specimens corresponded to the longitudinal axis of the diaphysis. The transverse axis of the specimen was parallel to the radius of the diaphysis.

TABLE I. Assignment of Specimens

Large Specimens ($n = 24$)
$n = 8$: used to examine Stress Levels Effect
$n = 8$: used to examine Specimen Size Effect
$n = 8$: used to examine Scan Rate Effect
Small Specimens ($n = 8$)
$n = 8$: used to examine Specimen Size Effect

The specimen assignment for tests are listed in Table I.

Dynamic Mechanical Tests

The two material properties measured using the DMA were the loss tangent (tanδ) and the storage modulus (E'). tanδ is an indicator of viscous energy loss within the material under conditions of dynamic loading, while E' is a measure of the elastic modulus.

Effects of Stress Levels. To accomplish the testing, first a static base stress was applied to the center of the specimen and then a cyclic dynamic stress was superimposed. In order to explore the effects of different applied stress levels on the measured quantities, six different levels of stress were applied sequentially to each of eight of the Large specimens. The combinations of applied stresses were (static stress, dynamic stress): (0.3, 0.2), (0.5, 0.4), (0.8, 0.7), (1.6, 1.4), (2.5, 2.1), and (3.0, 2.5) MPa. This stress range was chosen, because in preliminary experiments the measured values for tanδ and E' reached a plateau and became relatively constant for stress levels over (3.0, 2.5) MPa. Specimens were tested isothermally at 37°C for 10 min at each stress level, using a dynamic mechanical analyzer (DMA 7, Perkin–Elmer, CT) at a loading frequency of 1 Hz.

Test Duration. To investigate the influence of test duration on the measured values, isothermal tests were performed at 37°C for 150 min. The specimens were maintained at 37°C in an incubator before the tests. A dynamic stress of 4.0 MPa was applied to the Small specimens ($n = 8$), while 2.1 MPa was applied to the Large specimens ($n = 8$). The loading frequency was maintained at 1 Hz. The superimposed static stress was 5.0 MPa for the Small specimens and 2.5 MPa for the Large specimens to maintain a stable contact between the specimen and the supporting stage during the test. Since these stresses were small, it was unlikely that the specimens would have been damaged; nevertheless, the stresses were adequate to measure tanδ and E' without excessive noise in the signal. Data for tanδ and E' were collected at a sampling rate of 1 Hz. Each specimen was subjected to a series of four isothermal tests described below.

Specimen Moisture. Weight of the specimens was measured both before and after each test to clarify the role of

moisture in the dynamic mechanical analysis of bone. Prior to determining the weight of the wet specimens, excess surface moisture was removed using laboratory paper tissue. The experimental protocol for each specimen was as follows:

1. Wet bone specimens were tested in the DMA and then immersed in PBS for two days for rewetting.
2. The rewet specimens were tested in the DMA and then placed in an vacuum chamber with anhydrous calcium sulfate for five days for the Small specimens and eight days for Large specimens.
3. The vacuum-dried specimens were tested in the DMA and then immersed in PBS for two days to rehydrate.
4. The rehydrated specimens were tested in the DMA.

Effects of Scan Rate (Variable Temperature Tests). Eight Large specimens were scanned three times from 37–70°C with different temperature scanning rates. These scanning rates were 1.0, 3.0, and 5.0°C/min. These were nonisothermal tests and changes in tanδ were plotted as a function of temperature.

Statistical Analysis

tanδ was calculated by averaging the data from each isothermal test. All values were arranged as mean $\pm$ standard deviation (SD). The effect of geometry on tanδ and E' of bone specimens was statistically analyzed by a *t*-test. Further, a Repeated Measures ANOVA was performed to compare tanδ and E' values among the four different moisture conditions. Differences were considered statistically significant for $p < 0.05$.

RESULTS

Effects of Stress Levels

At low stress levels, the measured values for tanδ were low and also exhibited high noise-to-signal ratio. As larger stresses were applied, tanδ increased, but became relatively constant when more than 2.5 MPa of static and 2.1 MPa of dynamic stress were used. At this point, the noise level also decreased. The variations in tanδ as a function of stress levels are shown in Figure 1. Although not central to this study, the effects of stress on E' were also assessed. These were similar to those exhibited by tanδ. As larger stress was applied, larger E' values were obtained with less noise, and approached a constant value when the stress combination of (3.0, 2.5) MPa was applied.

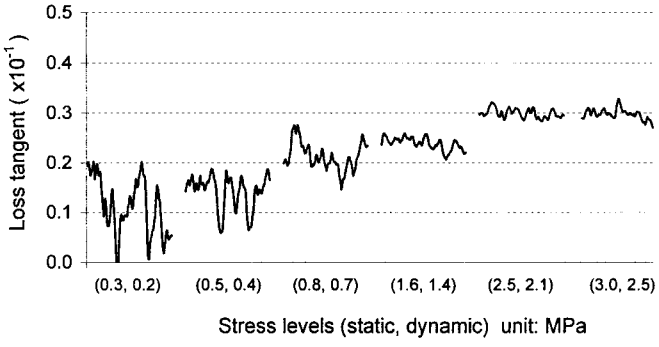

Figure 1. Loss tangent as a function of applied static and dynamic stress: (static, dynamic).

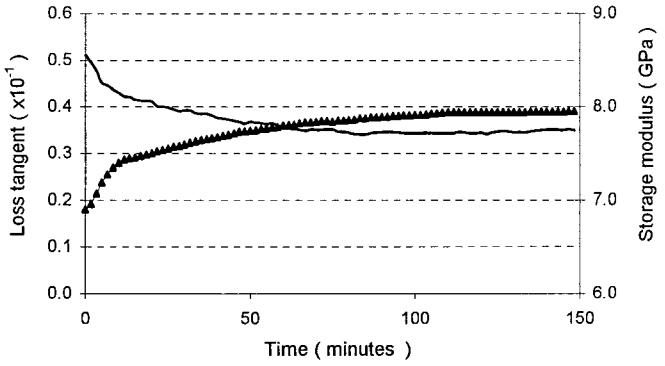

Figure 2. Loss tangent and storage modulus of wet bone as a function of time: (–) loss tangent; (△) storage modulus.

Effect of Specimen Size

The averaged values of tanδ and E' for the Small group were 0.042 ± 0.006 and 8.4 ± 1.7 GPa, respectively, while those for the Large group were 0.039 ± 0.008 and 10.3 ± 1.3 GPa. The results of the *t*-test detected no significant difference in tanδ values between the Small and Large specimen groups ($p > 0.05$) for wet bone; however, E' showed a significant difference between the two ($p < 0.05$).

Effect of Test Duration

No apparent difference was detected in the time-dependent change of either tanδ or E' between the two size groups. tanδ for the wet specimens decreased with time. A steep decline of tanδ was observed within the first 20 min of each isothermal scan, and a more gradual decrease thereafter. In most cases, tanδ remained relatively constant after 80 min (Fig. 2). In general, changes in the storage modulus were opposite to the trends exhibited by tanδ; a steep increase was detected in the first 20 min, followed by a slower rise. In the case of rewetted specimens, tanδ and E' changed with time in a manner similar to that exhibited by the wet specimens in both the Small and Large specimen groups. Table II shows the mean values of tanδ, E' and weight loss for each group. The averaged tanδ, E' and weight loss for the rewet group were almost the same as those for the wet group. However, there were significant differences between the wet and vacuum-dried specimens. For the first 30 min, tanδ of the vacuum-dried specimens decreased gradually and started to increase slowly thereafter. On the other hand, E' increased for the first 10 min but stayed constant thereafter (Fig. 3). The results for

TABLE II. Averaged Loss Tangent, Storage Modulus, and Weight Loss

Specimen Condition	Loss Tangent, tan δ			Storage Modulus, E' (GPa)			Weight Loss (%)		
	Small	Large	Both	Small	Large	Both	Small	Large	Both
Wet	0.042 (0.006)	0.039 (0.008)	0.041 (0.007)	8.4 (1.7)	10.3 (1.3)	9.4 (1.7)	3.4 (2.0)	3.6 (0.9)	3.5 (1.5)
Rewet	0.038 (0.003)	0.038 (0.005)	0.038 (0.004)	8.8 (2.0)	10.0 (1.3)	9.4 (1.7)	4.0 (2.0)	3.6 (0.3)	3.8 (1.4)
Vacuum-dried	0.036 (0.006)	0.022 (0.004)	0.029 (0.008)***	8.4 (1.9)	10.3 (1.3)	9.4 (1.8)	−2.2 (0.8)	−1.2 (0.4)	−1.7 (0.8)***
Rehydrated	0.048 (0.008)	0.036 (0.009)	0.042 (0.010)	8.5 (1.6)	9.6 (0.1)	9.1 (1.4)	4.0 (1.6)	3.4 (0.5)	3.7 (1.1)

[a] (Standard Deviation)
Small, smaller-sized specimen group; Large, larger-sized specimen group; Both, both groups.
p-value calculated by ANOVA and followed by Scheffe's Test, ***$p < 0.001$.

the rehydrated specimens were similar to those of the wet and rewet specimens.

ANOVA and multiple comparison test revealed that tanδ and weight loss of the vacuum-dried specimens showed significantly lower values ($p < 0.001$) among the four different specimen conditions (Table II). However, there was no significant difference in the storage modulus ($p > 0.05$).

Temperature

The tanδ response as a function of temperature was similar at scan rates of 1.0°C/min and 3.0°C/min. In both cases, tanδ increased with increasing temperature (Fig. 4). However, this relationship was not true at a scan rate of 5.0°C/min wherein the relationship between tanδ and temperature was not consistent across the samples tested. Great difference was found among the R-squared values of the linear regression curves for the scan rates (Table III). The R-squared value at a scan rate of 5.0°C/min was significantly low. The values for the low-rate scans were relatively high (approximately 0.9), with no obvious difference between the two lowest scanning rates. On the other hand, E' exhibited similar behavior for the three scan rates: E' decreased very gradually as temperature increased. However, there was a tendency for E' values to be smaller at a scan rate of 5.0°C/min compared to the other scan rates.

DISCUSSION

Dynamic mechanical analysis is a powerful technique frequently used in material science to characterize the viscoelastic behavior of materials. One advantage of using the DMA is that relatively small specimen sizes can be used; this is of great value in testing bone, where large specimens are difficult to obtain from human cadavers and from laboratory animals. To our knowledge, this is the first study to address in detail the use of a DMA for analyzing bone. Under controlled conditions, both the tanδ and E' values for bone obtained in this study showed small deviations and were highly reproducible.

The values of tanδ measured in this study with the DMA were about 0.04 for wet and 0.03 for vacuum-dried condition, which is relatively high compared to the previous data in the literature. Lakes et al.[18] measured tanδ for human tibial bone and bovine compact bone by torsional tests and reported the value of tanδ to be of the order of 0.01. Ramaekers[20] reported that the damping factor for bovine bone was approximately 0.02. This disagreement may be due to the difference in measuring techniques. The principal mode of the DMA used for this study was three-point bending, while the earlier studies tested specimens under shear loading. As described by Lakes et al.,[18] the viscoelastic properties of a material are very sensitive to stress fields. In addition, bone is an aniso-

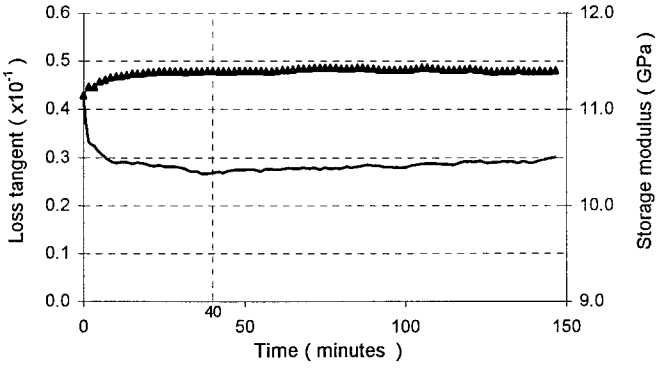

Figure 3. Loss tangent and storage modulus of vacuum-dried bone as a function of time: (–) loss tangent; (△) storage modulus.

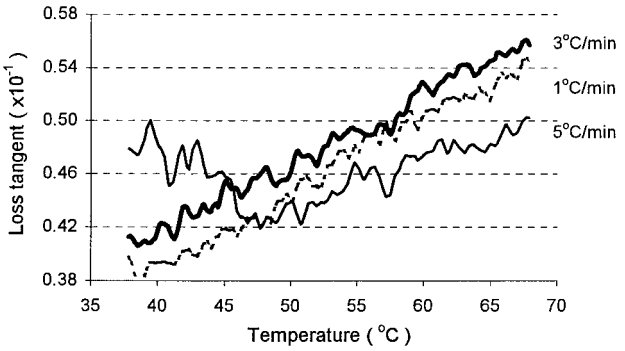

Figure 4. Typical patterns of the tanδ vs. temperature curves when different temperature scanning rates were used. The trend of the curve for 5°C/min was different from others.

TABLE III. R-Squared Values for Heating Rate Curves

	1 °C/min	3 °C/min	5 °C/min
Mean	0.91	0.94	0.47
S.D.	0.08	0.02	0.38

tropic material, and its mechanical behavior depends significantly upon the testing mode, specimen origin, and orientation. In the present study, all specimens were extracted from the same general location on the femur and their orientation was constant. The values of E' obtained in this study ranged from 7–10 GPa. These values were consistent with the data previously reported.[21]

As stated earlier, the mechanical testing mode used in this DMA study was three-point bending. To detect the phase lag between loading and deformation with sufficient sensitivity, adequate deflection was required at the center of the specimen gauge length (loading point). On the other hand, a smaller stress is preferable, because the test must be performed well under the elastic limit of the material. Thus, a threshold stress value, which provides adequate sensitivity and produces results that are stable with a low noise-to-signal ratio, needs to be determined by trial and error. In this study, such a threshold was obtained at 2.5 MPa of static stress and 2.1 MPa of dynamic stress for the larger of the two specimen sizes (Fig. 1). At low stress levels, the fluctuations in tanδ and E' were large. However, these fluctuations decreased proportionally as the applied stress levels were raised. Beyond the (2.5, 2.1) MPa stress combination, both tanδ and E' values were relatively constant for the range of stress levels evaluated in this study. It should be noted, however, that appropriate stress levels depend upon the chosen specimen geometry, because the gauge length of the specimen between the two supports under three-point bending varies with specimen size and the fixtures available. Thus, it is important to obtain correct stress levels via pilot studies.

Although the variable addressed here was the stress level, it is possible that there may have been some corresponding changes in the strain rate, because the test frequency was fixed at 1 Hz and, to change the stress level, the specimen was deformed to different degrees. When the (0.3, 0.2) MPa stress combination was applied to the Large specimens, estimated strain was about 0.004%. When the (2.5, 2.1) MPa stress combination was used, strain was nearly 0.024%. Larger strain occurred when larger stress was applied. Therefore, a higher strain rate would be observed under high strain because of the constant loading frequency of 1 Hz. Thus, strain rates became high as applied stress increased. Because strain rate affects the viscous behavior of materials,[18] it would be expected that the difference in strain rates would influence the tanδ in this study. From our calculations, the strain rates for the Small specimens turned out to be approximately twice those of the Large specimens. Nevertheless, in the results of statistical analysis for tanδ, no apparent difference was revealed between the two sizes. This suggests that the strain rate does not make a significant difference to the value of tanδ. However, more investigation is needed to elucidate the effect of the strain rate on bone viscosity.

Specimen geometry is critical for bone mechanical tests[22]; one reason being that bone is porous in nature. Although the porosity of compact bone is about 10%, which is much less than that of trabecular bone,[23] the effects of the porosity on the measurement of mechanical properties of even compact bone may become significant as the size of the test specimens is reduced. In this study, a significant difference was observed in E' between the two different specimen sizes. However, no significant difference was detected in tanδ between these sizes. As mentioned earlier, bone comprises water, organic, and mineral phases. Any changes in porosity would affect these three phases simultaneously. It is possible that tanδ is determined primarily by the inter-phase interactions between these phases and not so much by the macropores. Few reports have been published addressing the size effect on viscous property of cortical bone, but Linde et al.[24] measured the effect of geometry on mechanical properties of trabecular bone under compressive loading. They reported that the loss tangent does not significantly depend on specimen geometry, although the stiffness had a nearly linear relationship with specimen size. These findings are similar to the results of the present study, in which the measured stiffness increased as a function of specimen size, but tanδ was not affected.

One of the most interesting results of this study is that the loss tangent of the vacuum-dried bone was significantly lower than that of bone under wet conditions, including both the re-wet and rehydrated bone (Table II). Furthermore, the tanδ values for the vacuum-dried bone changed with time, exhibiting a trend opposite to those for wet bone; tanδ increased slowly with time for the vacuum-dried bone after approximately 40 min, whereas for the wet bone it decreased gradually during this same time period (Figs. 2 and 3). These results imply that the viscoelastic properties of bone depend on whether dry or wet conditions are used, and that moisture plays a significant role in determining tanδ for bone. Moreover, dimensional measurements indicated that the bone specimens shrank about 4% in volume when dried. It is possible that the viscoelastic properties are dependent on the organic phase of bone, which primarily consists of collagen. The viscous nature of a material is based on the relative molecular movement within the material.[25] The relative molecular movement of the collagen molecules and fibrils would be restricted when bone dries and shrinks. Such restriction on movement may decrease viscous energy loss in the material and may be one possible reason why smaller tanδ values were measured for the vacuum-dried condition. The weight loss for the specimens in the wet condition was about 3.5% after isothermal testing compared to the weight prior to testing (Table II). It is quite likely that this weight loss is caused by water evaporation. However, this drying was not found to be a linear process during the isothermal test. In a preliminary study, which measured weight change of the specimen with time under room conditions, wet bone specimens rapidly lost approximately 2.0% of their weight within the first 15 min. Over the next 50 min, weight decreased only another 1.0%,

and even more slowly thereafter. Taking this information into account, it is reasonable to assume that the steep decrease of tanδ within the first 20 min observed in the wet bone (Fig. 2) is due to rapid drying. Likewise, a plateau in tanδ values after 80 min of testing could be an indication of very little evaporation. Although water-absorption during testing could perhaps account for the slight increase in tanδ for vacuum-dried bone after approximately 40 min, it does not explain the initial steep decline (Fig. 3). It can be speculated that some manner of molecular relaxation of the organic phase may be responsible, but further study is needed to elucidate the exact mechanism.

Because the viscoelastic behavior of the vacuum-dried bone recovered after soaking in PBS was similar to that exhibited by bone in the original wet condition, it is possible that the evaporated moisture came primarily from the pores in bone. As shown in Table II, the loss of this water resulted in almost a 25% change in the tanδ values. As indicated by the results of this study, water content has a significant effect on tanδ. Therefore, when tanδ is measured for wet bone using a DMA, it is critical to take precautions to eliminate or minimize the effects of this parameter. For isothermal tests, we recommend the following:

1. Excess surface water should be removed before the test.
2. Tests should be run for at least 20 min to let tanδ values stabilize.
3. Values of tanδ averaged over several minutes should be regarded as the representative tanδ.

The thermal conductivity of the cortical bone is low; according to Biyikil et al.,[26] it is 0.3 W/m °C for wet cortical bone. It is, therefore, likely that thermal equilibrium is not achieved when the Large specimen is scanned at a high temperature scan rate. The specimens in this study were scanned from 37–70°C three times using different temperature scanning rates. No significant changes should be expected to occur in the mineral and organic phases under such low temperatures,[4,5] although there would be moisture vaporization effects as a function of time and temperature. Therefore, the absolute values for tanδ and E' may not be identical under different scanning rates, but the slope of the three curves may be compared. In our results, no obvious difference in the slope of E' was observed among the scan rates, indicating that E' may not be greatly affected by the scan rate. However, tanδ appeared to be influenced by this parameter. The curve for tanδ with the 5°C/min scan rate was rather unstable, and obviously different from others. When lower scanning rates were used, the curves were stable and repeatable. This implies that heating at 5°C/min was too fast to obtain thermal equilibrium in the specimen. For the specimen geometry used in the study, the scanning rate of 3°C/min worked well, because it gave consistent tanδ values in a reasonable amount of time. Therefore, low temperature scanning rates are recommended when the specimen is heated or cooled in the DMA. Also, when tanδ is measured at a specific temperature in an isothermal test, the specimen should be equilibrated at or near that test temperature prior to the actual test.

In summary, this study examined the feasibility of using a dynamic mechanical analyzer to investigate the viscoelastic properties of bone. It was determined that such a technique can produce repeatable and consistent data. However, while performing the test, the following test parameters need to be closely controlled: the applied stress level, specimen size, loss of moisture, and the temperature scan rate. Although not explored in this study, it would be expected that the loading frequency would also play a significant role.

The DMA uses relatively smaller specimens than most tensile or torsional tests, which is clearly an advantage when testing human bone, due to the paucity of bone stock available for testing. Thus, the DMA can contribute significantly to the study of the viscoelastic nature of bone. A recent study[27] has shown that the fracture properties of bone have a strong correlation with changes in the collagen. Due to its long chain structure, collagen would be expected to influence the viscoelastic properties of bone. The technique described in this study can be used to assess changes in such properties due to age or disease related deterioration of collagen in bone.

REFERENCES

1. Landis WJ. The strength of a calcified tissue depends in part on the molecular structure and organization of its constituent mineral crystals in their organic matrix. Bone 1995;16:533–544.
2. Burstein AH, Zika JM, Heiple KG, Klein L. Contribution of collagen and mineral to the elastic-plastic properties of bone. J Bone Joint Surg [Am] 1975;57:956–961.
3. Kronick PL, Cooke P. Thermal stabilization of collagen fibers by calcification. Connect Tissue Res 1996;33:275–282.
4. Bonar LC, Glimcher MJ. Thermal denaturation of mineralized and demineralized bone collagens. J Ultrastruct Res 1970;32: 545–548.
5. Raspanti M, Guizzardi S, De Pasquale V, Martini D, Ruggeri A. Ultrastructure of heat-deproteinated compact bone. Biomater 1994;15:433–437.
6. Privalov PL, Tiktopulo EI. Thermal conformational transformation of tropocollagen. I. Calorimetric study. Biopolymers 1970; 9:127–139.
7. Finch A, Gardner PJ, Ledward DA, Menashi S. The thermal denaturation of collagen fibers swollen in aqueous solutions of urea, hexamethylenetetramine, p-benzoquinone and tetra-alkylammonium salts. Biochimica Biophys Acta 1974;365:400–404.
8. Rochdi A, Foucat L, Renou JP. Effect of thermal denaturation on water-collagen interactions: NMR relaxation and differential scanning calorimetry analysis. Biopolymers 1999;50:690–696.
9. Posner AS, Beebe RA. The surface chemistry of bone mineral and related calcium phosphates. Semin Arthritis Rheum 1975; 4:267–291.
10. Lees S, Escoubes M. Vapor pressure isotherms, composition and density of hyperdense bones of horse, whale and porpoise. Connect Tissue Res 1987;16:305–322.
11. Pelker RR, Friedlaender GE, Markham TC, Panjabi MM, Moen CJ. Effects of freezing and freeze-drying on the biomechanical properties of rat bone. J Orthop Res 1984;1:405–411.
12. Yeni YN, Brown CU, Norman TL. Influence of bone composition and apparent density on fracture toughness of the human femur and tibia. Bone 1998;22:79–84.

13. Currey JD. Anelasticity in bone and echinoderm skeletons. J Exp Biol 1965;43:279–292.
14. Bowman SM, Gibson LJ, Hayes WC, McMahon TA. Results from demineralized bone creep tests suggest that collagen is responsible for the creep behavior of bone. J Biomech Eng 1999;121:253–258.
15. Parkkari J, Kannus P, Palvanen M, Natri A, Vainio J, Aho H, Vuori I, Järvinen M. Majority of hip fractures occur as a result of a fall and impact on the greater trochanter of the femur: a prospective controlled hip fracture study with 206 consecutive patients. Calcif Tissue Int 1999;65:183–187.
16. Robinovitch SN, Hayes WC, McMahon TA. Prediction of femoral impact forces in falls on the hip. J Biomech Eng 1991;113: 366–374.
17. Black J, Korostoff E. Dyanmic mechanical properties of viable human cortical bone. J Biomech 1973;6:435–438.
18. Lakes RS, Katz JL, Sternstein SS. Viscoelastic properties of wet cortical bone—I. Torsional and biaxial studies. J Biomech 1979; 12:657–678.
19. Fondrk M, Bahniuk E, Davy DT, Michaels C. Some viscoplastic characteristics of bovine and human cortical bone. J Biomech 1988;21:623–630.
20. Ramaekers JGM. The dynamic shear modulus and damping of compact bovine metacarpal bone in dependence on the topography along the bone shaft. Neth J Zool 1979;29:151–165.
21. Abe H, Hayashi K, Sato M. Data book on mechanical properties of living cells, tissues, and organs. Tokyo: Springer–Verlag; 1996. p 291–362.
22. Keaveny TM, Borchers RE, Gibson LJ, Hayes WC. Trabecular bone modulus and strength can depend on specimen geometry. J Biomech 1993;26:991–1000.
23. Martin RB, Burr DB, Sharkey NA. Skeletal tissue mechanics. New York: Springer–Verlag; 1998. p 29–78.
24. Linde F, Hvid I, Madsen F. The effect of specimen geometry on the mechanical behaviour of trabecular bone specimens. J Biomech 1992;25:359–368.
25. Askeland DR. The science and engineering of materials. Boston: PWS-KENT; 1989. p 526–531.
26. Biyikli S, Modest MF, Tarr R. Measurements of thermal properties for human femora. J Biomed Mater Res 1986;20:1335–1345.
27. Wang X, Bank RA, TeKoppele JM, Hubbard GB, Athanasiou KA, Agrawal CM. Effect of collagen denaturation on the toughness of bone. Clin Orthop 2000;371:228–239.