Apparent viscoelastic anisotropy as measured from nondestructive oscillatory tests can reflect the presence of a flaw in cortical bone

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Abstract: There is evidence that damage, viscoelastic stiffness properties, and postyield mechanical properties are related in bone tissue. Our objective was to test whether presence of a flaw would have an influence on the apparent viscoelastic properties of bone. Examining the effect of flaw orientation on apparent viscoelastic properties and utilization of dynamic mechanical analysis (DMA) as a nondestructive means for detection of damage were our secondary objectives. Cortical bone beams (2 × 2 × 19 mm) machined from the cranial cortex of the radii of six Warhill sheep were used. The specimens were placed in a DMA machine and baseline measurements of storage modulus (E’) and loss factor (tan δ), once for loads in the craniocaudal and once in the mediolateral directions, were performed using a three-point bending configuration for a frequency range of 1–10 Hz. Craniocaudal/mediolateral measurement ratio was calculated as a measure of anisotropy for tan δ and E’. After cutting a thin through-thickness macroscopic notch on the caudal surface at the center of each beam, oscillatory tests were repeated. Two-way repeated measures analysis of variance followed by Tukey’s test was used with group (craniocaudal, mediolateral, notched craniocaudal, and notched mediolateral measurements) and frequency as factors. Regression analysis and analysis of covariance were used for examining the relationship between viscoelastic parameters and frequency. Tan δ and E’ were not different between craniocaudal and mediolateral measurements before the flaw was introduced (p > 0.8 and p = 1, respectively). In the presence of the flaw, tan δ was significantly increased (p < 0.003) whereas E’ was significantly reduced (p < 0.001) for craniocaudal measurements. Tan δ and E’ were nearly isotropic in the tested directions before the introduction of a flaw into the bone tissue. Introduction of a flaw resulted in increased tan δ and E’ anisotropy. Presence of a notch resulted in a significant increase in tan δ anisotropy with increasing frequency. In conclusion, we have demonstrated that cortical bone tissue exhibits a different apparent viscoelastic behavior in the presence of a flaw and depending on the flaw’s orientation. Our finding that the presence of a notch and its orientation can be detected by nondestructive DMA suggests that in vivo techniques may be developed for detection of cortical bone damage. © 2004 Wiley Periodicals, Inc. J Biomed Mater Res 69A: 124 –130, 2004

Key words: cortical bone; dynamic mechanical analysis; viscoelastic properties; damage; crack; anisotropy

INTRODUCTION

There are naturally occurring flaws in bone tissue such as microcracks that are thought to appear as a result of fatiguing activities.1–5 Indeed, a number of in vitro experiments demonstrated that accumulation of microdamage in the form of microcracks is accompanied by degradation of cortical bone mechanical properties during fatigue loading.6–10 Left unrepaired, the presence of these microcracks predisposes the tissue for failure during subsequent loading by reducing its ability to withstand load or to dissipate energy.11–13

It has been demonstrated that reductions in strength and fracture resistance of cortical bone tissue attributed to the presence of such flaws can be monitored by measurements of stiffness changes.11 Furthermore, damage accumulation in bone is time dependent14,15 suggesting that microdamage is also associated with viscoelastic properties of the bone tissue. Consistent with this proposal, postyield mechanical properties of cancellous bone were related to the viscoelastic stiffness of the undamaged tissue in a recent study.16 Microcrack density alone, however, cannot account for the sensitivity of viscoelastic properties to damage.17 Consistent with this observation, it has been
Our primary objective was to test this hypothesis for cortical bone using a macroscopic flaw with a known size, shape, and orientation. Examining the effect of flaw orientation and utilization of DMA as a nondestructive means for detection of damage were our secondary objectives.

METHODS

Radii from six Warhill sheep (8 years old) were used. A 65-mm bone segment was cut out of each radius such that 25-mm proximal and 40-mm distal regions with respect to the center were included. The bone segment was clamped from the distal end and cortical bone beams (2 × 2 × 19 mm) were machined from the proximal piece using the Exakt cutting system (Exakt Technologies, Oklahoma City, OK) resulting in middiaphyseal specimens with the long axis of the beam being aligned with the long axis of the bone. One beam per bone was randomly selected from the cranial cortex of each radius. The experiment involved DMA of bone beams before and after the introduction of a surface flaw (Fig. 2).

The specimens were placed in a dynamic mechanical analyzer (DMA 7e; PerkinElmer, Norwalk, CT) and baseline viscoelastic measurements were performed in 0.9% saline solution at 37°C from oscillatory tests using a three-point bending configuration (15-mm span length). A calcium buffer in the saline solution which is recommended for long-term experiments was not used in this experiment. However, it is unlikely that the leaching of calcium would significantly affect the results because the total amount of time spent for measurements was <45 min. We have also verified in a separate study that there are no significant differences in cortical bone-tissue properties between two consequent measurements in our DMA system. A 550-mN static and 500-mN dynamic load waveform was used as the oscillatory input (Fig. 2), scanning a frequency range of 1–10 Hz (in 0.2-Hz increments). Storage modulus, E₁ (for dynamic tests, equivalent to Young’s modulus) and loss factor, tan δ (an indication of the amount of energy dissipated by viscous mechanisms relative to energy stored in the elastic component) were measured once for loads in the craniocaudal, mediolateral, and notched directions. Craniocaudal/mediolateral measurement ratio was calculated for tan δ and E₁ as a measure of viscoelastic anisotropy. The original geometry of the beams was used in calculations to simulate a situation in which the presence of a notch was unknown.

To simulate a flaw, a thin through-thickness notch (0.150 mm) was cut on the caudal surface at the center of each beam using a dental cutter (22-mm diameter SummaDisk; Shofu Dental Corporation, San Marcos, CA) attached to a CNC milling machine (Micromill 2000; Denford Inc., Medina, OH). The ratio of notch length to beam width (a/W) was 0.5. After the specimens were notched, oscillatory tests were repeated in the DMA machine (Fig. 2).

Two-way repeated measures analysis of variance was used with group (craniocaudal, mediolateral, notched craniocaudal, and notched mediolateral measurements) and frequency as factors. When significance was detected,
Tukey’s test was used to isolate group differences. The results are presented with $p$ values from analysis of variance when an effect is reported and from Tukey’s test when group differences are reported. To examine the general characteristics of the relationship between viscoelastic parameters and frequency, $f$, regression analysis was performed on measurements averaged from six specimens. Differences between groups were examined using analysis of covariance (ANCOVA).

**RESULTS**

tan $\delta$ was significantly different between measurement groups after allowing for effects of differences in frequency ($p < 0.001$; Fig. 3). tan $\delta$ was not different between craniocaudal and mediolateral measurements before the flaw was introduced ($p > 0.8$). Introduction of a flaw in the material as a craniocaudal cut significantly increased tan $\delta$ for craniocaudal measurements ($p < 0.003$). Tan $\delta$ for mediolateral oscillations, however, was not affected by the presence of the flaw ($p > 0.7$). Consequently, there was a significant difference in tan $\delta$ between craniocaudal and mediolateral oscillations in the presence of a flaw ($p < 0.005$). There was no interaction between frequency and groups, indicating that the effect of different levels of frequency did not depend on what level of “measurement” group was present ($p > 0.9$).

ANCOVA applied to the average data from each group revealed significant differences in the slope of tan $\delta$-frequency regressions between mediolateral and notched mediolateral and between craniocaudal and notched craniocaudal groups ($p < 0.007$ and $p < 0.048$, respectively; Fig. 3). However, percent differences in slopes between group averages were small (5–10% between groups as compared with 13–20% within groups).

Figure 2. Specimen flow during the experiment. Cortical bone beams were subjected to three-point bending DMA analysis in two orientations. The black dot marks the proximal end and the medial cortex of the bone beams so that beams are vibrated once in the craniocaudal and once in the mediolateral direction. A through-thickness notch was cut at the center of the beams on the caudal surface and the DMA analyses were repeated in the same orientations as prenotch measurements ($W = 2$ mm, $a/W = 0.5$, and $d = 150$ mm).

Figure 3. The relationship between tan $\delta$ and frequency is shown for each group. The increase in tan $\delta$ for craniocaudal bending due to the presence of a flaw is evident from the figure. The average from six specimens is plotted for each group. Error bars indicate the standard deviation for the six specimens at the corresponding frequency. Regression lines (for the average data) are extended to the axes for a better appreciation of the differences between groups.
There was no difference in $E_1$ between craniocaudal and mediolateral oscillations initially ($p > 0.01$; Fig. 4). In the presence of a flaw, however, $E_1$ was significantly reduced for craniocaudal oscillations ($p < 0.001$). Reduction in $E_1$ due to the presence of a flaw was not significant for mediolateral oscillations ($p > 0.18$). Accordingly, $E_1$ was significantly different between craniocaudal and mediolateral oscillations in the presence of a flaw ($p < 0.02$). Differences in $E_1$ between groups further depended on the frequency ($p < 0.001$).

The general shape of the $E_1$-frequency relationship conforms to a power-law in the form of $E_1 = a f^{m}$ where $a$ and $m$ are constants (Fig. 4). ANCOVA performed on the log-transformed average $E_1$ versus log-transformed frequency revealed that the power exponent $m$ was significantly different between before- and after-notch measurements in the mediolateral ($p < 0.02$; 8% difference) and craniocaudal directions ($p < 0.001$; 24% difference) as well as between mediolateral and craniocaudal measurements ($p < 0.001$; 28% difference and $p < 0.0563$; 6% difference before and after the notch, respectively). Compared to within-group variability (10–40%), percent differences in the power exponent between group averages were small (6–8%) in most comparisons and moderate (24–28%) for comparisons between the initial craniocaudal testing and the remainder of the measurements.

$\tan \delta$ and $E_1$ were nearly isotropic in the tested directions before the introduction of a flaw into the bone tissue (Figs. 5 and 6). Introduction of a flaw resulted in increased $\tan \delta$ and $E_1$ anisotropy. Although viscoelastic anisotropy increased with frequency ($p < 0.001$ in all cases), it was practically uniform in bones without the flaw over the frequencies tested. Presence of a flaw resulted in a significant increase in the dependence of $\tan \delta$ anisotropy on frequency ($p < 0.001$).

**DISCUSSION**

We have demonstrated that cortical bone tissue exhibits a different “apparent” viscoelastic behavior in presence of a flaw and depending on the flaw’s orientation. The reduction in the magnitude of the $E_1$ can be regarded as stiffness loss due to damage, indicating...
that the overall mechanical quality of bone tissue is degraded in the presence of damage.\textsuperscript{6-11,26} This is consistent with previous observations that mechanical damage causes property degradation in bone. However, the observations that: 1. frequency dependence of the elastic modulus and viscoelastic anisotropy are also altered, and 2. the tissue dissipated more energy by “viscoelastic” means in the presence of the flaw when subjected to the same apparent loads takes our understanding of the tissue-damage interaction much further.

The differences between the apparent viscoelastic behavior of damaged and nondamaged bone tissue may be a result of strain-rate amplification by the notch. Because bone mechanical properties are strain-rate dependent,\textsuperscript{27} strain-rate amplification by the flaw might result in local stiffening of the tissue that may be manifested as an alteration of apparent viscoelastic properties. The finding that there are more pronounced effects of the notch in craniocaudal bending is consistent with more concentration of strain by the notch in this orientation. A beam bending analysis treating the notch as a structural defect, or a geometrical discontinuity only, would estimate a 20-fold and 4-fold difference between the true modulus and the apparent modulus (when the notch is considered a material defect and geometry is ignored) in the craniocaudal and mediolateral bending, respectively. Our results (Fig. 4) indicate that the apparent modulus in the presence of a notch is much larger than that could be accounted for by the geometrical effect of the notch (about 1.5-fold reduction as opposed to 20-fold for the craniocaudal direction), further supporting the strain-rate stiffening effect by strain concentrations.

Strain-rate amplification suggested by apparent viscoelasticity changes in damaged bone may have biological implications. It has been reported that bone cells did not respond to strain levels that are typically measured at the continuum level\textsuperscript{28} and it was suggested that there were local strain peaks in the vicinity of microcracks that might stimulate bone cells.\textsuperscript{29,30} Strain rate has been proposed to be even a greater stimulant of the bone adaptive response than the strain amplitude.\textsuperscript{31} If true, strain-rate amplification by microcracks may provide a target for the remodeling units. Increased sensitivity to frequency for high frequencies may result in a remodeling response to impact loads that is different for damaged bone tissue from that of undamaged bone. Amplification of mechanical signals by flaw-shaped mechanosensory organs and the frequency sensitivity of these organs are not uncommon in other biological systems such as the slit sense organs in spider legs,\textsuperscript{32} cockroach sensilla,\textsuperscript{33} and fly wings\textsuperscript{34} supporting the plausibility of a damage-based mechanosignal amplification mechanism for bone tissue. Further experimental work to establish whether strain-rate stiffening is an active mechanism at crack tips in bone is warranted.

In a series of recent studies, it has been observed that estrogen deficiency (OVX) causes changes in the viscoelastic properties of sheep cortical bone that are similar to the changes caused by introduction of a flaw into the tissue observed in this study. Estrogen-deficient bone was viscoelastically more anisotropic than normal bone and the differences between normal and estrogen-deficient bone was more prominent at high frequencies.\textsuperscript{25,35} However, microstructural differences (porosity, osteonal architecture, and remodeling) or mineralization did not explain the viscoelastic property differences between normal and OVX bone.\textsuperscript{36} It may be a change in microdamage content that causes alterations in the viscoelastic behavior of OVX bone tissue.

Techniques based on vibrational or wave propagation principles that have in vivo applications have been successful in detecting age- and disease-related changes in bone properties,\textsuperscript{37,38} the effect of large structural defects on whole bone properties,\textsuperscript{39,40} and in predicting the effect of microstructure,\textsuperscript{41} bone mineral,\textsuperscript{42} and organic components\textsuperscript{43} on bone-tissue properties. The effect of mechanical damage on bone-tissue properties, however, has proven difficult to predict using similar means\textsuperscript{44} despite their usefulness in detecting damage in engineering materials.\textsuperscript{45} Our finding that the effect of a notch and its orientation can be detected by nondestructive DMA suggests that in vivo techniques may be developed for detection of cortical bone damage using frequency methods utilized in this study. Our results indicate that using two orthogonal
directions of testing will provide information on the nature of the damage as well.

In conclusion, the effect of damage on the apparent viscoelastic properties of cortical bone tissue was demonstrated. It was also found that frequency analysis of cortical bone tissue reveals the presence and the nature of the damage in the tissue; thus, DMA is proposed as a promising tool for analyzing cortical tissue damage noninvasively. Cortical bone tissue was subjected to a simulated flaw, in order to control damage morphology in this study. The nature of mechanical damage and its effects on bone viscoelasticity can be quite complicated. This study addresses an isolated form of damage (a defect with controlled geometry). The effect of damage introduced by physiological fatigue loads on the viscoelastic properties of the tissue and whether it can be measured by the frequency analysis of oscillatory vibrations should be further investigated.

References


