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Short communication

Anisotropic viscoelastic properties of cortical bone

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Abstract

Relaxation Young's modulus of cortical bone was investigated for two different directions with respect to the longitudinal axis of bone (bone axis, BA): the modulus parallel (P) and normal (N) to the BA. The relaxation modulus was analyzed by fitting to the empirical equation previously proposed for cortical bones, i.e., a linear combination of two Kohlraush–Williams–Watts (KWW) functions (Iyo et al., 2003. Biorheology, submitted):

$$E(t) = E_0\{A_1 \exp[-(t/\tau_1)^{\beta}] + (1 - A_1)\exp[-(t/\tau_2)^{\gamma}]\}, \quad [0 < A_1, \beta, \gamma < 1],$$

where E_0 is the initial modulus value $E(0)$. τ_1 and $\tau_2 (\gg \tau_1)$are characteristic times of the relaxation, A_1 is the fractional contribution of the fast relaxation (KWW1 process) to the whole relaxation process, and β and γ are parameters describing the shape of the relaxation modulus. In both P and N samples, the relaxation modulus was described well by the empirical equation. The KWW1 process of a P sample almost completely coincided with that of an N sample. In the slow process (KWW2 process), there was a difference between the relaxation modulus of a P sample and that of an N sample. The results indicate that the KWW1 process in the empirical equation represents the relaxation in the collagen matrix in bone and that the KWW2 process is related to a higher-order structure of bone that is responsible for the anisotropic mechanical properties of bone.

Keywords: Bone; Collagen; Structural anisotropy; Stress relaxation; Relaxation modulus; KWW function

1. Introduction

Bone is often regarded as a composite material that consists mainly of stiff hydroxyapatite (HAP)-like mineral particles embedded in a pliant matrix made of collagen fibers. Mineral particles have anisotropic shapes (Fratzl et al., 1992), which are thought to be the cause of the anisotropic mechanical properties of cortical bone. It is well known that the modulus of bone parallel to the longitudinal axis (bone axis, BA) is much larger than that normal to the bone axis (Reilly and Burstein, 1975; Bonfield and Gynpas, 1977; Lipson and Katz, 1984). On the other hand, as collagen fiber in bone matrix is viscoelastic, bone itself has noticeable viscoelasticity. Because of the anisotropic structure of bone at the mineral–collagen fiber level, the viscoelastic mechanical properties are also expected to be anisotropic. Anisotropy in the viscoelastic properties of cortical bone is important, for example, in the fixation of implant materials. Many researchers have investigated the anisotropy of bone, and several models of the anisotropy of Young's modulus of bone have been proposed (Bonfield and Gynpas, 1977; Currey, 1969; Piekarski, 1973; Sasaki et al., 1991; Wagner and Weiner, 1992; Hasegawa et al., 1994; Pidaparti et al., 1996). However, to the best of our knowledge, no empirical evidence of the anisotropic viscoelastic properties of bone has been presented.

The aim of the present work was to determine the effect of the structural anisotropy on the relaxation modulus of cortical bone. Stress relaxation experiments were performed using rectangular specimen plates whose longer edges had been cut parallel and perpendicular to the BA. In our previous paper, as a new empirical equation for description of stress relaxation of cortical bone, we proposed that stress relaxation of cortical bone could generally be described by a linear combination of two Kohlraush–Williams–Watts

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(KWW) functions (Iyo et al., 2003),

$$E(t) = E_0\{A_1 \exp[-(t/\tau_1)^{\beta}] + (1 - A_1)\exp[-(t/\tau_2)^{\gamma}]\}, \quad [0 < A_1, \beta, \gamma < 1], \tag{1}$$

where E_0 is the initial modulus value, $E(0)$. τ_1 and τ_2 ($\gg\tau_1$) are characteristic times of the relaxation processes, A_1 is the fractional contribution of the fast relaxation (KWW1 process) to the whole relaxation process, and β and γ are parameters describing the shape of the relaxation modulus. Relaxation modulus data were analyzed using Eq. (1). The effect of geometrical anisotropy on the relaxation modulus was quantified by parameters in Eq. (1).

2. Experimental

The bone samples used in this study were obtained from the mid-diaphysis of a 36-month-old bovine femur. Optical microscopic examination showed that all of the samples were generally plexiform but partly transformed into Haversian bone. The samples were cut using a band saw under tap water. In order to examine Young's moduli parallel and normal to the BA, we cut out specimen plates whose longer axes were parallel and normal to the BA, respectively. The cut sections were shaped into rectangular plates with approximate dimension of 0.5 cm (width), 3.2 cm (length), and 0.1 cm (thickness) by using emery paper under tap water. A plate with the longer edge parallel to the BA was coded specimen P, and a plate with the longer edge normal to the BA was coded specimen N. Fig. 1 shows the geometry of specimens.

The relaxation Young's modulus of the rectangular sample plate was measured by the three-point bending method. A Kyowa Electric Works (KEW) LTS-1KA strain gauge transducer was used as the force sensor. The LTS-1KA gauge was set on a SIGMA KOUKI Auto-microstage CTS-50X, and the auto-microstage was operated by a rectangular pulse generated by a SIGMA KOKI Co. Stage Controller Mark-12. By moving the microstage downward, a loading head attached to the force sensor probe pushed the specimen plate at the center to give the bending deformation. The deformation value was measured by a position detector equipped by the microstage. The force sensor detects the recovering force of the specimen. The electric signal generated in the LTS-1KA strain gauge was directly recorded by a KEW data logger, model UCAM10A, and finally sent to a computer through GPIB. According to stress–strain curves of bovine femora in the literature, yield strains are around 0.6% (Reilly and Burstein, 1975). Maximum strain less than 0.23%, which was estimated by using the beam equation, was applied within 0.03 s. The relaxation modulus measurement was performed for about 1×10^5 s. During the stress relaxation measurement, the bending deformation detected by the position detector was recorded, confirming that the deformation value was constant. All of the measurements were made in saline solution with 0.05% of thymol as an antibacterial agent. Measuring devices (LTS-1KA and CTS-50X) were placed in an incubator to keep the ambient temperature constant. The temperature of the specimen was maintained at $37 \pm 0.5^{\circ}$C by controlling the temperature of the incubator. Parameter fitting was carried out using Sigma Plot, a scientific graphing software program (SPSS Co.), the algorithm of which based on the Marquardt method of least squares. The suitability of the fitting was judged by both the coefficient of determination, R^2, and the mean square error, s. Analysis of variance (ANOVA) was used to compare E_0, $E_1(= A_1E_0)$, and E_2 $(=[1-A_1]E_0)$ in discussions about anisotropic mechanical properties of bone, where E_1 and E_2 are initial Young's modulus values for the KWW1 process and KWW2 process, respectively.

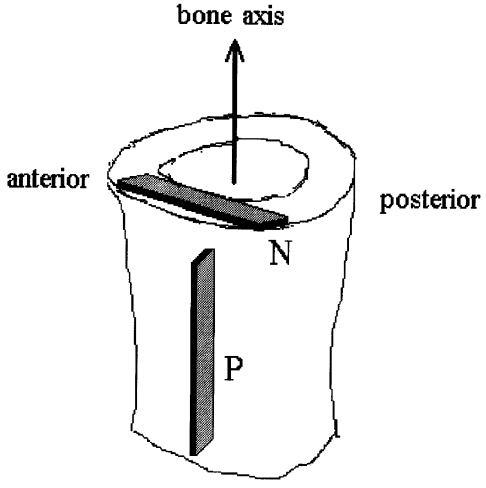

Fig. 1. Specimen plates examined. Plates cut parallel to a longitudinal axis of bone (bone axis, BA) were coded P. Plates cut normal to the BA were coded N.

3. Results

Fig. 2 shows the average values of relaxation Young's modulus, $E(t)$, plotted against time for specimens of P and N. At several points, standard errors are shown by vertical bars and are listed in Table 1. As mentioned above, stress relaxation of cortical bone has been revealed to be expressed by a combination of two relaxation processes according to Eq. (1): a fast process (KWW1 process) with a relaxation time, τ_1, no more than 100 s and a slow process (KWW2 process) with a relaxation time, τ_2, in the order of 10^6 s. In this experiment, fitting of the average data to Eq. (1) was

performed. The relaxation modulus results obtained were described well by Eq. (1). The relaxation parameters determined by the fitting, as well as the coefficient of determination, R^2, and the mean square error, s, are listed in Table 1. The average initial value of the relaxation Young's modulus, $E(0)$, of P was significantly larger than that of N ($p<0.05$, ANOVA).

In the figure, lines represent the relaxation modulus of the KWW1 process, $E_1(t)$, and that of the KWW2 process, $E_2(t)$, for P and N specimens decomposed from the data according to Eq. (1) using parameters of the best fit results listed in Table 1. Despite a difference in structural anisotropy of the specimens, the KWW1 relaxation process of a P specimen is indistinguishable from that of an N specimen at this magnification. In order to quantify the anisotropic mechanical properties of cortical bone, anisotropy ratio (AR) has been defined as the ratio of Young's modulus of bone in the direction parallel to the BA, E_P, against that normal to the BA, E_N, AR $=E_P/E_N$ (Hasegawa et al., 1994). AR values estimated from our results using average values are listed in Table 2, where the AR value for E_0 was listed as AR_0, and AR values for E_1, $AR_1 = E_{P1}/E_{N1}$, and E_2, $AR_2 = E_{P2}/E_{N2}$, were also estimated.

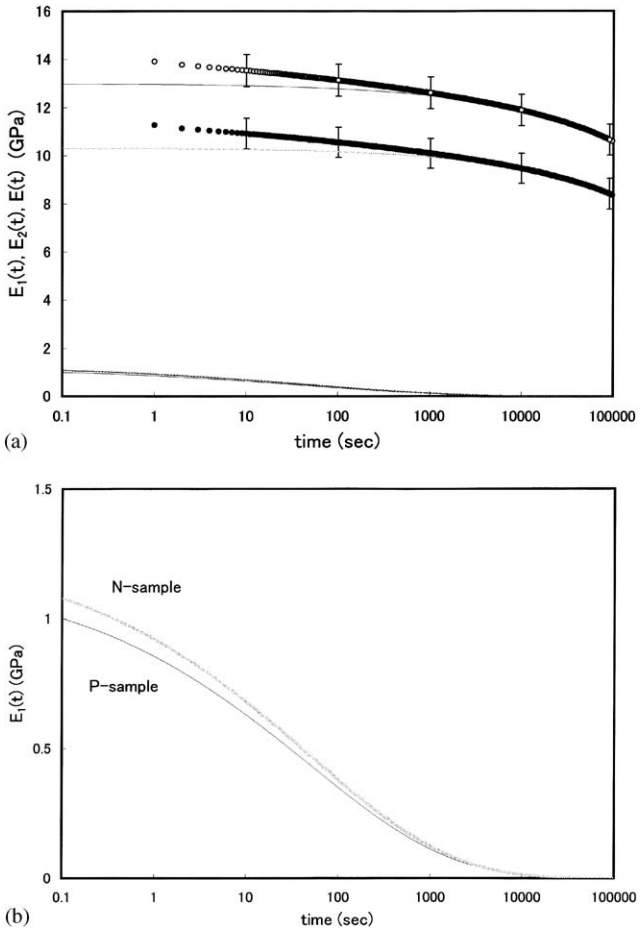

Fig. 2. (a) Average values of relaxation Young's modulus, $E(t)$, for bovine bone samples P(○), and N(●), plotted against time. The vertical lines indicate standard deviations. Lines represent the decomposition of data into the KWW1 process for P(——) and N(- - - - -) and the KWW2 process for P(——) and N(- - - - -), respectively. (b) Magnification of the KWW1 process for P(——) and N(- - - - -).

4. Discussion

4.1. KWW1 relaxation process

The E_1 value for a P specimen was almost equal to that for an N specimen ($p>0.6$, ANOVA), and AR_1 ($=0.93$) was close to 1, indicating that relaxation Young's modulus in the KWW1 process was insensitive to anisotropic morphology of bone. An elementary process of KWW1 relaxation processes was thought to be attributed to a component of bone that was mechanically isotropic. Hasegawa et al. (1994) reported that the calculated AR of demineralized bone material (bone collagen) was close to 1. They suggested that isolated collagen is more or less isotropic and that, by the impregnation of mineral, AR value of the collagen–mineral composite reaches that of whole bone. This fact leads to the conclusion that the KWW1 process in our experiment is attributed to a phase that is independent of the structural anisotropy of bone, that is, to collagen matrix.

The values of Young's modulus of collagen used in models to estimate the modulus of bone as a composite were 1.48 GPa (Hall, 1951), 1.24 GPa (Currey, 1964), and 1.41 GPa (Lees and Davidson, 1977). The initial values of the KWW1 relaxation modulus E_1 for P and N were about 1.19 and 1.28 GPa, respectively, as shown in Table 2. These values were very close to the Young's modulus values of collagen reported in the literature. The ratio of Young's modulus of bone with and without

Table 1
Relaxation parameters according to the empirical Eq. (1) determined for the average relaxation modulus curve

Sample code	Sample size	E_0 (GPa)	A_1	τ_1 (s)	β	τ_2 ($\times 10^6$ s)	γ	Standard error (GPa)					
								10 s	10^2 s	10^3 s	10^4 s	R^2	s
P	7	14.2	0.08	49	0.28	9.3	0.35	0.67	0.66	0.66	0.65	0.99989	0.0086
N	5	11.6	0.11	50	0.26	6.4	0.37	0.64	0.63	0.62	0.63	0.99986	0.0195

mineral, $X = E_{\text{demineralizedbone}}/E_{\text{bone}}$, has been reported to be about 0.13 (average) almost regardless of specimen anisotropy as shown in Table 3 (Lees et al., 1979; Hasegawa et al., 1994; Takano et al., 1996; Pidaparti et al., 1996). In our empirical formula, A_1 represents the fractional contribution of the KWW1 process to the whole bone relaxation processes. The value of A_1 in Table 1 was about 0.1 for both P and N specimens, very similar to X in the literature. The similar value of A_1 to that of X suggests that the KWW1 process represents the relaxation of the collagen matrix in bone.

Comparison of E_1 and related parameters, AR and X, with parameters of whole bone suggests that the KWW1 process originate from relaxation of the collagen matrix in bone. As shown in Table 1, other parameters related to the KWW1 relaxation process, τ_1 and β, of P specimens were also similar to those of N specimens. These parameters were also insensitive to structural anisotropy. In order to confirm the validity of the attribution of the KWW1 process, a comparison of the stress relaxation of collagen itself is necessary. Atkinson et al. (1999) compared the relaxation Young's modulus of a large fibril of a human patellar tendon with that of small fibril of a human patellar tendon. A tendon is a connective tissue that mainly consists of type 1 collagen. Fig. 3(a) shows the reproduced relaxation modulus of the large fibril of a human patellar tendon (Atkinson et al., 1999). The curves represent the KWW1-type

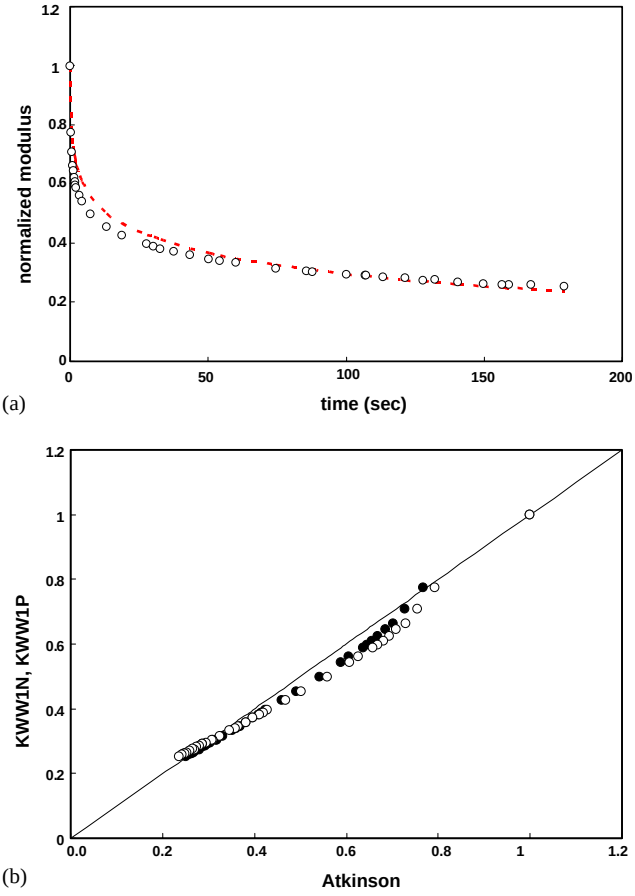

Fig. 3. (a) Relaxation modulus of human patellar tendon normalized by its initial value. Data points were reproduced from Atkinson et al. (1999). Dashed and solid lines represent, respectively, relaxation modulus of the KWW1 process for a P specimen normalized by its initial value (dashed line) and that for an N specimen (solid line), where values $\tau_1 = 49$ s and $\beta = 0.28$ in Table 1 were used for a P specimen and $\tau_1 = 50$ s and $\beta = 0.26$ in Table 1 were used for an N specimen. (b) Correlation plot of normalized modulus of empirical data for human patellar tendon by Atkinson et al. (1999) against KWW1 for P(○) and N(●) samples in this experiment. The solid line represents a complete correlation.

Table 2
Decomposition of initial Young's modulus value into those of the KWW1 and KWW2 processes and anisotropic parameters

Sample code	E_0 (GPa)	E_1 (GPa)	E_2 (GPa)	AR_0	AR_1	AR_2
P	14.2	1.19	13.0	1.22	0.93	1.26
N	11.6	1.28	10.3			

Table 3
Comparison of Young's modulus values of demineralized bones

Species	Condition	Method	Direction relative to BA	Young's modulus (GPa)		X	References
				Before demineralisation	After demineralisation		
Bovine femur	Wet	Ultasonic	Parallel	35.00	5.03	0.14	Lees et al. (1979)
	Wet	Ultasonic	Parallel	34.45	4.05	0.12	Hasegawa et al. (1994)
Canine femur							
	Wet	Ultasonic	Normal	21.36	3.84	0.18	
			Parallel	30.21	2.48	0.08	Hasegawa et al. (1994)
Human femur							
	Wet	Ultasonic	Normal	24.05	2.42	0.10	
	Wet	Ultasonic	Parallel	39.21	4.37	0.11	Takano et al. (1994)
Monkey femur							
	Wet	Ultasonic	Normal	25.15	3.70	0.15	
	Wet	Ultasonic	Parallel	33.90	3.86	0.11	Pidaparti et al. (1996)
Canine femur							
	Wet	Ultasonic	Normal	22.40	3.55	0.16	

relaxation of a P specimen (using parameters in Table 1, $\beta = 0.28$, $\tau_1 = 49$ s) and N specimen (using parameters in Table 1, $\beta = 0.26$, $\tau_1 = 50$ s). Fig. 3(b) shows the correlation plot of the normalized relaxation modulus of a patellar tendon with normalized KWW1 relaxation. The KWW1-type relaxation of bovine bone corresponded significantly to the relaxation data of the human patellar tendon presented by Atkinson et al. (1999). It is concluded that the KWW1 relaxation process represents stress relaxation of the collagen matrix in bone.

3.2. KWW2 relaxation process

AR_2 (=1.26) for the KWW2 process was similar to that of AR_0 (=1.22) for the whole bone, indicating that an elementary process of the KWW2 relaxation process originates from a component causing the anisotropy of the whole bone. The difference between the whole relaxation Young's modulus value of a P sample from that of an N sample is represented by the difference in the respective KWW2 relaxation modulus values. The relaxation time for the KWW2 process, τ_2, for P-specimen was larger than that for N-specimen. Values of γ for P and N specimens were similar but larger than β values. This indicates that the KWW2 process is attributable to a mode that is governed by the structural anisotropy in bone. The smaller modulus value of an N sample than that of a P sample is understandable on the basis of a preferred orientation of longer axes of mineral particles in the BA direction (Sasaki et al., 1991; Fratzl et al., 1992), indicating that the transmission of force along the longer axis in an N sample would be mediated more by the collagen matrix than that in the case of a P sample. τ_2 of an N sample was smaller than that of a P sample. We have reported that the longer relaxation time of bone, τ_2, increased with increase in the mineral fraction (Sasaki and Yoshikawa, 1993). This fact was attributed to an increase in reinforced collagen matrix by mineral particle with an increase in mineral content of the bone sample. Then, a smaller τ_2 value of an N sample than that of a P sample also suggests such geometry of a specimen plate that the reinforcement of collagen matrix by mineral particles may less effective in the longer axis direction of an N sample than that of a P sample. The KWW2 relaxation process was concluded to be originated from the mixed phase of collagen matrix and mineral particles in bone.

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