

## Measurement of Wave Velocity in Cortical Bone by Micro-Brillouin Scattering Technique: Effect of Bone Tissue Properties

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Hypersonic wave velocity was measured in the cortical bone of bovine femur using a micro-Brillouin scattering technique. Using thin plate specimens, wave velocities propagating in the bone axis direction were measured. Next, focusing on the hydroxyapatite (HAp), which is one of the main components of bone, we estimated the relationship between wave velocity and HAp content. The decalcification caused a clear wave velocity decrease from  $5.06 \times 10^3$  to  $3.28 \times 10^3$  m/s, showing the strong effects of HAp on the elasticity of bone. The micro-Brillouin scattering technique would be helpful for the evaluation of bone characterization in a small area. © 2012 The Japan Society of Applied Physics

The National Institutes of Health (NIH) consensus congress pointed out the necessity to evaluate not only bone mineral density (BMD) but also bone quality for bone diagnosis.<sup>1)</sup> The rather ambiguous term “bone quality” includes many factors such as bone microstructure, and bone turnover, which affect bone elasticity. The quantitative ultrasound (QUS) method can determine the wave properties *in vivo*, which are associated with the elastic properties. Moreover, previous studies have shown that QUS is useful for the assessment of osteoporosis using two parameters, i.e., the speed of sound (SOS) and broadband ultrasound attenuation (BUA).<sup>2,3)</sup> However, these parameters are affected by the structure, heterogeneity, and material properties of multi-scales. In particular, the bone material properties at the microscopic level are still poorly understood because it is difficult to investigate them without the effect of bone microstructure. In this study, using a micro-Brillouin scattering technique with a high spatial resolution, we measured wave velocity in the cortical bone without the effect of bone microstructure. In addition, focusing on the hydroxyapatite [HAp:  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ] crystallites, which are one of the main components of bone, we estimated the relationship between wave velocity and HAp content.

A ring-shaped cortical bone specimen was obtained from the midshaft of the left femur of a 31-month-old female bovine (Fig. 1). In the plane of the bone axis and radial directions, 36 slices of plate specimens were made. To obtain sufficient transparency, the thinly sliced specimens were polished using a polishing paper (Maruto N-100) to a thickness range of 32–77  $\mu\text{m}$ . In addition, another plate specimen perpendicular to the bone axis was prepared from the upper part of the specimen to check the conditions of decalcification using an X-ray diffractometer (Philips X-Pert Pro MRD).

Brillouin scattering measurement was performed using a six-pass tandem Fabry–Pérot interferometer (JRS Scientific Instruments).<sup>4)</sup> The micro-Brillouin scattering technique uses a solid state laser with a wavelength of 532 nm (Spectra Physics, Excelsior), and its system includes a microscope for Raman scattering. The actual spot diameter of the focused laser beam on the specimen was approximately 8  $\mu\text{m}$ , which enabled us to evaluate the wave properties without the effect of bone microstructure. In this system, the RI $\Theta$ A scattering geometry shown in Fig. 2 was used.<sup>5)</sup> The geometry enables the simultaneous observation of phonons propagating in the directions of the wave vectors of  $q^{\Theta A}$  and  $q^{180}$  in one measurement. In this study, the velocity of the wave

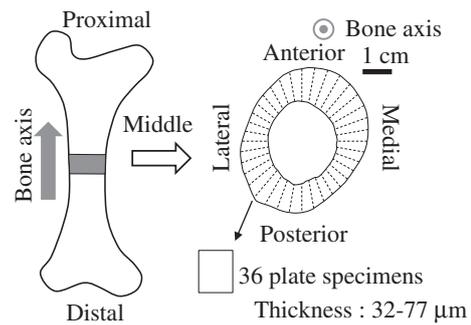


Fig. 1. Preparation of the 36 plate specimens. Thin plate specimens were cut out along the broken line from the ring-shaped cortical sample obtained from the midshaft.

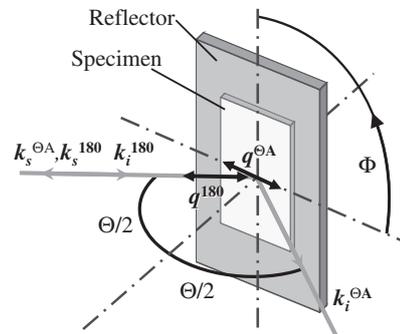
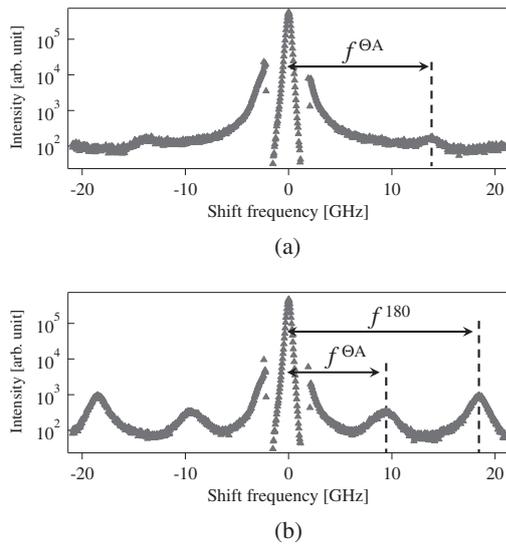


Fig. 2. RI $\Theta$ A scattering geometry.  $k_i$  is the wave vector of the incident light,  $k_s$  the wave vector of the scattered light,  $q$  the wave vector of the sound wave,  $\Theta/2$  the angle between the incident laser beam and the normal line of the sample surface, and  $\Phi$  the rotation angle in the plane.

propagating along the bone axis was measured at four different positions in each specimen, from the measurement of  $q^{\Theta A}$  phonons.

Decalcification was carried out using ethylenediaminetetraacetic acid (EDTA) solution.<sup>6)</sup> After the initial velocity measurement, all the specimens were immersed for 5 days in the solution at room temperature. The specimens were then washed for one day with running water, and velocity measurement was carried out again. To check the decalcification, X-ray diffraction analysis (X-ray source:  $\text{Cu K}\alpha$  under the tube conditions of 45 kV and 40 mA) was performed before and after the process.<sup>7)</sup> Before decalcification, an intense peak of diffraction was observed at  $25.8^\circ$ , which corresponds to the (0002) plane of HAp crystallites.<sup>8)</sup>

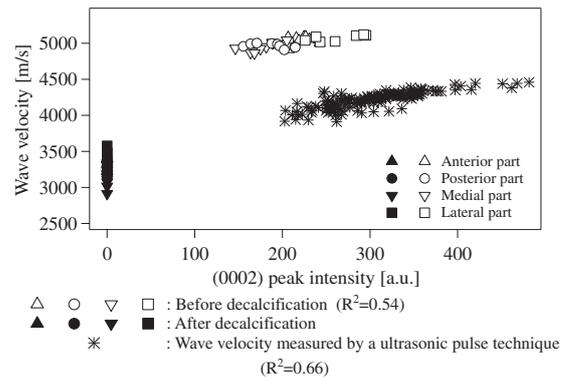


**Fig. 3.** Observed Brillouin spectra of the cortical bone. The shift frequencies  $f^{\Theta A}$  and  $f^{180}$  correspond to the directions of the wave vectors  $q^{\Theta A}$  and  $q^{180}$ , respectively. (a) Before decalcification bone. (b) After decalcification.

On the other hand, complete decalcification was verified from the fact that no (0002) peak was observed after decalcification. The integrated (0002) peak intensity is a good indicator of HAp content.

Figure 3 shows the Brillouin spectra (a) before and (b) after decalcification. After decalcification, Brillouin peak intensity became larger than that of the initial specimen, which results from the increasing transparency of the specimen. Accordingly, the wave velocity error for the estimation of peak frequency also decreased from  $0.07 \times 10^3$  to  $0.03 \times 10^3$  m/s. From the shift frequency of  $f^{\Theta A}$ , the obtained wave velocity in cortical bone was estimated in the range of  $(4.81\text{--}5.22) \times 10^3$  m/s. The average wave velocity was  $5.06 \times 10^3$  m/s with a standard deviation (SD) of  $0.12 \times 10^3$  m/s. The velocities are in the same range of those for cancellous bone.<sup>9)</sup> Because Brillouin scattering gives velocities in a small area of the bone matrix, this means that the properties of bone matrices in cortical and cancellous bones may be similar. On the other hand, the wave velocity for decalcified bone was in the range of  $(2.91\text{--}3.58) \times 10^3$  m/s. The average wave velocity was  $3.28 \times 10^3$  m/s with a SD of  $0.13 \times 10^3$  m/s. After decalcification, wave velocity became significantly lower than before (analysis of variance: probability value  $p < 0.01$ ). (0002) peak intensity also showed a significant decrease due to the decalcification. This indicates the possibility that wave velocity highly depends on HAp content of bone.

The relationship between wave velocity and (0002) peak intensity is shown in Fig. 4. There was a moderate correlation between wave velocity and (0002) peak intensity ( $y = 1.3x + 4726$ , correlation coefficient  $R^2 = 0.54$ ) before decalcification. Although the bone microstructure changes owing to the bone position,<sup>7)</sup> our data shows that the wave properties of the bone matrix do not strongly depend on bone microstructure but on HAp content. The (0002) peak intensity in the decalcified bone was almost zero, and we found a clear wave velocity variation. One reason for this



**Fig. 4.** Relationship between velocity and (0002) peak intensity. Velocities in the MHz range were obtained by an ultrasonic pulse technique.

seems to be the heterogeneity of the bone matrix, which mainly includes the elastic anisotropy of type I collagen.<sup>10–12)</sup> Previous ultrasonic studies have shown that the maximum-wave-velocity direction often slightly differs from the bone axis direction.<sup>7)</sup> In our decalcified bone, the direction of maximum wave velocity changes and may differ from the bone axis direction. This can induce a velocity variation in bone axis direction. Moreover, the velocities of the samples except lateral parts showed a moderate correlation ( $R^2 = 0.30$ ) before and after decalcification. This implies the effects of collagen on wave velocity. Figure 4 also shows the wave velocities for the bovine cortical bone determined by a conventional ultrasonic pulse technique in the MHz range.<sup>13)</sup> The wave velocity measured by the Brillouin technique was much higher than those obtained in the MHz range. One reason for this seems to be the frequency dispersion of velocity. Another reason is the difference in the measurement area between the pulse technique (a few mm) and the micro-Brillouin scattering technique ( $8 \mu\text{m}$ ). The wave velocity by the pulse technique includes the effect of bone microstructure. The micro-Brillouin scattering technique may give us elastic properties in a small area and thus is helpful for the characterization of bone matrices.<sup>14)</sup>

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