

Measurement of wave velocity in cortical bone by micro-Brillouin scattering technique.

-Effect of bone tissue properties.-

顕微 Brillouin 散乱法による皮質骨中の音速測定
-骨の組成が与える影響-

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1. Introduction

The NIH consensus congress pointed out the necessity to evaluate not only bone mineral density (BMD) but also bone quality for bone diagnosis [1]. The rather ambiguous term “Bone quality” includes many factors such as the bone microstructure, bone turnover, etc., which affects the bone elasticity. The quantitative ultrasound (QUS) method can derive the wave properties *in vivo*, which are connected to the elastic properties. Moreover, previous studies have shown that QUS are useful for the assessment of osteoporosis using two parameters, speed of sound (SOS) and broadband ultrasound attenuation (BUA) [2]. However, those parameters are affected by structure and heterogeneity from several scales. Especially, bone material properties at microscopic level are poorly understood because it is difficult to avoid the effect of microstructure.

In this study, using micro-Brillouin scattering technique with high spatial resolution, we measure wave velocity in cortical bone in the microscopic level. In addition, focusing on the amount of hydroxyapatite (HAp), which is one of the main compositions of bone, we estimate the relationship between wave velocity and HAp content.

2. Material and methods

2.1. Specimen

A ring-shaped cortical bone specimen was obtained from the mid-shaft of 30-month-old female bovine right femur (Fig. 1). In the plane of bone axis and radial direction, 36 plate specimens were sliced out and polished to the thickness of approximately 50 μm. In addition, another plate specimen perpendicular to the bone axis was prepared to check the condition of decalcification by X-ray diffractometer.

2.2. Brillouin scattering technique

Brillouin scattering measurement was performed by a six-pass tandem Fabry-Pérot interferometer. The micro-Brillouin scattering uses

a solid state laser ($\lambda_0:532$ nm), and system includes a microscope for Raman scattering. The actual spot diameter of the focused laser beam on the specimen was approximately 8 μm.

The RIA scattering geometry shown in Fig. 2 was used [3]. The geometry enables the simultaneous observation of phonons propagating in each direction of wave vector of $q^{\Theta A}$ and q^{180} in one measurement. In this study, focusing on the $q^{\Theta A}$ direction that propagates in the in-plane direction, we measured wave velocity propagating in the direction along the bone axis.

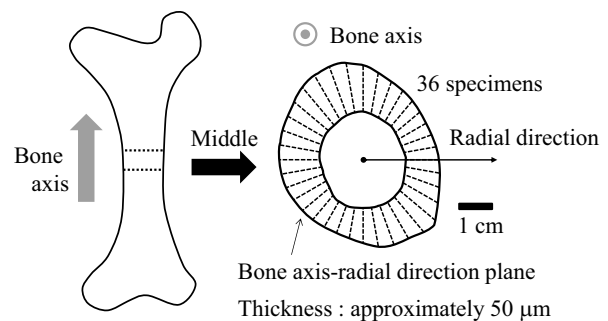


Fig. 1 Specimen preparation.

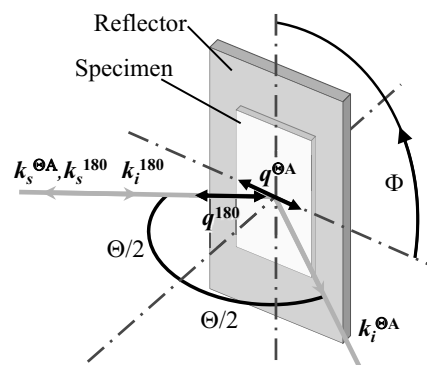


Fig. 2 The RIA scanning geometry.

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, Φ the rotation angle in the plane.

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2.3. Decalcification

Decalcification was carried out using lactic acid. After initial velocity measurement, all specimens were immersed for 5 days in lactic acid at room temperature. The lactic acid was not changed during the decalcification process. After this decalcification, velocity measurement was carried out again.

To make sure of the decalcification, X-ray diffraction (Philips, X-Pert Pro MRD, X-ray source: Cu-K α at a tube condition of 45 kV and 40 mA) was performed before and after the decalcification process. An intense peak of diffraction was observed at 25.8 $^\circ$, which corresponds to the (0002) plane. The amount of HAp was estimated by the integrated (0002) peak intensity.

3. Results and discussion

Figure 3 shows a Brillouin spectrum of (a) initial cortical bone and (b) decalcified cortical bone. After decalcification, Brillouin peak intensity became larger than initial cortical bone. This is because that transparency of the specimen increased by decalcification.

The range of wave velocity in cortical bone was 4.81-5.22 $\times 10^3$ m/s. The average value was 5.06 $\times 10^3$ m/s and the standard deviation was 0.12 $\times 10^3$ m/s. The velocity values are in the same range of the cancellous bone [4]. This means the wave velocity of cortical and cancellous bone are similar in the microscopic level. On the other hand, wave velocity in the decalcified bone was 2.94-3.54 $\times 10^3$ m/s. The average value was 3.28 $\times 10^3$ m/s and the standard deviation was 0.16 $\times 10^3$ m/s.

Figure 4 shows the average of wave velocity and (0002) peak intensity before and after decalcification. After decalcification, wave velocity became significantly lower than that before decalcification ($p < 0.01$). The (0002) peak intensity between two groups also showed a significant difference. This indicates the possibility that wave velocity highly depends on the content of HAp in bone

Bone mainly consist of collagen type I and HAp. Actually, velocity in the decalcified bone was very similar to the dry artificial collagen film (collagen type I) ; 3.20 $\times 10^3$ m/s [5], telling little effect of HAp.

4. Summary

Hypersonic wave velocity in the cortical bone was measured by a micro-Brillouin scattering technique. The velocity values were in the same range with those of cancellous bone. Before and after decalcification, wave velocity changed from 5.06 $\times 10^3$ m/s to 3.28 $\times 10^3$ m/s, telling strong effects of HAp on the elasticity of bone. Brillouin scattering gives us the elastic properties in the small area and would be helpful for the evaluation of bone characterization [6].

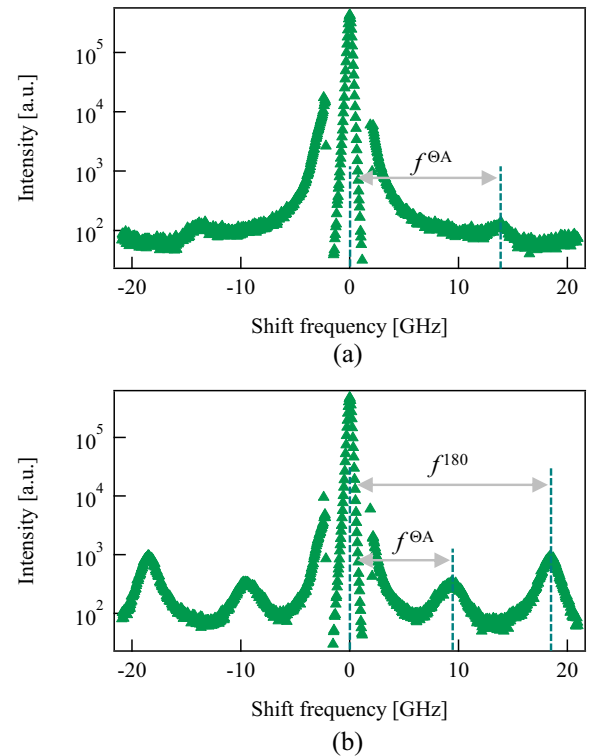


Fig. 3 Observed spectra by Brillouin scattering technique. (a) cortical bone, (b) decalcified cortical bone. The shift frequencies $f^{\Theta A}$ and f^{180} correspond to the direction of wave vectors $q^{\Theta A}$ and q^{180} , respectively.

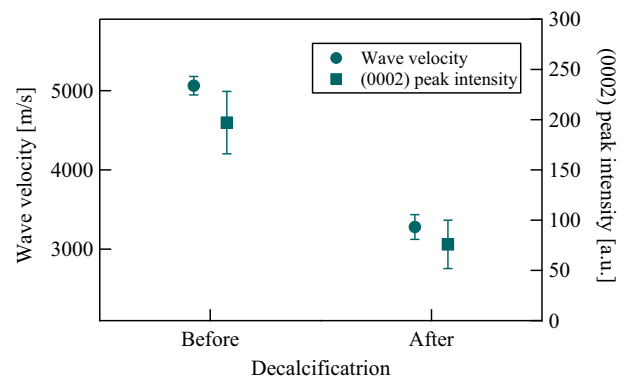


Fig. 4 Comparison of wave velocity before and after decalcification.

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