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Micro-Brillouin scattering study on the effect of water on wave velocity in cortical bone
骨中の水分が超高周波音速に与える影響

Yuki Imoto[1 ‡], Hiroko Tsuneda[1], Shinji Takayanagi[1], and Mami Matsukawa[1]
([1]Doshisha Univ)
井本有紀 [1‡]，常田裕子 [1]，高柳 真司 [1]，松川 真美 [1] ([1] 同志社大 波動セ)

1. Introduction

Bone is mainly composed of collagen and minerals such as hydroxyapatite (HAp). In order to evaluate bone strength, it is important to know not only bone mineral destiny (BMD) but also bone quality such as elastic properties and microstructure [1]. However, it is difficult to evaluate the elastic property of bone by ultrasonic methods in vivo because the results include effects of structure, heterogeneity and properties of bone and marrow. Nanoindentation and scanning acoustic microscopy (SAM) is useful for the evaluation of bone matrix [2]. But these measure properties only in the thickness direction. On the other hand, micro-Brillouin scattering technique can measure wave velocities of bone matrix in all in-plane directions [3]. In addition, the measured wave velocity shows good correlation with the acoustic impedance by SAM [4].

In this study, we have investigated the velocity anisotropy in the minute area of cortical bone using a micro-Brillouin scattering technique. Especially, we focused on the effect of water on the wave velocity in cortical bone. We then used decalcified samples to confirm the effects of water.

2. Material and methods

2.1. Specimen

A ring-shaped cortical bone specimen was obtained from the mid-shaft of 32-month-old female bovine right femur (**Fig. 1**). In the plane of bone axis and radial direction, 2 plate specimens (7 mm×4 mm) at the anterior and posterior parts were sliced out and polished to the thickness of approximately 70 μm.

2.2. Brillouin scattering technique

The Brillouin scattering measurement was performed by a six-pass tandem Fabry-Pérot interferometer. The micro-Brillouin scattering uses a solid state laser (λ_0:532 nm). The actual spot diameter of the focused laser beam on the specimen was approximately 10 μm.

In this study, the RIΘA scattering geometry was used [5]. This geometry enables the simultaneous observation of phonons propagating in each direction of wave vector of $\boldsymbol{q}^{\Theta\mathrm{A}}$ and $\boldsymbol{q}^{180}$ in one measurement. Focusing on the $\boldsymbol{q}^{\Theta\mathrm{A}}$ direction where wave propagates in the in-plane direction, we measured velocity anisotropy in the cortical bone. In order to evaluate the effect of the water on bone specimens, we also measured the velocity in dry and wet conditions before and after decalcification.

2.3. Decalcification

Decalcification to remove HAp was carried out using a lactic acid. After initial velocity measurements, specimens were immersed for 5 days in the lactic acid at room temperature. The lactic acid was not changed during the decalcification. After decalcification, we confirmed that there were no HAp crystallites by X-ray diffraction technique.

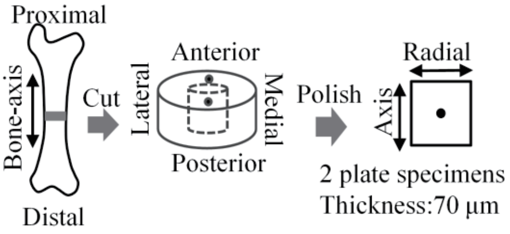

Fig. 1 Specimen preparation.

3. Results and discussion

Figure 2 shows typical Brillouin spectra of a cortical bone before and after decalcification. After decalcification, Brillouin peak intensity became larger. It is due to the increase of transparency. **Fig. 3** shows the velocity anisotropy in the anterior part of cortical bone under dry and wet conditions before decalcification. **Fig. 4** shows that in the anterior part and the posterior part after decalcification.

3.1. Wave velocity in dry condition

Before decalcification, the range of wave velocities in the anterior part was 4.85-5.04×10^3 m/s, and that in the posterior part was 4.83-5.00×10^3 m/s. After decalcification, the velocities decreased and were in the range of 3.22-3.54 ×10^3 m/s in the anterior part and 3.02-3.30 × 10^3 m/s in the posterior part. The results show that the wave

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mmatsuka@mail.doshisha.ac.jp

velocities in bone tissue strongly depend on the amounts of HAp crystallites.

3.2. Anisotropy in dry condition

Before decalcification, the bone anisotropies (difference between maximum and minimum velocity/maximum velocity) were around 3.8% in the anterior part (**Fig. 3)** and 3.5% in the posterior part. The maximum velocities were always found near the bone-axis direction which was the direction of collagen alignment. After decalcification, the anisotropies increased up to 9.2% in the anterior part and 8.6% in the posterior part. Because there were no HAp crystallites after decalcification, the anisotropic character seems to mainly depend on the collagen. Thus, the strong contribution of collagen to the anisotropic elastic properties in bone was confirmed.

3.3. Effect of water on wave velocity and anisotropy

When we dropped water of 40mL, the bone specimens were fully wet. In the wet condition before decalcification, wave velocity in the anterior part decreased in the range of 1.8-3.1% (**Fig. 3**), and that in the posterior part, it was in the range of 1.9-3.5%. After decalcification, the wave velocity also decreased depending on the amount of dropped water. In addition, the velocity decreases due to the water were larger in the anterior part than in the posterior part (**Fig. 4**). The results suggest that the water in bone effects on the elastic properties and it depends on the position.

As for the velocity anisotropy changes due to the water content, the anisotropy increased up to 4.3% in the anterior part and up to 4.0% in the posterior part before decalcification. After decalcification, we found more increase in wet condition. These data show that the absorption of water in bone affects both velocities and anisotropy.

4. Conclusion

The longitudinal wave velocities in cortical bone under dry and wet conditions were measured by a micro-Brillouin scattering technique. In the wet condition, the wave velocity decreased and the anisotropies increased. The results show that the water content effects on the elastic properties in bone. Especially, the velocity changes due to the water content depended on the position in the bone and collagen.

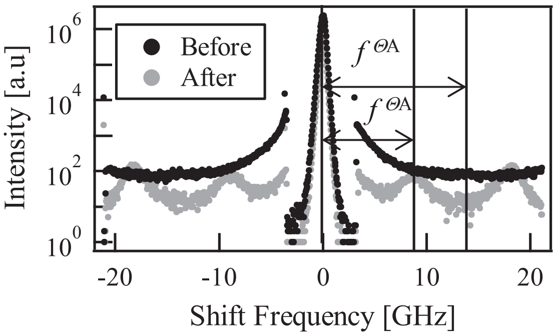

Fig. 2 Observed spectra before and after decalcification. The shift frequencies $f^{\Theta A}$ corresponds to the direction of wave vectors $\boldsymbol{q}^{\Theta A}$.

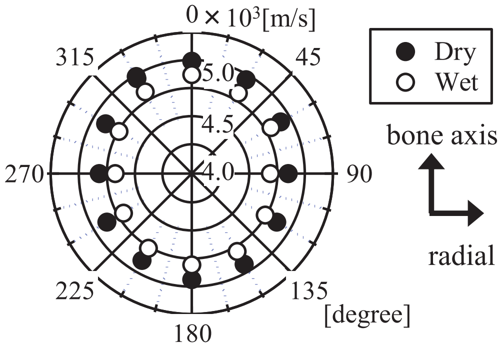

Fig. 3 Anisotropy in the anterior part of cortical bone under wet and dry conditions before decalcification.

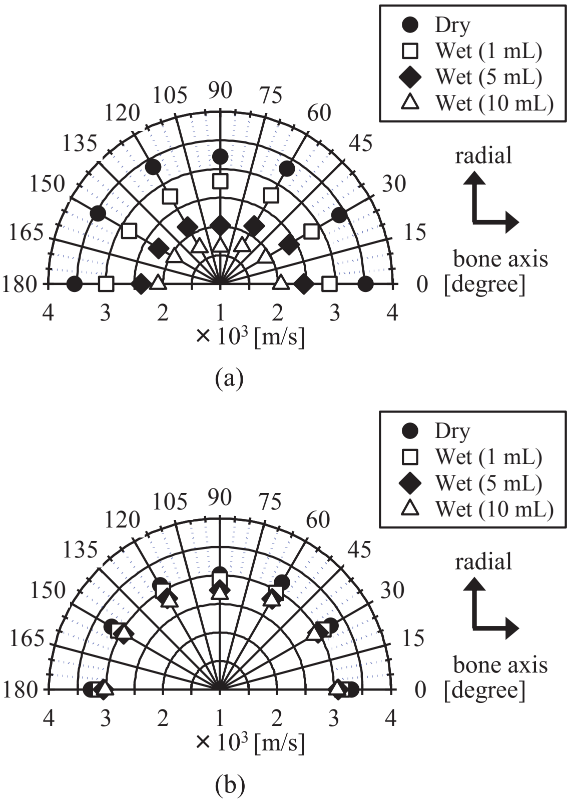

Fig. 4 Anisotropy under dry and wet conditions after decalcification. (a) the anterior part, (b) the posterior part.

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