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Effect of collagen on wave velocity in bone measured by micro-Brillouin scattering

顕微 Brillouin 散乱法を用いたコラーゲンが骨中の縦波音速に与える影響の評価

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1. Introduction

The diabetic disease and adynamic bone disease weaken bone, although the bone structure and bone mineral density (BMD) appear normal [1,2]. Therefore, U.S. National Institutes of Health (NIH) consensus development panel has pointed out the necessity to evaluate not only BMD but also bone quality. The ambiguous term “bone quality” includes several possible features of bone such as micro- to macroscopic structure, bone turnover, and material properties which affect bone elasticity. In order to retrieve bone elastic properties, quantitative ultrasound (QUS) methods in the MHz range have been used in vivo. Moreover, previous studies have shown that QUS is useful for the assessment of osteoporosis using speed of sound and broadband ultrasound attenuation. However, these parameters are affected by structure, heterogeneity and material properties in the large area through which the ultrasound waves passed. On the other hand, evaluating the mechanical properties of the bone without the effect of the microstructure remains difficult.

In this study, using micro-Brillouin scattering technique with high spatial resolution, we measured wave velocity in the cortical bone. Focusing on the collagen especially, we estimate the relationship between wave velocity and the effect of water in bone.

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 left femur (**Fig. 1**). In the plane of bone axis and radial direction, plate specimens at the lateral part was sliced out and polished to the thickness of approximately 70 µm.

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 (λ_0:532 nm). The system includes an optical microscope for Raman scattering. The actual spot diameter of the focused laser beam on the specimen was approximately 10 µm.

The RIΘA scattering geometry shown in **Fig. 2** was used. The geometry enables the simultaneous observation of phonons propagating in each direction of wave vector of $\boldsymbol{q}^{\Theta A}$ and $\boldsymbol{q}^{180}$ in one measurement. In this study, focusing on the $\boldsymbol{q}^{\Theta A}$ direction that propagates in the in-plane direction, we measured wave velocity propagating along the bone axis or radial directions. In order to evaluate the effect of the water, we measured the velocity in dry and wet conditions. The velocity in the specimen was defined as the average velocity obtained from the data at 5 points as shown in **Fig. 1**.

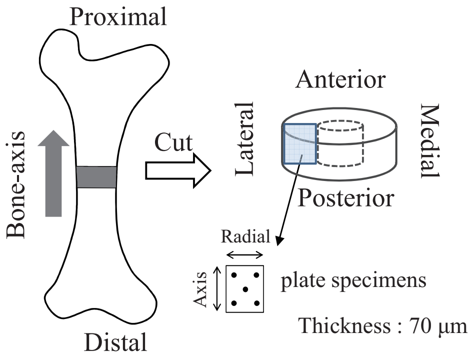

Fig. 1 Specimen preparation.

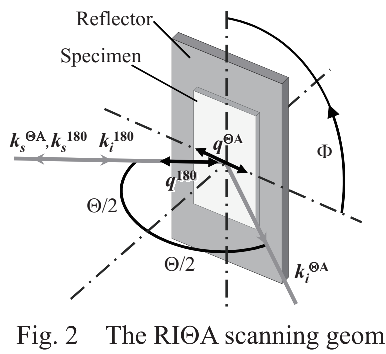

Fig. 2 The RIΘA scanning geometry.
$\boldsymbol{k}_i$ is the wave vector of the incident light, $\boldsymbol{k}_s$ the wave vector of the scattered light, $\boldsymbol{q}$ the wave vector of the sound wave, Θ/2 the angle between the incident laser beam and the normal line of the sample surface, Φ the rotation angle in the plane.

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2.3. Decalcification

Decalcification was carried out using a lactic acid. After initial velocity measurement, a specimen was immersed for 5 days in the lactic acid at room temperature. The lactic acid was not changed during the decalcification process. After this decalcification, velocity measurements in dry and wet conditions were carried out.

3. Results and discussion

Figure 3 shows Brillouin spectra of **(a)** initial cortical bone and **(b)** decalcified cortical bone. After decalcification, Brillouin peak intensity became larger because transparency of the specimen increased.

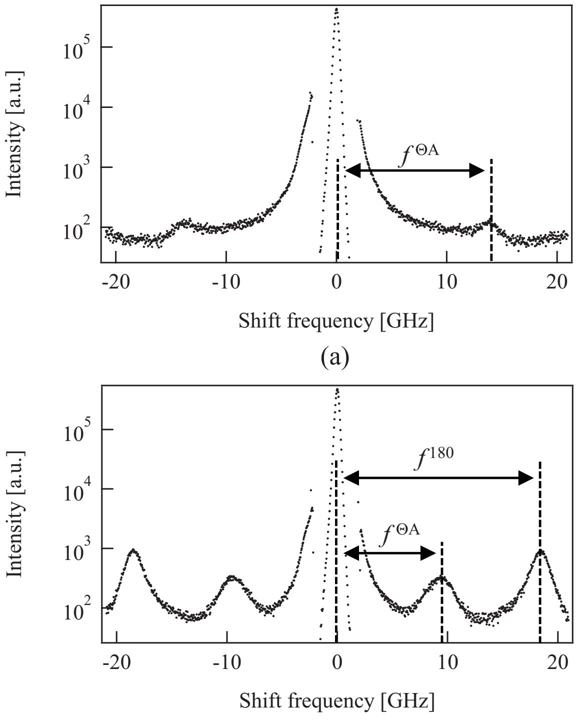
The range of wave velocity in the dry cortical bone was 4.81-5.04×10^3 m/s. The velocity values are in the same range of the trabeculae in cancellous bone [3]. This means the bone properties of cortical and cancellous bone are similar in the small area. On the other hand, wave velocity in the decalcified bone was 2.96-3.54×10^3 m/s. The velocity values were similar to the dry artificial collagen film (collagen type I) ; 3.20×10^3 m/s [4].

Figure 4 shows the average values of wave velocity in dry and wet conditions. The velocity in wet condition was significantly lower than that of dry conditions ($p<0.01$). The density in the wet specimen increased 7 % as much as that of dry specimen. The increase of density possibly causes the velocity decrease of 3 %. On the other hand, the difference was not statistically significant due to the propagating direction in cortical bone.

Figure 5 shows the average values of wave velocity in dry and wet conditions after decalcification. There was a significant difference in the velocity between dry and wet condition ($p<0.01$). In addition, the anisotropy clearly appeared in specimen after decalcification. The velocity in specimen seems to depend on the property of Type I collagen. The effect of the water might increase due to the characteristics of collagen.

4. Summary

A micro-Brillouin scattering technique was used to measure wave velocity in cortical bone tissue under dry and wet conditions. The velocities in wet condition decreased around 3 %. In after decalcification, the decrease was more than 6 %. The result show the water in collagen might change the bone property. Brillouin scattering gives us the elastic properties in the micrometer scale and would be helpful for the evaluation of bone characterization.



(a)

(b)

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 $\boldsymbol{q}^{\boldsymbol{\Theta A}}$ and $\boldsymbol{q}^{\boldsymbol{180}}$, respectively.

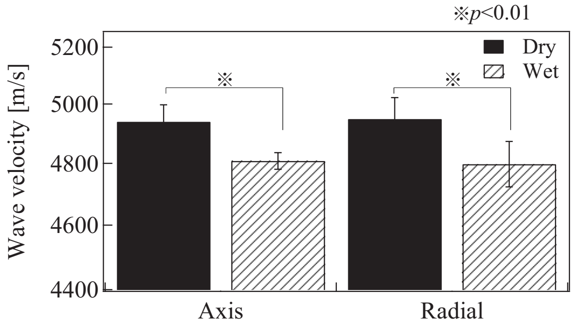


Fig. 4 Comparison of wave velocity in dry and wet conditions in samples before decalcification.

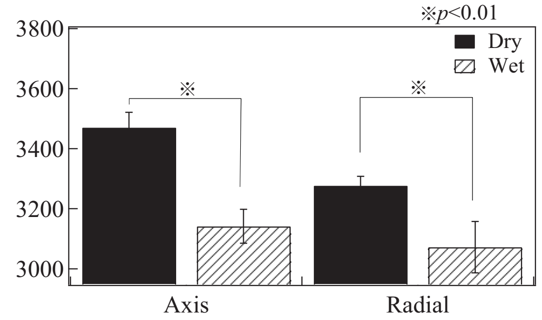


Fig. 5 Comparison of wave velocity in dry and wet conditions in samples after decalcification.

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