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Laser ultrasonic study on the wave velocity in bone with abnormal collagen crosslinks

レーザー超音波法を用いた骨中の縦波音速評価 -コラーゲン中の悪玉架橋が与える影響-

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1. Introduction

The National Institutes of Health (NIH) consensus development panel has noted that the bone strength depends on not only bone mineral density (BMD) but also bone quality [1]. Actually, diabetic patients are often found to have high bone fracture risk, despite of the normal BMD. Without reduction of BMD and deterioration of bone structure, the diabetic diseases also weaken bones [2]. In diabetic patients, bone quality deteriorates due to the excessive formation of advanced glycation end products (AGEs) in collagen [1 - 3]. Currently, bone strength is estimated based on BMD measurement, and the X-ray method is widely used as an evaluation method for BMD. However, since the collagen cannot be measured by the X-ray method, the changes of bone strength due to collagen cannot be evaluated. On the other hand, the methods using ultrasonic waves can evaluate the elastic properties of bones, and the results include the effects of collagen. In the GHz range, Imoto et al, have reported that the longitudinal wave velocities in cortical bone with artificial glycation were lower than those of cortical bone without glycation [4]. However, the velocity decrease in the MHz range has not been confirmed yet.

In this study, we prepared bone samples with artificial glycation, and evaluated the longitudinal wave velocity in bone in the MHz range.

2. Material and Methods

2.1 Fabrication of bone samples

Figure 1(a) shows the preparation steps of the bone samples. We used the mid shaft of left bovine femur (31 months). We processed the shaft into disk-shaped samples. Normal directions of the disks were axial, radial and tangential. The diameters and thicknesses of these samples were 10.0 mm and 1.00 ± 0.03 mm, respectively.

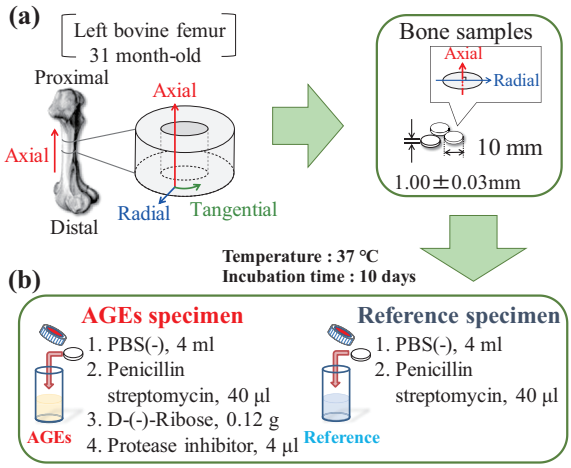

Fig. 1 (a) Preparation of bone samples. (b) The process of glycation.

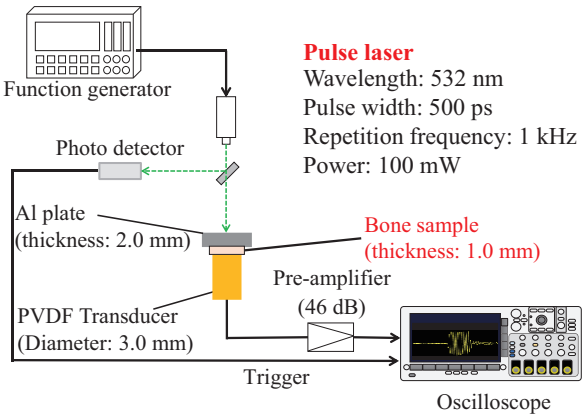

Fig. 2 Experimental setup used.

2.2 Incubation for glycation

Figure 1(b) shows the preparation method of glycated bone samples. The samples were incubated in a mixture of phosphate buffered saline (PBS; 166-23555; Wako, Osaka, Japan), D-(-)-Ribose (R9629; SIGUMA-ALDRICH, St. Louis, USA), Protease Inhibitor Cocktail Set Ⅲ (without EDTA; 539134-; Calbiochem, San Diego, USA) and Penicillin-Streptomycin (15140; gibco, Carlsbad, USA) [4]. Reference sample were set in a mixture of PBS and penicillin-streptomycin. All sample were kept in an incubator at 37℃ for 10 days.

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2.3 Experimental procedure

Figure 2 shows the experimental set up used. A thin aluminium plate was set on the bone sample as a sound source for the laser ultrasonic method. A short laser pulse (wavelength: 532 nm, pulse width: 500 ps, repetition frequency: 1 kHz, power: 100 mW) was irradiated onto the surface of the aluminium plate to generate ultrasound. The longitudinal ultrasonic waves that passed through the aluminium and the bone sample were received by a PVDF transducer (handmade, diameter: 3 mm). The received signal was amplified 46 dB with a preamplifier, and observed by an oscilloscope. Glycerin was used for the interface layers between aluminium and bone, and bone and transducer. The wave velocity in the bone was calculated from the difference of arrival times between the waveforms that only passed through the aluminium and that passed through both aluminium and the bone sample. The wave velocity of each bone sample was estimated as the average of the velocities measured at five points in the sample.

3. Results and Discussion

Figure 3 shows the observed waveforms received by the PVDF transducer. The top wave is the one which passed only through aluminum, and the waves below show those passed though the aluminum and bone sample. Due to the attenuation at the interfaces and bone, the amplitudes of the bone waves were much smaller.

Figure 4 shows the results of wave velocities. In both samples before and after the glycation, the anisotropy of the wave velocity was confirmed. The wave velocity was the highest in the bone axis direction and the lowest in the radial direction. This tendency was in good agreement with the previous results by Yamato et al [5]. In addition, the wave velocity decreased in the glycated samples in all directions ($p < 0.05$). The decrease rate were 1.7 % (axial direction), 3.4 % (radial direction) and 6.0 % (tangential direction). These results suggest that glycation may decrease velocity of ultrasonic wave in the cortical bones, indicating the possible decrease of elasticity. Yasui et al. have reported that the wave velocity in bone decreased in diabetic model rats (SDT rats) by comparing the healthy model rats (SD rats) and that the rate of decrease was larger in the tangential direction than in the axial direction in the GHz range[6]. In this study, similar results were observed in the MHz range. However, the reason of the different velocity decrease due to the direction is not clear. In the next step, the anisotropy of the wave velocity in bone with abnormal collagen crosslinks should be investigated in detail.

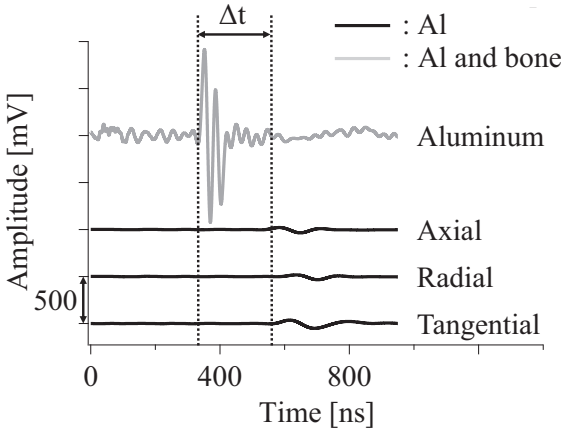

Fig. 3 Observed waveforms by PVDF transducer (Before glycation, reference specimen).

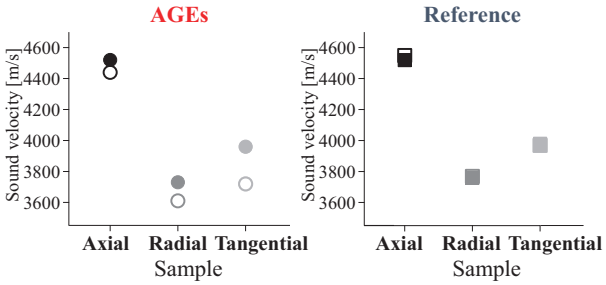

Fig. 4 Observed longitudinal wave velocities
Black : glycated samples. White : normal samples.

4. Conclusion

In this study, we investigated the effects of glycation on the longitudinal wave velocity in the cortical bone using a laser ultrasound technique. Consequently, the wave velocity decreased after glycation. This result possibly indicates that abnormal collagen crosslinks reduce the longitudinal wave velocity and elasticity of cortical bone.

References

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