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Effects of crosslinking condition of collagen on ultrasonic wave properties in bovine cortical bone
ウシ皮質骨中の架橋状態が超音波伝搬特性に及ぼす影響

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1. Introduction

The mainstream of recent clinical evaluation of bone is the dual-energy X-ray absorptiometry (DXA). DXA enables to measure bone mineral density (BMD), which accounts for 80% of bone weight. However, after 1990's, some fracure cases have also been reported from patients with sufficient amount of minerals. One reason for this phenomenon is the abnormal crosslinks between molecules of the collagen, which occupies about 50% of bone volume. The abnormal crosslinks called advanced glycation end products (AGEs)[1] generates due to saccharification and oxidation. DXA cannot measure the amount and quality of collagen. Therefore, new techniques which can noninvasively evaluate the abnormality of bone collagen are strongly required. The Quantitative UltraSound technique (QUS) can be a good candidate for this purpose. It enables the measurement of bone elastic properties in vivo. However, there are few studies on the effects of AGEs on the bone ultrasonic properties.

In this study, we have investigated the effects artificial AGEs crosslinks in bovine cortical bone on the ultrasonic wave properties.

2. Material and Methods

A left femur was obtained from a 27-month-old bovine. A ring-shaped cortical bone sample was obtained from the mid-shaft. Four spherical specimens (diameter 9 mm) were fabricated from the anterior and posterior parts in the ring sample as shown in Fig. 1.
Three dimensional anisotropy of ultrasound wave velocity has been investigated in such spherical bone specimens.[2] For AGEs formation, they were incubated in a phosphate buffered saline (PBS) solution, with D-(-)-Ribose, Protease Inhibitor Cocktail Set III (without EDTA) and Penicillin-Streptomycin for 30 days at 37℃ (AGEs specimen)[3]. Reference specimens were also incubated in pure PBS solution for 30 days at 37℃.

Measurements of longitudinal wave velocities were performed using a conventional ultrasonic pulse system in Fig. 2. A PVDF focus transmitter and a flat PVDF receiver were used in this experiment. Both PVDF transducers were mounted coaxially with distance of 60 mm in degassed water at 25.0±0.1 ℃. A single sinusoidal signal with a center frequency of 1 MHz and amplitude of 50 Vp-p was applied to the focus transducer. The longitudinal wave propagated through water, specimen and water. The flat transducer received the wave, and converted it into the electrical signal. The signal was amplified by a pre-amplifier and visualized in an oscilloscope. The measured specimen was placed in the focal zone of the sound field. The measurements were performed at each rotation angle θ of 5 degrees in the Axial-Radial (A-R), Axial-Tangential (A-T) and Radial-Tangential (R-T) planes. The velocity and attenuation were estimated from the arrival time and amplitude spectra of observed waves.

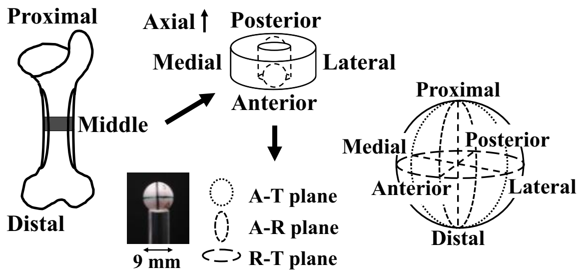

Fig. 1 Spherical specimen.

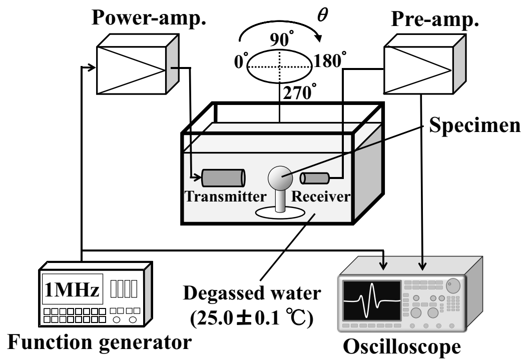

Fig. 2 Ultrasonic measurement system.

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3. Results and Discussion

Figure 3 shows velocity changes in axial-tangential plane (A-T plane) of anterior or posterior part specimens. Here, 0 degree indicates the axial direction (A) and the 90 degree indicates the tangential direction (T) in the spherical specimen. In the anterior part, the velocity changes of AGEs specimen were higher than those of reference. On the other hand, the velocity changes of AGEs specimens in the posterior part were similar. One possible reason for these different tendencies seem to result from bone microstructure at the microscopic level. In the posterior part, cylindrical haversian structure is dominant, whereas the brick like plexiform structure is dominant in the anterior part.

Figure 4 shows velocity changes in axial-radial plane (A-R plane). The 0 degree indicates the axial direction (A) and the 90 degree indicates the radial direction (R) in the spherical specimen. The velocity changes of AGEs specimen were higher than those of reference in most parts and directions. Because of the small number of the specimens used, the more quantitative evaluation of AGEs crosslinks seems necessary. However, the increase of velocity by AGEs formation is obvious, possibly telling that the abnormal crosslinking results in the hard but brittle bone properties.

4. Conclusion

The effects artifical AGEs crosslinks in bovine cortical bone on the ultrasonic wave velocities were investigated. In most cases, velocity of AGEs specimen increased, showing dependences on the microstructure.

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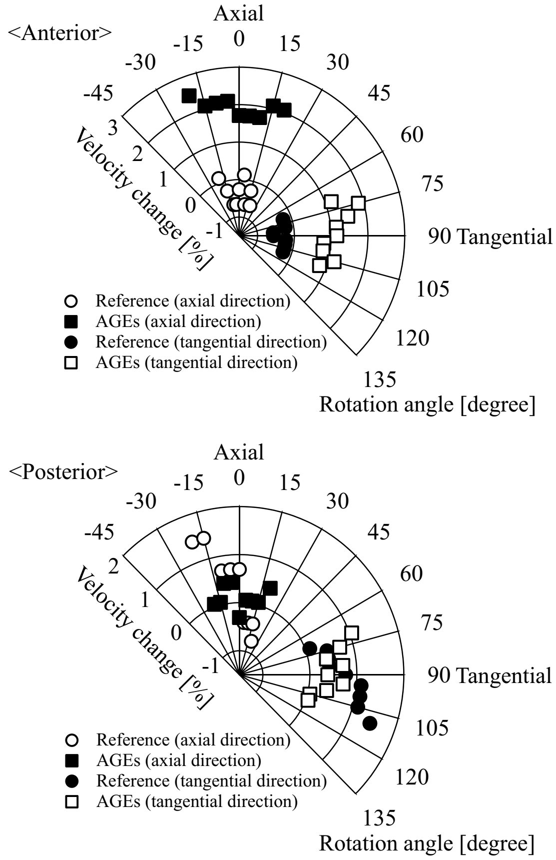

Fig. 3 Velocity changes in axial-tangential plane.

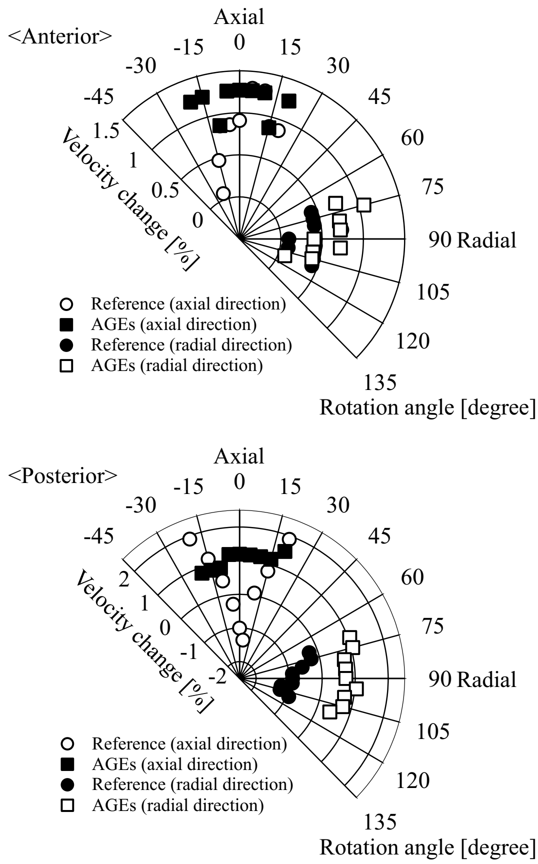

Fig. 4 Velocity changes in axial-radial plane.