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Influence of abnormal collagen cross-links on ultrasonic velocity in bone
骨コラーゲン中の悪玉架橋が超音波音速に与える影響

Ryohei Ueda[1†], Mami Kawase[1], and Mami Matsukawa[1]
([1]Doshisha Univ. Wave Electronics Research Center)
上田　涼平 [1†], 川瀬　麻実 [1], 松川　真美 [1]
([1] 同志社大波動エレクトロニクス研究センター)

1. Introduction

Recently, the increase of the bone fracture risk by the osteoporosis and diabets has become a problem.

The present mainstream of the diagnosis of the osteoporosis is the dual-energy x-ray absorptiometry (DXA). This technique can diagnose minerals in bone, which account for about 80 percent of bone weight. However, there are some cases of bone fracture, in spite that the mineral amounts stay normal. One possible reason for this is the formation of advanced glycation end products crosslinks [1,2]. They are generated by the saccharification and oxidation due to osteoporosis or diabetes. The effects of the glycation on the bone mechanical strength and elasticity are still unknown. The DXA cannot diagnose the collagen, and glycation. Then, we have proposed QUS (Quantitative Ultrasound) methods which can quantitatively evaluate the elastic properties of bone.

In this study, we investigated the effects of glycation on the velocity changes in bone using ultrasound in the MHz range.

2. Bone sample

Four cortical bone samples were obtained from a diaphysis of a swine femur (175 days old). The samples were processed into plates (20 × 5 × 0.5 mm^3) (**Fig. 1**). The long side direction of the plates are equal to the bone axis direction. Two bone samples were immersed in the incubation liquid (Phosphate buffered saline (PBS), D-(-)-Ribose, Protease Inhibitor Cocktail Set III, Penicillin-Streptomycin) in order to form the abnormal collagen cross-links (glycation) [3]. As reference samples, two other bone samples were immersed in the liquids of PBS and Penicillin-Streptomycin. The two glycated samples are named as samples A and B, the other two reference samples are named as samples C and D. These bone samples were kept in the incubator at 37 °C during the incubation period (14 days). In addition, we prepared samples, which were obtained from diaphysis of a bovine radius (28 month old). These samples were processed and incubated as with the swine samples.

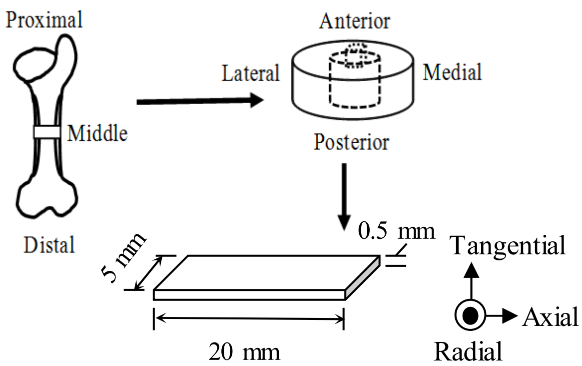


Fig. 1 Preparation of samples.

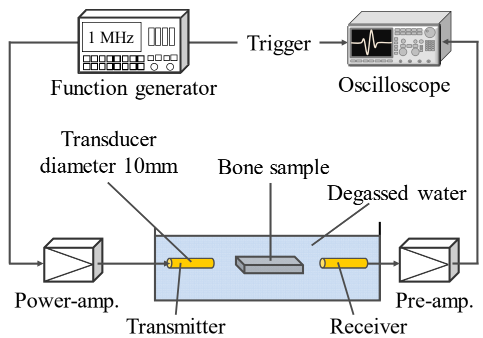


Fig. 2 Experimental system.

3. Experiments

In this experiment, measurements were performed using an ultrasonic pulse technique by self-made PVDF transducers (diameter 10 mm). They were placed coaxially with a distance of 60 mm. Signals from a function generator (Agilent, 33250A) was amplified 20 dB by a power amplifier (NF, HSA 4101), and delivered to the transmitter, then converted into the ultrasonic waves. One cycle of sinusoidal wave at 1 MHz and 70 Vpp was applied to the transmitter. Ultrasonic waves that passed through the sample were received at the receiver and converted into electrical signals. The signals ware amplified 40 dB by a preamplifier (NF,

mmatsuka@mail.doshisha.ac.jp

BX-31) and observed by a digital oscilloscope (Tektronix, 33250A). Longitudinal wave velocity in the axial direction of bone was estimated by the arrival time differences of the propagated wave with bone or without bone in water at 24 °C.

After these ultrasonic measurements, we conducted the three-point bending test by using a compact universal testing machine (Shimadzu, EZ test), in order to measure the bending elastic modulus and strength of swine bone samples.

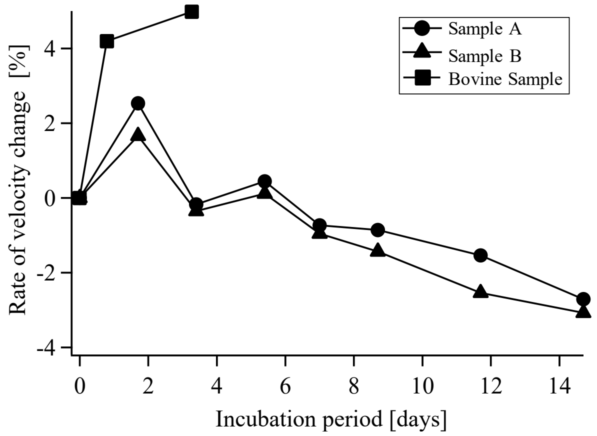
4. Results and discussion

We have already studied the effects of the glycation on the cortical bone by Brillouin scattering and pointed that the hypersonic velocity in the GHz range decreased after the initial small increase [4]. In this study, velocity change in the MHz range was studied as a function of the incubation period (**Figs. 3 (a)** and **(b)**). The glycated samples showed a small initial velocity increase from 0 to 2 days, the velocity then monotonously decreased. The final decrease of the velocity was around -2.7 %. On the other hand, the velocity of reference samples only monotonously decreased. The final velocity decrease was around -4.2 %. The velocity decreases of glycated samples were always smaller than those of reference samples. Although there are velocity difference between the swine bone and the bovine bone, the bovine sample also exhibits the initial increases. This tendency was similar to the results in the GHz range [4].

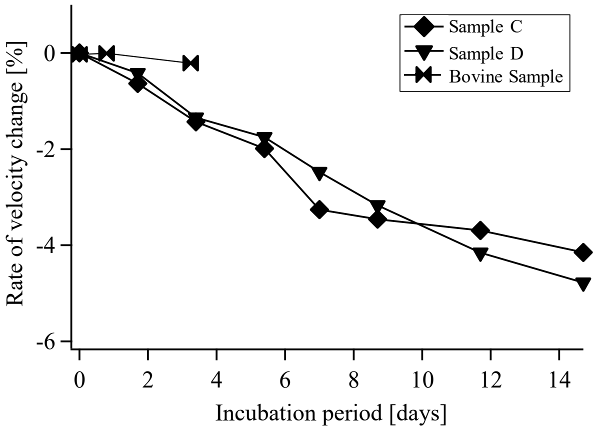
As for the results of three-point bending test, the reference sample was broken at the load of about 19 N. On the other hand, the glycated sample was broken at the load of about 14 N. The apparent bending elastic modulus of the reference sample was about 23 GPa. On the other hand, the apparent bending elastic modulus of the glycated sample was about 20 GPa. These data tell us that the bone strength was deteriorated by the glycation.

5. Conclusion

The effects of glycation on the swine and bovine cortical bones were investigated. An ultrasonic pulse technique in the MHz range was used. The velocities of glycated samples showed initial increases and then decreased. The results suggest that the elastic properties of bone increases at the initial stage of glycation. By the three point bending test of samples after 14 days incubation, we found that the bone strength was deteriorated by the glycation.



(a)



(b)

Fig. 3 Changes of velocity as a function of incubation period.
(a) Velocity changes of the glycated samples.
(b) Velocity changes of the reference samples.

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