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Decrease of longitudinal wave velocity in glycated collagen
糖化コラーゲン中の縦波音速の低下

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1. Introduction

Decrease of bone strength is one of the factors of bone fracture risk. The National Institutes of Health (NIH) has reported that the bone strength depends on not only bone mineral density (BMD) but also bone quality [1]. 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. Diabetic patients often have a high bone fracture risk despite of the normal BMD. One reason seems to result from the deterioration of collagen which occupies 50 % of the total volume of bone [2]. Yasui, et al. have reported decrease of elastic modulus of diabetic rat bone due to hyperglycemia [3]. In case of diabetes, the amount of insulin decreases and Advanced Glycation End Products (AGEs) cross-links tends to be generated. The abnormal collagen crosslinks in bone are expected to affect bone elasticity. However, the collagen elasticity cannot be evaluated by BMD. In contrast, ultrasonic techniques, which are non-invasive, inexpensive, and safe, can provide information of not only BMD but also elasticity.

In this study, using a micro-Brillouin scattering technique, we studied changes of wave velocity in a collagen due to glycation with high spatial resolution.

2. Material and methods

2.1. Specimen

Figure 1 shows a single layer uniaxial collagen film (10 mm×10 mm, Collagen Type-I, Atree). The film thickness was approximately 30 μm. The films were in wet or dry conditions to investigate water content effects on elasticity. We measured the velocities of longitudinal waves which propagate in parallel and vertical directions to the collagen fiber orientation. After incubation for glycation, velocity measurements were carried out. **Figure 2** shows measurement points under the wet condition. The averaged velocity value measured at 9 different positions was obtained from each film.

2.2. Incubation for glycation

For glycation, the film was incubated in a mixture of Phosphate buffered saline (PBS), D-(-)Ribose, Protease Inhibitor Cocktail Set III and Penicillin-Streptomycin. A reference film was put in the mixture of PBS and penicillin-streptomycin. All films were kept in an incubator at 37°C for 10 days.

2.3. Brillouin scattering technique

Brillouin scattering measurements were carried out with a six-pass tandem Fabry-Pérot interferometer using a solid state laser with wave length of 532 nm. The system included an optical microscope. The actual spot diameter of the focused laser beam on the specimen was approximately 10 μm.

For Brillouin scattering measurements, the Reflection Induced θ Angle scattering geometry was used [4]. This geometry enables to observe ultrasound waves propagating in 2 directions ($q^{\theta A}$ and q^{180}). Here, we focused on the in-plane $q^{\theta A}$ direction. From following equation, we can obtain wave velocity.

$$v^{\theta A} = f^{\theta A} \frac{\lambda_0}{2\sin(\frac{\theta}{2})}$$

Here, $v^{\theta A}$ is the wave velocity, $f^{\theta A}$ is the measured shift frequency and λ_0 is the incident light wave length.

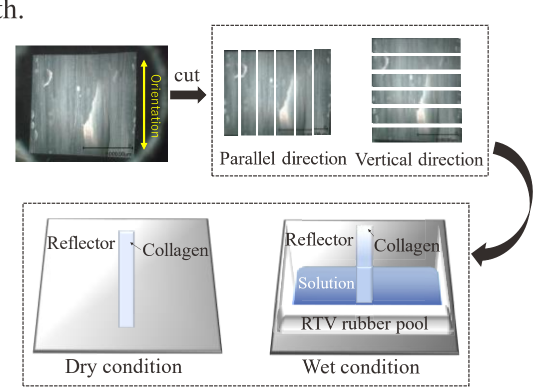

Fig. 1 Specimen preparation.

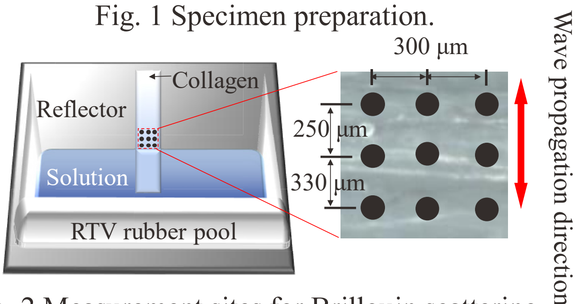

Fig. 2 Measurement sites for Brillouin scattering.

3. Results and discussion

Figure 3 shows a typical Brillouin spectrum obtained from a wet glycated film in the parallel direction. From this shift frequency, the longitudinal wave velocity was estimated as 1.93×10^3 m/s.

Figure 4 shows changes of the wave velocity due to distance from solution surface. Wave velocities did not change until around 300 μm from the surface. Therefore, the wave velocity of wet condition was measured at points 100 μm above the water surface. In the wet area, the velocity was about 1140 m/s lower than those in fully dried area. The results suggest that water content in the collagen decreases the elasticity.

Figure 5 shows changes of longitudinal wave velocity in the wet glycated film due to the incubation time. In the dry condition, changes of the velocity showed a decrease. Imoto, et al. have also reported similar decrease of longitudinal wave velocity in the artificially glycated dry bone [5].

Figure 6 shows the wave velocities after the incubation of 10 days. In the dry condition, the velocities in the glycated films were 6.0 % (parallel direction) and 5.2 % (vertical direction) lower than those in the reference film without glycation (*$p<0.01$). The decrease of velocity in the glycated films were 5.7 % (parallel direction) and 9.1 % (vertical direction) in the wet condition (*$p<0.01$). The decrease of velocity in the vertical direction in the wet film was the highest in all conditions. The different decrease may come from abnormal collagen crosslink direction. These results may suggest that the glycation of collagen in bone also reduces the elasticity of bone and the effects AGEs are anisotropic.

4. Summary

Longitudinal wave velocities in the dry and wet collagen glycated films were investigated by a micro-Brillouin scattering technique. The velocities in wet condition were lower than those of dry conditions. The wave velocities in both dry and wet films decreased due to glycation. The decrease shows the decrease in elasticity.

References

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2. M. Saito, et al., Curr Osteoporos Rep, 2, 554 (2018).
3. H. Yasui, et al., Calcified tissue Int, in press (2020).
4. J. K. Krüger, et al., J. Phys. D., 31, pp. 1913-19 (1998).
5. Y. Imoto, et al., Glycative Stress Research, 2, pp.101-107 (2015).

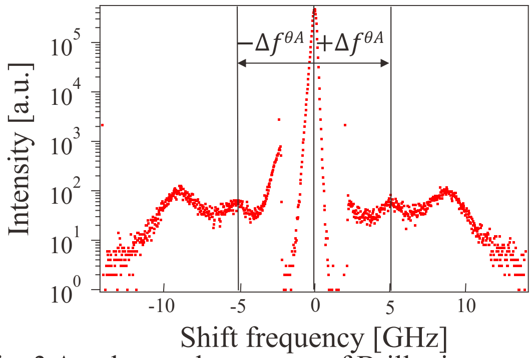

Fig. 3 An observed spectrum of Brillouin scattering from a wet glycated collagen film in the parallel direction to the collagen fiber orientation.

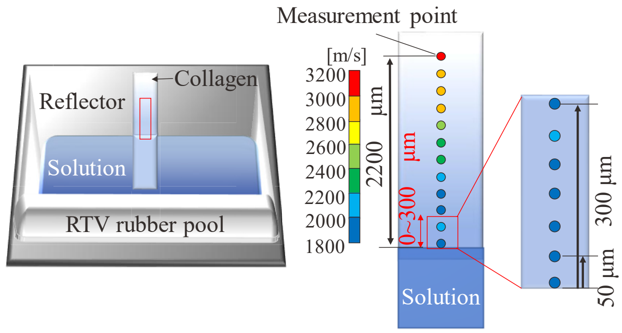

Fig. 4 Wave velocity changes due to the distance from solution surface.

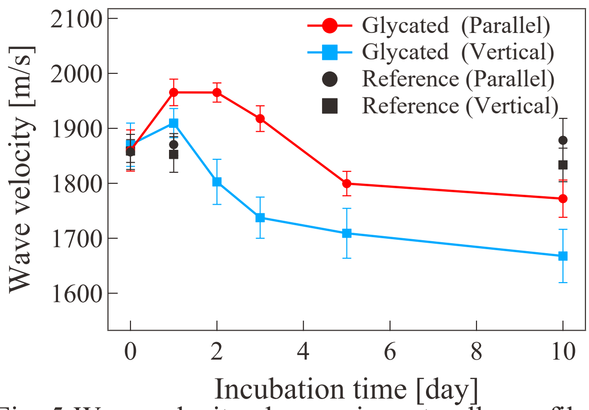

Fig. 5 Wave velocity changes in wet collagen films due to incubation.

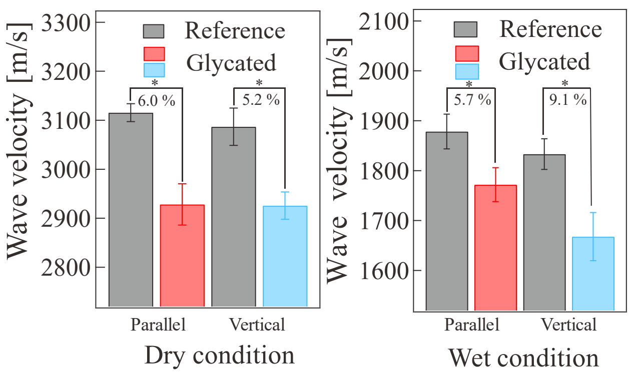

Fig. 5 Averaged wave velocities in parallel and vertical directions in the collagen films after incubation of 10 days.