# Photoacoustic waves from rat tibia

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# 1. Introduction

The number of diabetic patients is increasing due to lifestyle changes and aging society <sup>[1]</sup>. There are cases where diabetes decreases bone strength and increases the risk of fracture. According to National Institute of Health, bone strength depends on bone mineral density (BMD) and bone quality <sup>[2]</sup>. Bone quality includes factors such as bone remodeling, collagen, microstructure, microcracks, crystalline orientation etc. Diabetes possibly increases fracture risk due to bone quality deterioration.

To investigate the effects of diseases on bone, we should know the changes of bone matrix as well as microstructure. One interesting idea is the application of the Brillouin scattering technique <sup>[3]</sup> which can measure wave velocity in a small area, making use of the focused laser light. Another idea is the photoacoustic (PA) technique.

Therefore, this study focuses on the PA technique that can evaluate the properties of bone matrix. PA uses a short laser pulse whose electromagnetic energy is absorbed and converted into heat in the material, exciting ultrasonic waves due to thermoelastic expansion. In the case of appropriate light wavelength, soft tissue transmits laser light so that the PA technique can be used for in vivo evaluation of bone in the body.

### 2. Samples and experimental method

Sprague-Dawley (SD) rats were used as measurement samples. The cortical bone specimens were extracted from tibias and polished to the thickness of approximately 150  $\mu$ m. (4.0×4.0 mm<sup>2</sup>, thickness direction: radial). For comparison, a collagen sheet (10×10 mm<sup>2</sup>, thickness: 150 mm, A-tree) and artificially synthesized and polished hydroxyapatite (HAp) film (2.0×2.0 mm, thickness: 150  $\mu$ m, HOYA Technological) were also prepared. The measurement specimens are shown in **Fig.1**.

The photoacoustic measurement system used is shown in **Fig. 2**. The pulsed laser beam (Torus XS, Cobolt, repetition frequency 1 kHz, wavelength 1064 nm, pulse width  $2.5\pm1.0$  ns) was focused (spot diameter 40 µm). The beam passed through the

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Fig. 1 Optical images of specimens. (a) tibia bone (b) HAp sheet and (c) collagen



Fig. 2 Experiment system.

center of a hollow concave ultrasonic transducer (Toray techno, custom made, focal length 15 mm) in degassed water. The photoacoustic wavegenerated by the laser beam was received by the concave transducer, amplified by 46 dB using an amplifier (SA-420F5, NF), and measured by an oscilloscope (DPO7254C, Textronix). Distribution of photoacoustic waves in the specimens were measured by moving the laser irradiation position with interval of 100  $\mu$ m.

# 3. Results and Discussion

Typical PA waveforms obtained from the tibia bone, collagen and hydroxyapatite films are shown in **Fig. 3**. **Figure 4** presents the distribution of the averaged peak-to-peak (p-p) amplitudes of the PA waves from the tibia bone at 50 different positions. The results show two peaks. For comparison, **Fig.5** shows the distribution of averaged p-p amplitudes of the PA waves from collagen and HAp films at 20

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different positions, respectively. The p-p amplitudes of photoacoustic waves from HAp in **Fig.6** were 52% lower than those of collagen. The data tell the dependence of PA amplitudes on the specimens. Because bone includes both HAp and collagen, the results in **Fig.5** are reasonable. The PA wave amplitude is known to be proportional to light absorbance <sup>[4]</sup>. We check transmitted light of collagen and HAp thin films. Transmitted light of the collagen film was 16 % lower than that of the HAp film. The result is possibility indicating that collagen absorbs light more than HAp in the near infrared region.

**Figure 7** shows the distribution of the peak-topeak amplitudes of the waveforms and the optical microscope image of the bone surface. Several point of high amplitudes (around 70 mV) represent the effect of bone surface microstructure because previous study represents that the amplitude of the PA wave increased especially near of the small pores made by the vessel<sup>[5]</sup> Other increase and decrease in amplitude is likely due to the heterogeneity the bone matrix containing collagen and HAp.

### 4. Conclusion

PA waves from the tibia bone surface were investigated. The results showed that amplitude of PA signals can be used to judge the effects of the bone components, HAp and collagen.

In the future, we would like to conduct detailed measurements of PA waves considering calcium content and microstructures.

#### References

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Fig. 3 Observed photoacoustic waveforms. (a) tibia bone and, (b) HAp sheet and (c) collagen film.



Fig. 4 Amplitudes of waves from tibia bone.



Fig. 5 Amplitudes of waves from collagen and HAp specimens.



Fig. 6 The average p-p amplitudes of the PA waves from the collagen and HAp specimens.



Fig. 7 Distribution of amplitudes.