# Three dimensional anisotropy of ultrasonic wave velocity in bovine cortical bone

- Effects of HAp crystallites orientation and microstructure -

ウシ大腿骨皮質骨中の3次元音速異方性

-HAp 結晶と微細構造が及ぼす影響-

Tomohiro Nakatsuji<sup>1‡</sup>, Kazufumi Yamamoto<sup>2</sup>, Keisuke Yamashita<sup>1</sup>,

Takahiko Yanagitani<sup>3</sup>, Mami Matsukawa<sup>1</sup>, Kaoru Yamazaki<sup>4</sup> and Yukihiro Matsuyama<sup>2</sup> (<sup>1</sup>Doshisha Univ.; <sup>2</sup>Hamamatsu Univ. Sch. Med.; <sup>3</sup>Nagoya Inst. Tech.; <sup>4</sup>Iwata City Hospital)

中迁 知宏<sup>1‡</sup>, 山本 和史<sup>2</sup>, 山下 圭祐<sup>1</sup>, 柳谷 隆彦<sup>3</sup>, 松川 真美<sup>1</sup>, 山崎 薫<sup>4</sup>, 松山 幸弘<sup>2</sup> (<sup>1</sup>同志社大;<sup>2</sup>浜松医大;<sup>3</sup>名工大;<sup>4</sup>磐田市民病院)

# 1. Introduction

Quantitative ultrasound (QUS) is a good method to measure elastic properties of bone in vivo, which is expected to be an innovative technique to evaluate bone quality. However, this method has comparatively low measurement accuracy, which results from the complicated wave propagation properties in bone. Actually, bones are usually heterogeneous and anisotropic<sup>[1,2]</sup>. At the microscopic level, we can see haversian and bricklike (Plexiform) structure in the cortical bone. At the nanoscopic level, the cortical bone mainly consists of hexagonal hydroxyapatite (HAp) crystallites and type I collagen. These tissue structures have the characteristic velocity anisotropies and affect the elastic properties of bone<sup>[3]</sup>. Therefore, this anisotropy is considered as a clue to clarify the mechanical characteristics. Moreover, velocity values and degree of anisotropy varied at different parts. In this study, three demensional anisotropies of ultrasound velocity are investigated in detail, using spherical specimens obtained from the bovine corical bone. Then, we investigate the effects of HAp crystallites orientation and microstructure on the velocity.

## 2. Materials and Methods

A left femur was obtained from a 30month-old bovine. A ring-shaped cortical bone sample was obtained from the mid-shaft. Two spherical specimens (diameter 9 mm) were taken from anterior and posterior parts in the ring specimen as shown in **Fig. 1**.

Measurements of longitudinal wave velocities were performed using a conventional ultrasound pulse technique as shown in **Fig. 2**. A PVDF focus transmitter (diameter 20 mm, focal length 40 mm) and a flat PVDF receiver (diameter 10 mm) were used in this experiment. Both PVDF transducers were mounted coaxially with distance of 60 mm in degassed water at  $25.0\pm0.1$  °C. A single sinusoidal signal with a center frequency of

mmatsuka@mail.doshisha.ac.jp

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 40-dB preamplifier and visualized in an oscilloscope. The measured specimen was placed in the focal zone of the sound field. The direction of the incident ultrasonic wave was put to the center of the spherical specimen. By changing the incident angle, we obtained the distribution of the longitudinal wave velocity. The measurements were performed at each rotation angle  $\theta$  of 5 degrees in the Axial-Radial (A-R), Axial-Tangential (A-T) and Radial-Tangential (R-T) planes.

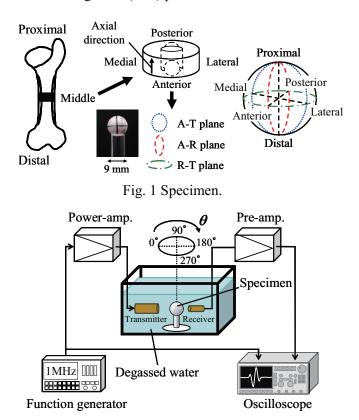


Fig. 2 Ultrasound measurement system.

The crystallites orientation of the specimen was determined by XRD pole figure analysis (Philips, X-Pert Pro MRD). X-ray source (Cu-K $\alpha$ , generated at a tube conditions of 45 kV and 40 mA) irradiated the specimen surface through the parallel beam optical system with 0.3 mm x 3.0 mm slit. The X-ray irradiated area was the same with the focal point in ultrasound measurement.

#### 3. Results and Discussion

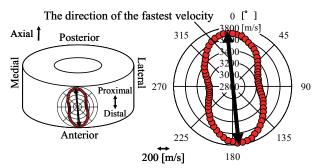
Figure 3 shows the velocity data of a specimen obtained from the anterior part. The velocity clearly changed due to the rotation angle. In all planes, we found the velocity anisotropies. In the A-T and A-R planes, velocity was higher in the axial direction. However, the direction of the fastest velocity was inclined from the axial direction. Figure 4 shows the pole figure measured at the anterior part. From this pole figure, the highly concentrated pole was clearly observed. This pole concentration indicates that the *c*-axes of HAp crystals are oriented in that direction. We found a small tilt of *c*-axes direction from the bone axis, which was in agreement with the velocity data. Figure 5 shows the relationships between the velocity and the intensity of HAp crystallites *c*-axes in each part. A significant correlation was observed and the HAp crystallites orientation seems to affect the anisotropy of ultrasound velocity. In the posterior part, however, there is comparatively less correlation. The main reason seems to be the effect of bone microstructure. Actually, most posterior parts show haversian structure, whereas the other parts show mainly Plexiform structures. In the R-T plane, on the other hand, there was no correlation between the velocity and HAp crystallites orientation. One possible reason for the velocity anisotropy in R-T plane seems to result from bone microstructure at the microscopic level.

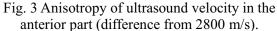
## 4. Conclusion

We have investigated the velocity anisotropy and HAp crystallites orientation in bovine cortical bone. The direction of the fastest velocity showed a small tilt from the bone axis, which was in good agreement with a small tilt of HAp crystallites *c*-axes direction. A significant correlation between the velocity and HAp crystallites orientation was observed in the A-T and A-R planes. In the R-T plane, there was no correlation between the velocity and HAp crystallites orientation. The velocity seems to be dependent on the microstructure in this plane.

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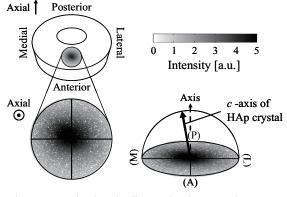
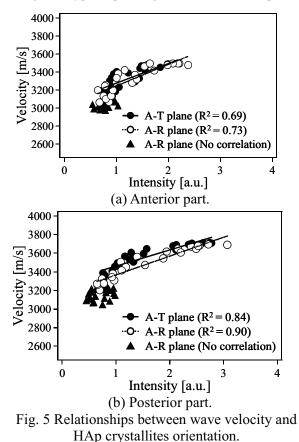


Fig. 4 A typical pole figure in the anterior part.



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