Dependence of ultrasonic wave velocity on the HAp crystallites orientation and bone mineral density

皮質骨中の超音波音速と HAp 結晶配向及びミネラル量の関係

Daisuke Suga<sup>1†</sup>, Kazuma Ikeda<sup>1</sup>, Shinji Takayanagi<sup>1</sup>,

Mami Matsukawa<sup>1</sup>, Takahiko Otani<sup>1</sup>, Kazufumi Yamamoto<sup>2</sup>and Takahiko Yanagitani<sup>3</sup> (<sup>1</sup>DoshishaUniv.; <sup>2</sup>JA Shizuoka Kohseiren Enshu Hospital; <sup>3</sup>Nagoya Inst. Tech.) 菅大輔<sup>1‡</sup>, 池田一真<sup>1</sup>, 高柳真司<sup>1</sup>, 松川真美<sup>1</sup>, 大谷隆彦<sup>1</sup>, 山本和史<sup>2</sup>, 柳谷隆彦<sup>3</sup> (<sup>1</sup>同志社大; <sup>2</sup>JA 静岡厚生連遠州病院; <sup>3</sup>名工大)

# **1.Introduction**

Bone strength is considered to depend on not only bone mineral density (BMD), but also bone quality. The concept of bone quality includes calcification, metabolic turnover, microstructure and elasticity etc.

The mainstream of recent evaluation of bone is the dual-energy X-ray absorptiometry (DXA). DXA enables to measure BMD, which is still the gold standard for bone diagnosis. On the other hand, the Quantitative Ultrasound technique(QUS) can measure the elastic properties of bone in vivo, because the obtained ultrasonic wave properties directly reflect the longitudinal elasticity. The present problem of QUS is comparatively low measurement accuracy, due to the complicated wave propagation in bone. Actually, bones are usually heterogeneous and anisotropic<sup>[1,2]</sup>. At the</sup> microscopic level, we can see two types of microstructure, plexiform and haversian, in the cortical bone. At the nanoscopic level, the cortical bone mainly consists of hexagonal hydroxyapatite (HAp) crystallites and type I collagen. These multi-scale structures of bone affect the elastic properties<sup>[3]</sup>.

We have then investigated the relation between the velocity and the HAp crystallites orientation in bovine femur cortical bone.As a result,velocities in axial direction were affected by nanoscopic HAp crystallites orientation, in addition to the structure and BMD. In this study, dependence of ultrasonic wave velocity on the HAp crystallites orientation and BMD wasnext investigated using the bovine metacarpal corical bone. Then, we report interesting results which are different from those of bovine femur cortical bone.

## 2. Materials and Methods

A left metacarpal was obtained from a 30month-old female bovine. A ring-shaped bone sample with 8mm thick were obtained from the mid-shaft as shown in **Fig. 1**. From observation by an optical microscope, the bone sample showed two types of microstructure, plexiform and haversian.

mmatsuka@mail.doshisha.ac.jp

Measurements of longitudinal wave velocity were performed using a conventiona lultrasonic pulse system as shown in **Fig.2**. A couple of PVDF transducers with diameter of 3 mm were used. A single sinusoidal signal with a center frequency of 5 MHz and amplitude of 16 Vp-p was applied to one transducer. The transmitted longitudinal wave propagated through water, material and water.Other transducer then received the wave, and converted it into the electrical signal. The signal was amplified by a preamplifier and visualized in an oscilloscope. The measurement point on ring-shaped sample was accurately decided using a precision stage. This measurement was performed in degassed water at  $25.0\pm0.1$  °C.

The HAp crystallites orientation in the bone sample was determined by using an X-ray diffractometer (Philips, X-Pert Pro MRD). X-ray source (Cu-K $\alpha$ , generated at a tube conditions of 45 kV and 40 mA) irradiated the material surface through the parallel beam optical system with 0.3 mm x 3.0 mm slit. The X-ray irradiated area was equal to the measurement points in ultrasonic measurement.

Forelimbs Metacarpal bone Ring-shaped bovine bone







Fig. 2Ultrasonic measurement system.



Fig. 3Distribution of velocity, peak intensity and BMD in the mid-shaft of bovine metacarpal.

Measurement of BMD was performed by using DXA system (Hologic Co, QDR-1000).

## **3.Results and Discussion**

**Figure 3** shows the distribution maps of longitudinal wave velocity, integrated (0002) peak intensity and BMD of the sample. The integrated (0002) peak intensity represents the HAp crystallites which align in the bone axis direction. The velocities in the medialand lateral parts seem to be higher than those in the anterior part, despite that the micro structures were all plexiform in these parts. Both plexiform and haversian structures were observed in the posterior part, where velocities were slightly low. The distribution of HAp crystallites orientation also shows a similar tendency with velocity. In contrast, the values of BMD are lower in the medial and lateral part.

Figure 4 shows relationship between velocity and peak intensity. The velocity was strongly correlated with the intensity ( $R^2=0.70$ ). On the other hand, Figure 5shows relationship between velocity and BMD. The velocity was not correlated with BMD ( $R^2=0.21$ ).

**Figure 6** shows relationship between velocity and HAp intensity devided by BMD (HAp/BMD). BMD is proportional to the total amount of HAp crystallites, then, HAp/BMD shows the ratio of HAp crystallites oriented in axial direction. The correlation with the velocity becomes lower than that of HAp intensity. These results mean that the longitudinal wave velocity was influenced strongly by the amount of HAp crystallites oriented in axial direction rather than the total amount of bone mineral.

### 4. Conclusion

In the bovine metacarpal cortical bone, the longitudinal wave velocity was affected strongly by the HAp crystallites orientation rather than BMD. This result shows that the elastic property does not depend on the amount of BMD, but on HAp crystallites orientation. It also tells that the present BMD standard of bone diagnosis is not appropriate for the evaluation of bone quality.





Fig. 5 Relationships between velocity and BMD.



#### References

- 1. S. F. Lipson, J. L. Katz: J Biomech 17 (1984) 241-249.
- 2. R. B. Martin, D. B. Burr:Springler-Verlag(1980).
- Y. Yamato, M. Matsukawa, *et al.*: Calcif. Tissue Int. 82(2008)162-169.