Evaluation on mechanical properties of a single trabecular in bovine femur

S. Enoki¹, M. Higashiura², M. Sato², K. Tanaka² & T. Katayama² ¹Department of Mechanical Engineering, Nara National College of Technology, Japan ²Department of Biomedical Engineering, Doshisha University, Japan

Abstract

The increase of patients with osteoporosis is a social problem. Osteoporosis decreases bone strength and increases the risk of fracture. The finding of prevention and treatment methods is an urgent issue. Since cancellous bone is metabolically more active than cortical bone, cancellous bone is used for diagnosis of osteoporosis. There are a lot of studies about stress analysis of cancellous bone, and quantities and orientation of trabecular bone that make up the cancellous bone. These studies reported that the trabecular structures contribute to the mechanical properties of cancellous bone. It is considered that the mechanical property of trabecular is based on the assumption that it is constant regardless of the direction and site orientation. However, literature evaluating the mechanical properties of a single trabecular yields a wide dispersion of the results; the mechanical properties of trabecular bone has not been clarified yet. It is necessary to reduce the dispersion of test results to allow for quantitative evaluation of the mechanical properties of trabecular bone and is essential to evaluate the strength of cancellous bone. In this study, single trabecular specimens were polished to bending test specimens in rectangular shape. X-ray μ CT was used to obtain shape of trabecular bone specimens. Moreover, three point bending tests were conducted on these specimens and the bending elastic modulus and strength were obtained. Bending elastic modulus and bending strength of the trabecular bone were almost the same value regardless of the direction of each axis. Mechanical properties of trabecular by the bending test do not depend on the orientation of cancellous bone.

Keywords: trabecular, cancellous bone, bending modulus, bending strength, X-ray μ CT.



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1 Introduction

Recently, the rising incidence of patients with osteoporosis is becoming a social problem. Osteoporosis decreases bone strength and increases the risk of fracture. Such diseases have a negative impact on quality of life, making it an urgent issue to find its prevention and treatment methods. Patients with osteoporosis are mainly measured by bone mineral density (BMD), such as dualenergy X-ray absorptiometry (DXA) [1], quantitative X-ray computed tomography (OCT) [2] and others. According to the report of National Institute of Health (NIH) in 2000, in order to predict bone strength, bone density alone is not sufficient and it is important to explore other parameters such as "bone quality" serving as an indicator for bone strength measurement [3]. In order to clarify bone quality, it is important to make research on mechanical properties of bone. Bone is a hierarchically structured material and is distinguished into the cortical and cancellous bone. Since cancellous bone is metabolically more active than cortical bone, cancellous bone is used for diagnosis of osteoporosis [4]. There are a lot of studies about stress analysis of cancellous bone, and quantities and orientation of trabecular bone that make up the cancellous bone [5]. These studies reported that the trabecular structures contribute to the mechanical properties of cancellous bone. It is considered that the mechanical property of trabecular is based on the assumption that it is constant regardless of the direction and site orientation. However, literature evaluating the mechanical properties of a single trabecular yields a wide dispersion of the results [6-8]; the mechanical properties of trabecular bone has not been obtained. The cause to these dispersion are considered to be due to the trabecular specimens being obtained and tested without polishing to the proper shape. It is considered that the dispersion in the specimen size cause dispersion in the results of the elastic modulus. The specimen geometry of trabecular had been evaluated in the order from several millimeters to several tens of micrometers by using 2D image of optical microscopy or micrometer. However, since trabecular bone of several hundred micrometers in diameter is a small and irregular in structure, it is considered the shape of the trabecular bone specimen evaluation should be carried out in 3D observation. It is necessary to reduce the dispersion of test results to allow for quantitative evaluation of the mechanical properties of trabecular bone and is essential to evaluate the strength of cancellous bone. In this study, single trabecular specimen were polished to bending test specimens in rectangular shape by using specially designed Jig and X-ray µCT was used to obtain shape of trabecular bone specimens. Three point bending tests were conducted on these specimens and the influence of direction of the trabecular bone specimen on the bending elastic modulus and strength were discussed.









2 Materials and methods

2.1 Specimen preparation

Trabecular bones were obtained from cancellous bones of a bovine femur, at the Z-axis direction in parallel to the major axis of the femur, the X-axis and Y-axis direction in perpendicular to the major axis of the femur as indicated in fig.1. Single trabecular specimen on each axis was extracted from the cancellous bone in length of 2mm to 3mm and in diameter of 200µm to 500µm. Trabecular bone has been processed into a rectangular. Trabecular bone was polished to the thickness of the trabecular bone to 100µm as shown in fig.2 by using Speed Lap (Maruto ML-521-d). Speed Lap is a double-sided polishing machine flake type automatic lap polishing to create a slice. Side polishing was performed by using the sandpaper (600grit) to form the width of 250um of trabecular bone. Cantilever jig (Jig A, shown in fig.3) [9] and Both ends supported jig (Jig B, shown in Fig.4) were used to support specimens duting polishing. Three dimension images of each trabecular bone specimen (3D images) was obtained by using micro focus X-ray system (Shimadzu, SMX-160CTS). Width and thickness of specimens were obtained from the average value of three points of 3D image.





Figure 2: X ray µCT-imaged of trabecular bone specimen after speed lapping.



Figure 3: Cantilever jig (Jig A).



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2.2 Bending test of trabecular

Elastic bending tests were performed by using Micro Material Tester (Shimadzu, MMT-11NV-2) with a load cell capacity of 10N. Each specimen was placed on the jig as shown in fig.5 and span length was set to 1.1mm. Knife-edge indenter of 0.1mm in tip radius was used as an indenter within the three point bending test. The positioning of specimen was conducted by XY stage in resolution of 0.01mm. Elastic bending test was carried out at a crosshead speed of 0.001mm/sec, and maximum displacement was 0.02mm. The following equation [10] was used to calculate the elastic modulus for each specimen.

$$E = \frac{(P/\delta)L^3}{4bh^3} [1 + 2.85(h/L)^2 - 0.84(h/L)^3]$$

- E: Elastic modulus
- P/δ : Load displacement curve
- *L*: Distance between the fulcrum
- b: Width of the specimen
- h: Thickness of the specimen

Bending strength test was performed and carried out at a crosshead speed of 0.001mm/sec, and maximum displacement was 1mm. The following equation was used to calculate the bending stress for each specimen.

$$\sigma_b = \frac{M}{I} \times \frac{h}{2} = \frac{PL/4}{bh^3/12} \times \frac{h}{2}$$

 $b_{e:}$ Bending strength

- M: Maximum bending moment
- *I*: Second moment of area

P: Load

- L: Distance between the fulcrum
- b: Width of the specimen
- *h*: Thickness of the specimen.





Figure 5: Three point bending tester.

3 Results and discussion

3.1 Influence of supporting jig during polishing process on specimen dimensions and bending tests

Figs. 6 and 7 show the cross-sectional, and the overall view of the specimens which were prepared by using Jig A and Jig B described in Section 2.1. Twenty trabecular bone specimens obtained from the Y-axis direction were used. The side of the specimens shown in fig.7 has been polished more uniformly than fig.6. Fig.8 shows a comparison of the width dimension of the specimens polished by Jig A and Jig B. According to fig.8 width dimension by using Jig A yields $256 \pm 22.7 \mu$ m with relative standard deviation at 8.87%; meanwhile width dimension by Jig B yields $250 \pm 7.73 \mu$ m and relative standard deviation at 3.09%. Jig B can polish the side of the specimens better than Jig A. Fig.9 shows the bending results to specimens polished by using Jig A and Jig B. The elastic modulus of the specimens by Jig A yields 7.58 ± 1.96 GPa, with relative standard deviation at 25.9%; meanwhile the elastic modulus by Jig B yields 7.77 ± 0.765 GPa with relative standard deviation at 9.85%. Relative standard deviation



Figure 6: Trabecula specimen polished by using Jig A.

polished by using Jig B.

for the specimens by Jig B has decreased 16.1% compared to Jig A. The accuracy of the width dimensions improved that of elastic modulus. By using the both ends supported jig (Jig B), the bending elastic modulus can be obtained with small standard deviation.



3.2 Bending test

All specimens were prepared by using the Jig B. Fig.10 shows the comparison of the elastic modulus of ten trabecular bone specimens obtained from each of the X. Y and Z axis. Elastic modulus of X. Y and Z axis was 7.42 ± 0.657 GPa. 7.77±0.765GPa and 7.45±0.861GPa respectively. There was no significant difference in the elastic modulus. It is estimated that the bending elastic modulus of trabecular bone does not depend on the orientation of cancellous bone. Fig. 11 shows the comparison of the elastic strength of ten trabecular bone specimens obtained from each of the X, Y and Z axis. Bending strength of X, Y and Z axis was 247±17.1MPa, 251±14.8MPa and 247±16.2MPa respectively. There was no significant difference in bending strength. It is estimated that the bending strength of trabecular bone does not depend on the orientation of cancellous bone. On the other hand, it was reported that for compression test in the X, Y and Z axis direction of the cancellous bone, there was a significant difference to elastic modulus and to the orientation of the trabecular bone that make up the cancellous bone [9]. According to the reported results and our study, structural parameters of trabecular bone such as thickness, weight, orientation and connectivity rather than characteristics of the trabecular is related to the anisotropy of the cancellous bone structure.





Figure 10: Elastic modulus of trabecula specimen on each X, Y, and Z axis obtained from elastic bending test.





4 Conclusions

Single trabecular samples were polished to bending test specimens in rectangular shape by using specially designed Jig and X-ray μ CT was used to obtain shape of trabecular bone specimens. Three point bending tests were conducted on these specimens and the influence of direction of the trabecular bone specimen on the bending elastic modulus and strength were discussed. The investigation yields the following conclusions.

1. By using the both ends supported jig, the bending elastic modulus and strength were obtained with small standard deviation.

2. There were no significant difference in the elastic modulus and strength of the trabecular bones which were extracted in parallel and in perpendicular to the major axis of a femur.



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