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Short communication

Micro-cantilever bending for elastic modulus measurements of a single trabecula in cancellous bone

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ABSTRACT

Mechanical tests performed on small bone specimens such a single trabecula remain challenging because their isolation, fixation, and precise loading are complicated. Hence, we describe a novel experimental method to measure the elastic properties of a single trabecula using micro-cantilever bending (MCB) testing. The method does not require specimens to be completely separated from the cancellous bone, and the specimen can be easily fixed during the test. In total, 10 trabecular specimens taken from the proximal epiphysis of an adult bovine femur were used in the present study. Measurements were conducted using a small testing device comprising a 1-axial stage, load cell, optical microscope, and small plate with a taper bore for applying load at the edge of the specimen. Each specimen was positioned at the edge of the bore and was deformed by displacing the stage. The deflection of the specimen was observed by optical microscopy. The elastic modulus of the specimen was calculated on the basis of the force–deflection relationship, assuming that the shape of the specimen was a vertical circular cylinder. As a result, an average elastic modulus of 9.1 ± 5.4 GPa was obtained for a single trabecula, including the values in literature. Thus, the MCB test is a novel simple method for biomechanical analysis of a single trabecula.

1. Introduction

The cancellous bone is organized into a three-dimensional network of single trabeculae, and the apparent elastic modulus depends on this network. The mechanical properties and nanostructure of each trabecula are important factors in determining the mechanical properties of the cancellous bone. Accordingly, small bone specimens such as a single trabecula must be investigated to understand the impact of aging, osteoporosis, and/or medicines on the risk of cancellous bone fractures. However, few studies have performed mechanical tests on such specimens (Carretta et al., 2013a; Lucchinetti et al., 2000) because such studies remain technically challenging.

Tensile tests were performed on a single trabecula in previous studies (Yamada et al., 2014; Carretta et al., 2013b, 2013c; McNamara et al., 2006; Hernandez et al., 2005; Bini et al., 2002; Rho et al., 1993). Although tensile tests present some advantages, it is difficult to completely isolate, fix, and examine small bone specimens. We recently examined trabecular specimens (at least 3 mm in length) by tensile testing; however, the specimens were larger than the standard size and existed in the edges of the cancellous bone (Yamada et al., 2014). Three-point bending tests (Carretta et al., 2013b, 2013c; Hambli and Thurner, 2013; Szabó et al., 2011; Jungmann et al., 2011; Busse et al., 2009) were also performed on a single trabecula. However, such tests generally require complete isolation of the specimen, deflection measurements at high resolution, and very precise loading, which are usually difficult to obtain. Therefore, a simpler and more reliable experimental method is required for biomechanical analysis of a single trabecula.

Here we demonstrate a novel experimental method to investigate the elastic properties of a single trabecula on the basis of cantilever bending, as shown in Fig. 1. The micro-cantilever bending (MCB) test does not require the specimen to be isolated completely from the cancellous bone, and the specimen can be fixed easily during the test.

2. Materials and methods

2.1. Specimen preparation

A total of 10 trabeculae were dissected from the proximal epiphysis of an adult bovine femur (two years old) as shown in Fig. 2. First, the proximal epiphysis was sliced vertically to the longitudinal axis of the femur (bone axis; Fig. 2a), and the bone

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marrow was removed by brief water jetting (Fig. 2b). Second, plate-like cancellous bone samples were cut out from the slices using a low-speed diamond wheel saw (model 650, South Bay Technology Inc., USA). Third, a specific single trabecula of about 1 mm in length aligned in the plane of each plate-like sample was randomly selected and isolated from the cancellous bone, while keeping one extremity connected to the other trabeculae (Fig. 2c). Fourth, the remaining attached cancellous bone portion was shaped into a small rectangle such that its height corresponded to the depth of a specimen holder (Fig. 2d). The specimen was placed into the holder almost vertically and fixed by embedding the cancellous bone portion in epoxy resin (Fig. 2e).

2.2. Trabecular axis and morphology

The longitudinal orientation of each trabecula within the femoral epiphysis was visualized by scanning the plate-like samples (Fig. 2c) using a microfocus X-ray CT instrument (inspeXio SMX-90CT, Shimadzu Corporation, Japan) at a tube voltage of 90 kV, tube current of 110 μA, and voxel size of 0.092 mm/voxel. The trabecular orientation, α, was defined as the angle between the longitudinal direction of the trabecula and bone axis (Fig. 3).

The shape of the trabecula was determined by high-resolution scanning of the specimens fixed to the jigs (Fig. 2e) using a voxel size of 0.009 mm/voxel. The area (A), circularity, and aspect ratio of the cross-sections were analyzed using ImageJ software.

2.3. MCB

The MCB tests were conducted using a small testing device (Fig. 4), comprising an acrylic plate to apply the displacement and load on the specimen, a 1-axial stage (ALS-4011-G1M, Chuo Precision Industrial Co., Ltd., Japan), and a load cell (LVS-1KA, Kyowa Electronic Instruments Co., Ltd., Japan). The tests were conducted under optical microscopic observation at a 3-μm resolution (VH-5000, Keyence Corporation, Japan). Each specimen was positioned at the edge of a taper bore drilled into the acrylic plate, with the contact position in close proximity with the free end. The plate was displaced horizontally in a stepwise manner using the stage, and microscopic images were captured vertically (Fig. 4a). The deflection, d, was defined as the horizontal displacement of the contact position in the direction of plate displacement and the load, F, in the same direction was measured by the load cell connected to the plate. The maximum d and F values were 178 ± 16 μm and 0.68 ± 0.75 N, respectively. A drop of water-based ink was applied to the edge of the bore, and the contact position at the surface of the specimen was observed by optical microscopy after the test. Then, the distance, l, between the fixed end of the specimen and contact position was measured for the calculation of the elastic modulus. Specimens were loaded and then unloaded three times under air-dried conditions.

The shape of the specimen was assumed to be a vertical circular cylinder of orthotropic material and the shear stress was considered to be negligible during loading. Then, the elastic modulus, E, in the longitudinal direction of the single trabecula was calculated as in Eq. (1) from the plane curve of the bending axis (e.g., Lekhnitskii, 1963).

$$E = \frac{l^3}{3I}\frac{F}{d} \tag{1}$$

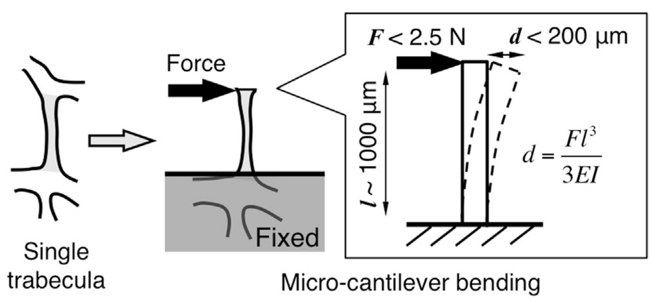

Fig. 1. Schematic of micro-cantilever bending for a single trabecula.

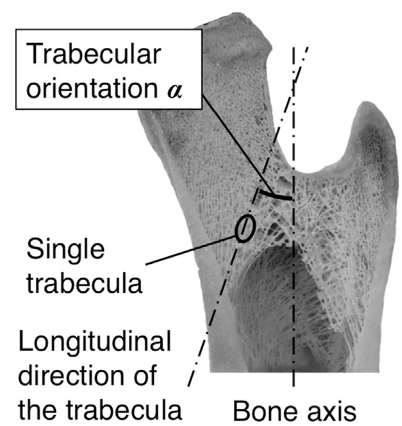

Fig. 3. Definition of the trabecular orientation α.

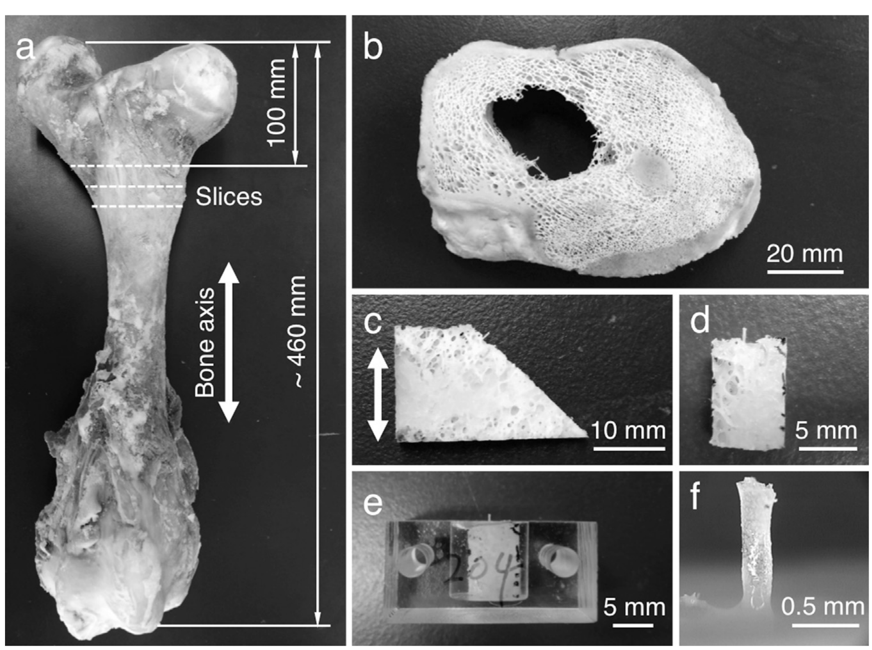

Fig. 2. Specimen preparation: the proximal epiphysis of a bovine femur (a) was sliced vertically to the longitudinal axis of the femur (bone axis) and the bone marrow was removed using a water jet (b). Plate-like cancellous bone samples were cut out from the slices, and a specific single trabecula of about 1 mm in length aligned in the plane of each plate-like sample was randomly selected. The cancellous bone surrounding the target single trabecula was removed (c). The remaining cancellous bone portion was shaped into a small rectangle (d). The specimen was placed into a holder almost vertically and fixed by embedding the cancellous bone portion into epoxy resin (e). The trabecular specimen was observed by optical microscopy (f). White arrows indicate the bone axis.

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