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Mechanical properties of rat epiphyseal cancellous bones studied by indentation testing

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Twenty-six pairs of rat femoral heads, distal femurs, proximal tibiae, and humeral heads were tested using an indentation test with a flat-ended cylindrical indentor. A useful mechanical data set including ultimate load, stiffness, and ultimate strength has been generated. Differences were found between the cancellous bones at different locations. Good correlations have been obtained between indentation depth (at 50 N load) and ultimate strength ($R = -0.937$, $p < 0.05$), which means that with an increase of ultimate strength the indentation depth or deformation decreased proportionally. Based on the experimental results and the comparison with other methods in the literature, the simplicity and usefulness of this indentation test to evaluate compressive mechanical properties of rat epiphyseal cancellous bone are apparent.

1. Introduction

The morphological characteristics of rat cancellous bones and the mechanical properties of rat diaphyseal bones have been well documented. Unlike the number of reports on the mechanical properties of human, canine, bovine bones and even rat diaphyseal bones, there have been no reports on the mechanical properties of epiphyseal cancellous bones of rats, which are widely used as models for bone growth, fracture healing, osteoporosis and osteopenia. Study of the mechanical properties of rat epiphyseal cancellous bones is needed to provide a better experimental design.

Indentation testing is a type of compression test where an indentor is driven into a sectional surface of a bone specimen. Although the failure mechanisms are more complicated and less clear than the conventional compression test, it has been used for examining the mechanical properties of cancellous bones of different species [1–6]. The purpose of this study is to generate a mechanical data set for rat epiphyseal cancellous bones using the indentation test. In this study, the application of this indentation method on rat cancellous bone is described and the simplicity and usefulness of this method are discussed.

2. Materials and methods

Twenty-six pairs of femurs, tibiae and humeri of adult female Sprague-Dawley rats (275 ± 25 g) used as healthy controls from other protocols were studied. The bones were collected within one hour of death, stripped of all soft tissues, wrapped in normal saline soaked paper towel, and frozen in airtight plastic bags at $-20\,^{\circ}$C until mechanical testing. At the time of testing, the bones were thawed in normal saline and kept moist. The rat bones were ground on a rotating grinder to an approximate depth of less than 1 mm to expose the subchondral cancellous bone at different epiphyseal locations (Fig. 1) and then potted in dental stone for mechanical testing. The surface to be tested was chosen in a plane perpendicular to an approximate line of weight-bearing, in order to get comparable values of different locations.

A mechanical test machine (MTS System 810, Minneapolis, MN) was operated in a displacement control (calibrated using an extensometer) for the indentation test. The machine displacement transducer had been previously calibrated using an extensometer. The platform holding the specimen was leveled to ensure that the loading was perpendicular to the specimen surface to be tested. A cylindrical stainless steel indentor 1.31 mm in diameter with a flat bone-contacting surface was used. After the specimen was positioned on the platform and the indentor adjusted close to the specimen surface, the indentor was driven into the bone at a constant rate of 1 mm min^{-1}. The loading was stopped when the curve obviously dropped down after the ultimate load was reached (the highest point of the curve).

The curve of load–displacement was recorded using a chart recorder. No preloading was used. A stiffness measurement was obtained by measuring the slope of the linear portion on the load–displacement curves.

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Since the test machine was controlled with a linear displacement rate (monitored by a built-in linear variable displacement transducer), the time base of the recorder could be converted to displacement. Indentation depth (or deformation) (μm) at 50 N was measured from the load–displacement curve. Ultimate strength was calculated using the formula [7]

$$\sigma = 4P/\pi d^2$$

where P is the ultimate indentation load (N) and d is the diameter of the indentor (mm). An assumption is made in order to use the above equation, that rat epiphyseal cancellous bones have an isotropic two phase porous structure.

Statistical analyses with ANOVA were used to determine if any differences existed between different locations. Pearson correlation coefficients were calculated between indentation depth and elastic modulus or ultimate strength.

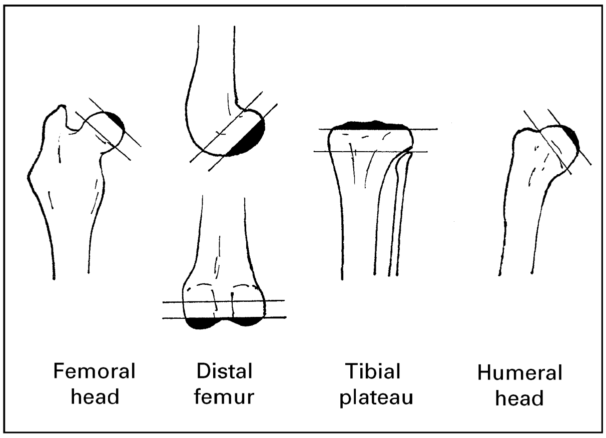

Figure 1 The surfaces to be tested were in a plane perpendicular to the line of weight-bearing. The femoral head was ground perpendicular to the centre line of the femoral neck from the centre of femoral head to a depth of 0.75 to 1.0 mm. The distal femoral condyle was ground from weight-bearing surface of the condyles to a depth of 1 mm (This surface had a 20 degree angle with the distal femoral diaphysis). The tibial plateau was ground from the upper joint surface to a depth of 1 mm. The humeral head was ground perpendicular to the centre line of the humeral neck from the centre of humeral head to a depth of 1 mm. The bones were then embedded in dental stone with the surfaces to be tested in a horizontal position.

3. Results

The average indentation depth was around 200 μm. It is not deep enough to reach growth plates since the thickness between the test surface and the growth plates ranged from at least 1.5 mm for femoral head or upper tibia to 2.0 mm for femoral condyle or humeral head (according to X-ray images). So, no adverse effect on the growth plate was seen.

The ultimate load, stiffness, ultimate strength (ultimate stress), and indentation depth (at 50 N load) of the rat bones were obtained directly or calculated from the load–deformation curve (Table I). The ultimate strengths were 71 ± 15 MPa for the femoral head, 45 ± 9 MPa for the medial distal femoral condyle, 39 ± 11 MPa for the lateral distal femoral condyle, 50 ± 10 MPa for the medial tibial plateau, 38 ± 9 MPa for the lateral tibial plateau and 44 ± 9 MPa for the humeral head. Good correlation was obtained between indentation depth (at 50 N load) and ultimate strength ($R = -0.937$, $p < 0.05$), which means that with the increase of ultimate strength the indentation depth or deformation decreased proportionally.

4. Discussion

To the best of our knowledge, this is the first report on measuring mechanical properties of rat cancellous bones using indentation testing. A useful mechanical data set has been generated. Differences were found between epiphyseal cancellous bones at different locations. The reason for this phenomenon is the functional difference between the different locations tested in the cancellous bones [8]. For example, the humeral head bears less load compared to the femoral head, so the ultimate strength of the cancellous bones of the humeral head is less than that of the femoral head. Good correlations have been obtained between indentation depth (at 50 N load) and ultimate strength ($R = -0.937$, $p < 0.05$), meaning that with the increase of ultimate strength the indentation depth or deformation decreased proportionally.

Sumner *et al.* [6] has verified that the data obtained from the indentation test correlated well with that from the conventional compressive test. Although the ultimate strength and elastic modulus of different cancellous bones from different subjects are different, they generally fall into a certain range, like that of

TABLE I The results of indentation testing

Bones	Ultimate load (N)	Stiffness ($\mathrm{N\,mm^{-1}}$)	Ultimate strength (MPa)	Indentation depth at 50 N load (μm)
Femoral head	95 ± 21	490 ± 230	71 ± 15	125 ± 54
Medial femoral condyle	61 ± 13	223 ± 96	45 ± 9	263 ± 98
Lateral femoral condyle	52 ± 14	249 ± 81	39 ± 11	216 ± 70
Medial tibial plateau	67 ± 14	239 ± 65	50 ± 10	223 ± 64
Lateral tibial plateau	52 ± 12	259 ± 77	38 ± 9	209 ± 67
Humeral head	60 ± 12	242 ± 75	44 ± 9	223 ± 64

Mean ± SD, $n = 48$ to 52 at each epiphyseal location.

compression tests. According to the data pooled from the literature [1–6] and the data generated from this study, the ultimate strength of cancellous bones obtained by the indentation test ranges from 38 to 71 MPa. The wide range of these values is not surprising and are due to different subjects and different locations. Even with the conventional compressive test, the range of elastic modulus was much larger, ranging from several MPa to 3000 MPa [8]. Trabecular bone modulus can vary 100-fold from one location to another even within the same metaphysis [8].

Indentation testing may be more suitable to the *in vivo* condition (a constrained compression test). A similar testing procedure, penetration test described by Hvid *et al.* was used to detect cancellous bone strength during knee arthroplasty [9]. The mechanical properties (mostly obtained from compression testing) of a cube or cylinder bone sample separated from the bone such as the femur or tibia are not the same as when the cube or cylinder are in the bone tissue, which can be only obtained by indentation test or penetration test. Also, indentation testing is simpler than compression testing. Only a flat surface of the sample is needed for testing, and is less invasive than the conventional compression test. The indentation test makes testing on smaller bones such as the rat bones feasible since the diameter of the indentor can be designed as small as 1.31 mm.

There have been no reports to our knowledge of attempts to study rat bones using compression testing or any other methods, possibly because the bone size is too small. Since the structure of cancellous bone is anisotropic, which is more apparent for smaller bones, indentation testing may be more appropriate. Also, fewer variables are involved with indentation testing compared to compression testing. When the conditions of the test machine are the same, with indentation test only the specimen deformation and the surface area of the indentor are needed, while using compression test with a cylindrical sample the length (which cannot easily be controlled), end surface area, and the deformation have to be known.

In conclusion, the ultimate load, stiffness, and ultimate strength of the rat epiphyseal bones were obtained by using the indentation test. A useful mechanical data set has been generated. Differences were found between the cancellous bones at different locations. Based on the results and comparison with conventional compression testing, the simplicity and usefulness of this indentation test for small bone samples are superior.

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References

1. G. K. AITKEN, R. B. BOURNE, J. B. FINLAY, C. H. RORABECK and P. R. ANDREAE, *Clin. Orthop.* **201** (1985) 264.
2. J. C. BEHRENS, P. S. WALKER and H. SHOJI, *J. Biomech.* **7** (1974) 201.
3. J. B. FINLAY, R. B. BOURNE, W. J. KRAEMER, T. K. MOROZ and C. H. RORABECK, *Clin. Orthop.* **247** (1989) 193.
4. R. G. JOSEFCHAK, J. B. FINLAY, R. B. BOURNE and C. H. RORABECK, *ibid.* **220** (1987) 192.
5. S. SAITOH, Y. NAKATSUCHI, L. LATTA and E. MILNE, *Skeletal Radiol.* **22** (1993) 425.
6. D. R. SUMNER, T. L. WILLKE, A. BERZINS and T. M. TURNER, *J. Biomech.* **27** (1994) 1095.
7. Y. NAKABAYASHI, H. W. WEVERS, T. D. V. COOKE and M. GRIFFIN, *J. Arthroplasty* **9** (1994) 307.
8. S. A. GOLDSTEIN, *J. Biomech.* **20** (1987) 1055.
9. I. HVID, K. ANDERSEN and S. OLESEN, *Eng. Med.* **13** (1984) 73.

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