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Young's modulus repeatability assessment using cycling compression loading on cancellous bone

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Abstract: For various applications, precision of the Young's modulus of cancellous bone specimens is needed. However, measurement variability is rarely given. The aim of this study was to assess the Young's modulus repeatability using a uniaxial cyclic compression protocol on embedded specimens of human cancellous bone. Twelve specimens from 12 human calcanei were considered. The specimens were first defatted and then 1 or 2 mm at the ends were embedded in an epoxy resin. The compression experiment consists in applying 20 compressive cycles between 0.2 per cent and 0.6 per cent strain with a 2 Hz loading frequency. The coefficient of variation of the current protocol was found to be 1.2 per cent. This protocol showed variability similar to the end-cap technique (considered as a reference). It can be applied on porous specimen (especially human bone) and requires minimal bone length to limit end-artifact variability. The current method could be applied in association with noninvasive measurements (such as ultrasound) with full compatibility. This possibility opens the way for bone damage follow-up based on Young's modulus monitoring.

Keywords: biomechanics, repeatability, elasticity, calcaneus, trabecular bone

1 INTRODUCTION

In the past decades, apparent elastic properties of cancellous bone have been obtained through various experimental protocols tested on different anatomic sites; from Carter and Hayes [**1**] to Lievers *et al.* [**2**], including (non-exclusively) Linde and Hvid [**3**], Røhl *et al.* [**4**], and Keaveny *et al.* [**5**], the variety of experimental protocols, more or less easy to implement, is large.

Røhl *et al.* [**4**] focused on the tensile and compressive properties of cancellous bone. Parallelepipedic specimens ($9 \times 9 \times 20\,mm^3$), taken from human tibiae, were embedded between 2-cm thick layers of epoxy resin, penetrating about 2 mm into the bone. Tensile and compressive Young's moduli were determined, by the output of an extensometer fixed directly on the trabecular bone, from the slope of the (fifth-order polynomial) fitted loading curve at 0.18 per cent strain.

Keaveny *et al.* [**5**] used cylindrical specimens of trabecular bone coming from different anatomic sites to assess the measurement precision as a function of the testing protocol. Young's modulus of the specimens, determined using each of the testing protocols, is compared to the reference method defined as the *four-extensometer* technique, which can only be used on relatively dense specimens. For the protocol involving brass endcaps, approximately 8 mm of bone was embedded in each endcap, leaving approximately 16 mm of bone free to be tested. Young's modulus was determined using the maximum slope of the stress–strain curve on compression test between 0 and 0.3 per cent strain for human specimen (0.4 per cent for bovine bones).

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More recently, Lievers *et al.* [**2**] tested the influence of specimen length on apparent modulus and concluded that cancellous bone specimen length (in the range assessed, 10–22 mm) has no effect on apparent elastic modulus measurements made using an epoxy-embedded end-constrained testing protocol. Approximately 6 mm of bone at each end was embedded in epoxy resin and Young's modulus was determined from tensile testing using Røhl *et al.*'s [**4**] protocol.

From these studies, in terms of macroscopic characterization of cancellous bone, it can be concluded that the international literature is relatively consensual: the use of an end-constrained specimen to carry out tensile or compression tests is needed so that frictional effects between the specimen and the compression plates are minimized [**5**] and so that the mechanical properties are not underestimated when using sufficient dimensions for the tested specimen (a 10 mm diameter cylinder for [**2**]). The use of the end-constrained method also simplifies the experimental measurement while the mean values of the determined parameter property remain close to the ones obtained with an extensometer positioned directly on the specimen [**5**].

But, whatever the aim of determining mechanical properties of cancellous bone, precision of measurements has to be assessed. As elastic properties of cancellous bone depend on the tested specimen, the true value remains unknown and cannot be used to estimate the precision errors; their evaluation relies on the assessment of measurement repeatability. Keaveny *et al.* [**5**] calculated the coefficient of variation (CV) in modulus for each tested specimen (CV ranged from 0.56 per cent to 1.33 per cent) but did not estimate the repeatability of the experimental technique.

This technical note proposes an experimental protocol using embedded specimens to assess the measurement repeatability of Young's modulus estimated on human cancellous bone. The chosen mechanical test was a uniaxial cyclic compression.

2 MATERIALS AND METHODS

2.1 Specimen preparation

Twelve calcanei from six male and six female donors were used (mean age 80.6 years, 1SD = 8.7 years). All cadaver calcanei were obtained with legal authorization from the Department of Anatomy (Service du don des corps, Université Paris Descartes, Paris, France). All bones were cleaned of soft tissue and marrow, and defatted using the Supercrit® technique (BioBank, Paris, France), which eliminates fats while protecting the organo-mineral structure of bone tissue and its mechanical properties [**6**]. This cleaning process enables specimens to be kept at room temperature and is used to secure bone grafts before surgery. Specimens were cut into parallelepipedic samples (around $15 \times 15 \times 24\ mm^3$) to have their principal trabecular orientation approximately aligned with the loading axis [**7**]. Before testing, sample ends were embedded in 2 mm thickness Araldite® epoxy resin washers; resin penetration into bone was about 1 mm (Fig. 1) and the extremities were milled to obtain plane and parallel faces. Specimens were moisturized in a saline solution 24 hours prior to experimentation. Their height and section were measured with a digital calliper with a precision of 0.01 mm.

2.2 Mechanical testing

Repeatability tests were carried out at room temperature applying cyclic compression loading with an Electropuls E1000 testing machine (Instron Ltd, High Wycombe, UK) instrumented with a 2 kN load cell (accuracy 0.5 per cent). Deformations were recorded as close as possible to the sample with a 50 mm gauge length extensometer (accuracy 1 per cent) (Instron Ltd., High Wycombe, UK) attached to the compression device. The specimens were positioned between two parallel compression platens (Fig. 2).

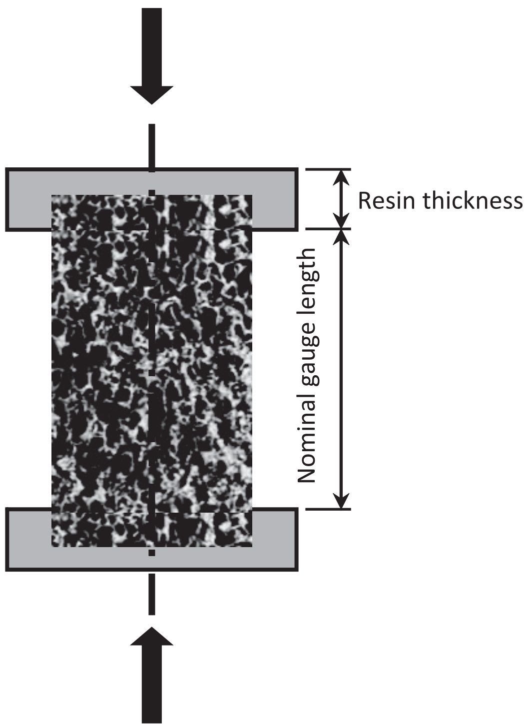

Fig. 1 Calcaneus specimen embedded in epoxy resin

A 30 N compression preload was applied to ensure full contact. The test sequence consisted of 20 deformation cycles between 0.2 per cent and 0.6 per cent with a 2 Hz loading frequency (close to gait frequency). The test sequence was repeated three times for the 12 specimens, with complete take-off of the specimen and of the extensometer between two sequences (full-repositioning test). The three measurements were carried out within the same day for a given specimen. Loading of the specimen during 20 cycles allowed us to assess that the specimens were not damaged (no modulus reduction, no residual strain). Young's modulus was calculated using 40 per cent to 90 per cent of the loading part of the stress–strain curve (Fig. 3).

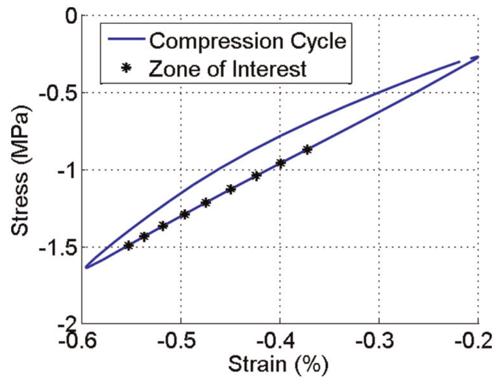

Fig. 3 Stress–strain curve and zone of interest (black stars) used for the Young's modulus calculation

2.3 Evaluation of precision of the technique

The assessment of repeatability was performed using the method proposed by Glüer *et al.* [**8**]: the coefficient of variation (CV_{SDj}) of repeated measurements was calculated for each specimen, noted j (with $j = 1,..,12$ in the current study), using

$$CV_{SD_j} = \frac{SD_j}{\bar{x}_j} \cdot 100 \text{ per cent} \qquad (1)$$

where x_{ij} is the result of the ith measurement for specimen j ($i = 20$ in the current study), $\bar{x}_j$ is the mean of all x_{ij} and SD_j is the standard deviation of n_j repeated measurements

$$SD_j = \sqrt{\sum_{i=1}^{n_j} \frac{(x_{ij} - \bar{x}_j)^2}{n_j - 1}} \qquad (2)$$

The global coefficient of variation (CV_{SD}) is used to evaluate the repeatability over the whole group of specimens and can be written as

$$CV_{SD} = \left(SD / \sum_{j=1}^{m} \bar{x}_j / m \right) \cdot 100 \text{ per cent} \qquad (3)$$

with m the number of specimens ($m = 12$ in the current study) and with SD defined by

$$SD = \sqrt{\sum_{j=1}^{m} SD_j^2 / m} \qquad (4)$$

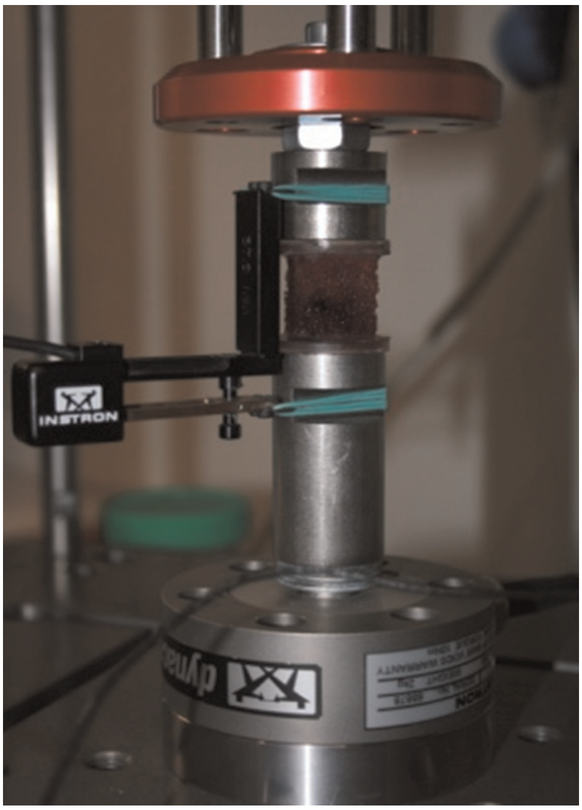

Fig. 2 Calcaneus specimen in the compression device with extensometer close to the specimen and the load cell at the bottom of the setup

3 RESULTS AND DISCUSSION

The mean age of the specimens was 80.6 years old which is similar to *in vitro* studies using samples from bodies donated to science. This age might limit extrapolation of the current results to specimens from younger subjects.

The three values of Young's modulus for the 20th cycle were used for the calculation of mean Young's modulus of each specimen. Results are presented in Table 1. Standard deviation was computed and the coefficient of variation of each specimen was evaluated. The global coefficient of variation of the proposed experimental protocol was 1.2 per cent.

Table 1 Mean Young's modulus, standard deviation and coefficient of variation (per cent) of each specimen

Specimen	E_j (MPa)	SD_j (MPa)	CV_{SDj} (per cent)
18G	82.16	0.91	1.11
20G	155.35	0.91	0.59
47D	88.21	3.22	3.65
47G	79.83	0.75	0.94
61D	230.52	2.80	1.22
61G	183.71	3.48	1.89
63D	215.27	3.98	1.85
63G	368.46	1.23	0.33
84D	343.86	0.79	0.23
84G	193.90	0.93	0.48
122D	345.50	5.18	1.50
122G	332.97	3.47	1.04

For various applications a correct evaluation of the measurement precision is needed in testing cancellous bone (e.g. to detect early damage evolution of cancellous bone submitted to fatigue loading).

The technique used in the current study was a platen compression method with epoxy-embedded ends. This technique was preferred to the one currently most often used (brass end-cap, glued specimen, [**5**]) because the current biomechanical protocol has to be coupled with an ultrasonic one [**9**] avoiding fixation of the specimen extremities in a steel end-cap. The current protocol allows repeated Young's modulus measurements alternatively with nondestructive measurements (such as ultrasound). For instance, Moreschi *et al.* [**9**] proposed a dynamic acousto-elastic testing method for the estimation of trabecular bone microdamage that can be applied as an intermediate measurement.

Keaveny *et al.* [**5**] attempted to study the variability in Young's modulus determination of trabecular bone using compression testing and different measurement methods (end-cap technique compared to platens method). They calculated the coefficient of variation for the platen methods of one bovine tibial specimen (CV = 15.6 per cent) and one bovine humeral specimen (CV = 8.6 per cent). In that paper, they also evaluated the coefficient of variation of end-cap technique using ten bovine tibial specimens tested six times. The CV ranged from 0.56 per cent to 1.33 per cent. Our current protocol provides a CV close to the one obtained by Keaveny *et al.* [**5**] using the end-cap technique, confirming the usefulness of the epoxy-embedded ends. The current protocol could be an alternative solution to the end-cap technique in all the cases where the height of the specimen is limited.

Recently Lievers *et al.* [**2**] used also an end-constrained protocol on bovine cancellous bone, but with an extensometer placed directly on the bone sample. This can only be done on relatively dense specimens. The current technique can be applied whatever the specimen porosity. The 25 mm length in mean for the bones used in the current study can also be decreased; the size of the specimens was conditioned here by a non-invasive measurement method associated to the mechanical measurement which needed a 22 mm minimum length.

The proposed method applied on calcaneus cancellous bone enables the global repeatability in the Young's modulus determination to be evaluated with a complete take off of the specimen and measurement materials. It can be useful, for example, when the experimental protocol includes intermediate measurements.

4 CONCLUSIONS

The proposed protocol allows end-constraint of the specimens and repeatable measurements with a variability of 1.2 per cent. This protocol showed variability similar to the end-cap technique (considered as a reference). It can be applied on porous specimens (especially human bone specimens) and requires minimal bone length to avoid end-artifact variability. The current method could be applied in association with non-invasive measurements (such as ultrasound) with full compatibility.

CONFLICT OF INTEREST

There are no conflicts of interest related to the work in this paper.

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