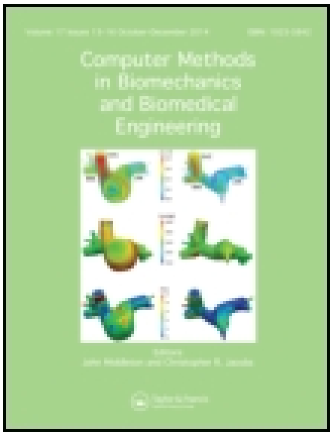

Computer Methods in Biomechanics and Biomedical Engineering
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gcmb20

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Published online: 30 Jul 2014.

To cite this article: S. Jaramillo-Isaza, P.-E. Mazeran, K. El-Kirat & M.-C. Ho Ba Tho (2014) Effects of bone density in the time-dependent mechanical properties of human cortical bone by nanoindentation, Computer Methods in Biomechanics and Biomedical Engineering, 17:sup1, 34-35, DOI: 10.1080/10255842.2014.931090

To link to this article: http://dx.doi.org/10.1080/10255842.2014.931090

Computer Methods in Biomechanics and Biomedical Engineering, 2014
Vol. 17, No. S1, 34–35, http://dx.doi.org/10.1080/10255842.2014.931090

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Keywords: cortical bone; density; nanoindentation; time dependent

1. Introduction

Cortical bone is a composite and biological material with a multiscale hierarchical architecture (Ho Ba Tho et al. 2012). Effects of density in the mechanical response of cortical and cancellous bone have been widely characterized at the macro-scale (Hodgskinson and Currey 1992; Ammann and Rizzoli. 2003; Vanleene et al. 2008). Bone density is related to its mineral and organic composition and thus to their time-dependent mechanical properties.

At the micro-scale, mechanical properties are commonly assessed by nanoindentation. However, the classical Oliver–Pharr method used to calculate the mechanical response is based on an elastic-plastic model (Oliver and Pharr 1992). Thus, time-dependent mechanical response cannot be assessed. However, specific protocols have been developed for time-dependent mechanical properties in organic and biological materials by nanoindentation (Oyen 2006; Mazeran et al. 2012).

This study aims to assess the effects of different bone densities in the time-dependent mechanical response of human cortical bone by nanoindentation. The mechanical model reported by Mazeran et al. (2012) will be used to compute the time-dependent mechanical response.

2. Methods

2.1 Sample preparation

Three samples of human femoral cortical bone (F1, F2 and F3) were used. Sample densities were measured previously by Archimedes principle and an accurate balance. The samples were grounded with abrasive paper (P800, P1200, P2400 and P4000), and polished on micro-cloths with alumina suspensions (particle size of 1, 0.3 and 0.04 μm). Finally, the samples were cleaned using ultrasounds in distilled water to eliminate all debris.

The values of bone density used in this study are summarized in Table 1.

2.2 Experimental protocol

A Nano Indenter® G200 (Agilent Technologies, Santa Clara, CA) was used. Fifty indentations per sample were performed in the longitudinal direction of the cortical shell. Indentations were carried out in the interstitial lamellae. The indentation protocol is composed of four steps:

(1) Load stage at constant load-rate/load untilan indentation depth of 3000 nm
(2) Hold time 300 s
(3) Unload to 50% of the $L_{\max}$ at constant unload-rate/load
(4) Hold time 300 s.

2.3 Assessment of the mechanical properties

The indentation data was fitted by a mechanical model composed of elastic, viscoelastic, plastic and viscoplastic components. It allows one to compute an elastic modulus (E_{elast}), two viscoelastic modulus (E_{ve1}, η_{ve1}, E_{ve2}, η_{ve2}), hardness (H) and viscoplasticity (η_{vp}) (Mazeran et al. 2012) (Figure 1).

To assess the apparent elastic modulus (E_{app}), the following equations were used:

First, to compute an equivalent elastic modulus for a given strain rate

$$\frac{1}{E_{equiv}} = \frac{1}{E_{elast}} + \frac{1}{E_{ve1} + \dot{\varepsilon} \times \eta_{ve1}} + \frac{1}{E_{ve2} + \dot{\varepsilon} \times \eta_{ve2}}$$

Then, to assess the apparent elastic modulus

$$E_{app} = E_{equiv} \times (1 - v^2)$$

where ν is the Poisson's ratio of bone.

To compare the results, non-parametric statistical analyses (Kruskal–Wallis and Dwass–Steel–Critchlow–Fligner) were performed.

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Table 1. Values of the apparent bone density measured for each sample.

	F1	F2	F3
Densities (kg/m^3)	1892 ± 30	1650 ± 275	1221 ± 161

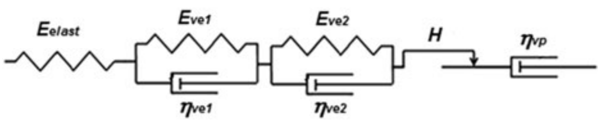

Figure 1. Model of the mechanical behaviour of bone used in this study.

3. Results and discussion

The results of the time-dependent mechanical properties are presented in Figure 2.

The results show different mechanical responses in the interstitial lamellae. The elastic response E_{app} (Figure 2(a)) shows different values for all samples. This fact could be associated with the EVEPVP method, which considers the effect of the viscoelasticity and the strain rate in the elastic response of bone. Nevertheless, these results are in agreement with previous values reported by Ho Ba Tho et al. (2012). For E_{elast} and E_{ve1} (Figure 2(b) and 2(c)) values were found to have statistically significant differences ($p < 0.05$) but do not follow a monotonic law. As previously reported by Vanleene et al. (2008), this phenomenon could be related to variations of bone porosity, the carbonate and protein weight. For the viscous component η_{ve1} (Figure 2(d)), differences were found only between samples F1 and F3 probably due to the high variance of data, especially for the low density in sample (F3). Hardness and viscoplasticity (Figure 2(e) and 2(f)) decrease with density but only statistical differences ($p < 0.05$) were found in hardness between F2 and F3.

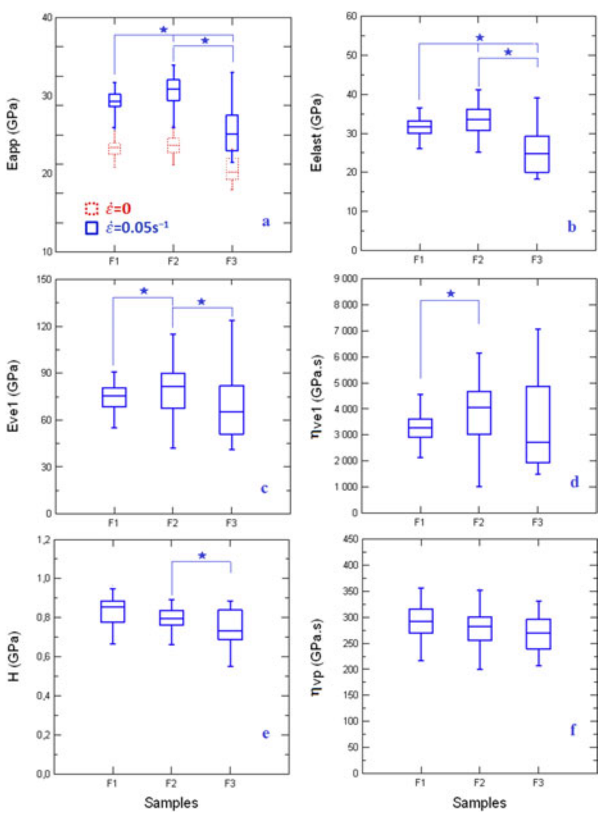

Figure 2. The time-dependent mechanical response as a function of bone sample (statistically significantly differences (*) $p < 0.05$).

4. Conclusions

This experimental investigation attempts to quantify changes in the time-dependent mechanical behaviour of cortical bone as a function of bone density. Because of the monotonic relation found, density could be correlated to the hardness and viscoplasticity but not with the elastic response. Nevertheless, degradation of bone mechanical response cannot be attributed completely to one element. Further investigations in the Haversian system, the micro- and nanoporosity and in the interactions of organic and mineral components in the bone matrix should highlight better interpretations.

Acknowledgements

This project is co-financed by the European Union engaged in Picardie with the European Regional Development Fund and CNRS (grant Collegium UTC CNRS INSIS).

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