# Macro-micro Characterization of Human Cortical Bone Using Ultrasound and Nanoindentation Technique

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## Abstract

Mechanical properties of human cortical bone were assessed at different scale of the tissue level using an ultrasonic technique and nanoindentation.
Our results suggest that elastic properties on cortical bone at the tissue level (haversian system) reflects the homogenised elastic properties of the osteon at the microscopic level with values closed to that of mineralised osteon lamellae. It should be noted that variation of elastic properties is higher (40%) at the microscopic level than the macroscopic level which is about 15%. The variation at the microscopic level (reflecting the remodeling process) does not seemed to alter the macroscopic elastic properties values.

## Introduction

Mechanical properties of human bone have been assessed for more than two decades at different levels (organ, tissue). Experimental techniques were based on wave propagation analysis, conventional mechanical testing. According to our knowledge no multiscale measurements had been assessed in order to quantify the relationship between the macroscopic and microscopic mechanical behaviour of human cortical bone.
In the present study, mechanical properties of human cortical bone has been assessed at different scale of the tissue level. The objectives were to compare the elastic properties of the cortical bone obtained by a transmission ultrasound technique and the elastic properties of lamellae osteon by nanoindentation technique.

### Methods

Six cubic samples (representative volume of 64-125mm$^3$) were obtained from a cadaveric femur (male 70 years of age). At the macroscopic level, an ultrasonic transmission technique developed in our laboratory allowed velocities measurements to be measured. Bar wave propagation mode was used allowing the direct assessment of Young's modulus obtained by the relation between velocity and density (ρ):

$$V_{bar} = \sqrt{\frac{E}{\rho}} \qquad (1)$$

Calibration setup is performed on materials with known values of velocities and elastic properties. Impulse waves were generated by a function generator (HP 3312A) and ultrasonic waves produced by the transducers (75kHz, Massa Products) were recorded and analyzed by a numerical scope (Tektronics 2232) (Figure 1). Acoustic and elastic properties were assessed in the axial direction.

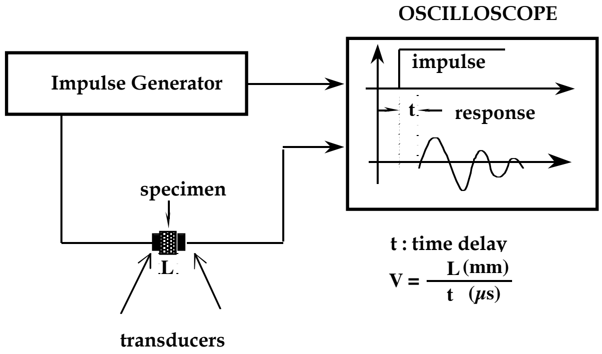


Figure 1. Illustration of the ultrasonic transmission technique setup.

Nanoindentation (Hysitron Inc., Minneapolis, MN, USA) were performed on the same specimens in order to assess the elastic properties at the microscopic level (osteon lamella, interstitial lamella). Three types of osteons have been selected through scanning electron pictures (XL 30 ESEM - FEG); white (mineralised osteon), grey (intermediate mineralisation) and dark (low mineralisation) osteons. Three indentations were performed on the thick lamella located in the middle of the osteon. The nanoindenter is constituted with a Berkovich diamond tip and the mechanical test induces 3 holding and unloading with a constant holding during 10 seconds in order to decrease the effect of the viscoelasticity. The maximal force is 2500μN, which induces a maximal depth of about 400nm. The reduced modulus (Er) is obtained from the load – displacement experimental curve with the relation:

$$E_r = \frac{\sqrt{\pi}}{2} \cdot \frac{S}{\sqrt{A}} \qquad (2)$$

A is the contact area and S is the stiffness obtained from the slope on the last unloading. The calibration of the tip area has been performed with indents on fused silica.

Statistical tests (Statgraphics V5.0, Sigma plus) were performed to compare the difference between elasticity measured at different scale of the cortical bone tissue.

**Results**

The elastic moduli measured at the macroscopic level was respectively were 20±5 GPa (N=6). At the microstructural level of the same samples, axial elastic moduli of the interstitial lamella was found to be equal to 22±3. Axial elastic modulus for the white (N=61), grey (N=17) and dark osteons (N=39) were found to be statistically different ($p<0.05$) with respective values of 21.30 ± 3.00 GPa, 19.27 ± 1.78 GPa and 12.95 ± 2.66 GPa. The range of variation of elastic properties within the same osteon lamella is 0.2GPa to 8GPa (mean value of 2.6 ± 1.7) (figure 2).

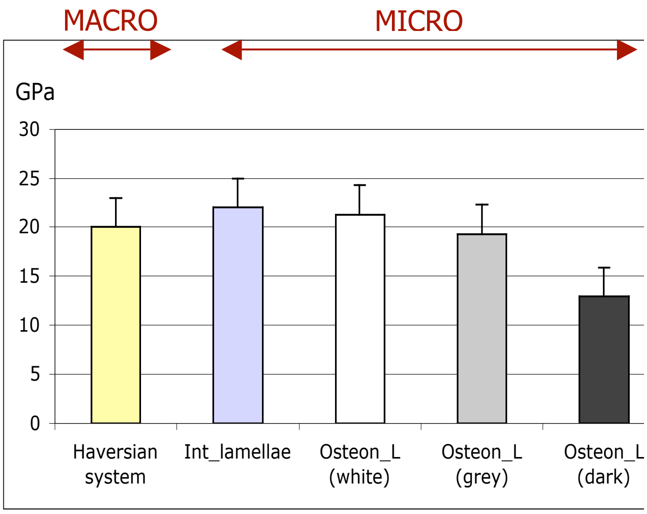


Figure 2. Values of elastic properties of the cortical bone measured at the macro and microscopic level.

**Discussion**

At the macroscopic level, values are within the range found in previous work ([1], [2]).
At the microscopic level, values of elastic moduli obtained with interstitial lamellae are slightly lower than that found by previous authors for cortical femoral and tibial bone (about 25-27 GPa) [4,5]. For osteon lamellae elastic properties values are in agreement with that found in the literature. One should note that in the literature, the different degree of mineralization of the osteon are not specified in opposite with our study.
One should note that at the macroscopic level, the bone volume is considered homogeneous (basic assumption of a continuum media for the ultrasonic measurement). It means that quantitatively, the ultrasonic measurements reflect the homogenized elastic properties, which seemed to be consistent with the present study.
Data performed on the specimen demonstrated that the elastic properties at the microscopic level varied from 13GPa to 25GPa reflecting different degree of mineralization of the osteons or remodeling process. These variations (heterogeneities) at the microscopic level which represents a variation of 40% reflects the dynamic process occurring at that level, meanwhile at the macroscopic level variation are about 15% [2].
These results suggest that elastic properties at the macroscopic level are not altered by the process of mineralization or remodeling as it reflects the homogenized elastic properties.

**Conclusions**

Our results suggest that elastic properties on cortical bone at the tissue level (haversian system) reflect predominantly the elastic properties of the mineralised osteon at the microscopic level with similar quantitative values. As the ultrasonic measurements reflect homogenized elastic properties measured at the microscopic level, this would suggest that mineralized osteon and interstitial lamellae are predominant.
Our results also suggest that a significant decrease of elastic properties at the tissue level may be related to significant macro porosity and alterations of the material properties of the mineralised osteon at the microscopic level simultaneously or a dense bone with predominant osteon in process of mineralization.
These results are of importance for a better understanding of the contribution of the elastic properties of the osteons at the tissue level and for modeling multiscale mechanical behavior of cortical bone.

**References**

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