INTRODUCTION: For over two decades, studies have been performed at the tissue level in order to determine the elastic properties of human cortical bone using ultrasound and mechanical techniques. At the microscopic level, the nanoindentation technique has been performed in order to determine the elastic moduli in the axial and transverse directions. Axial elastic modulus for osteon (19.1-22.5 GPa) lamellae was found to be lower than that of interstitial lamellae (23-25 GPa) [1-2]. No significant difference has been found between younger and older cortical bone, but most inner thick lamellae have greater elastic properties (20.8 GPa) than the outer lamellae (18.6 GPa) [3]. The purpose of this study was to measure the axial elastic modulus of thick lamellae of osteons exhibiting different degree of mineralization.

METHODS: Six femoral human cortical samples (5x4x4mm) have been obtained from a cadaveric femur (male 70 years of age) and cut parallel to the axis of the femur in the lateral and medial sides between 30% to 70% of the shaft. The samples were kept dry in the freezer at the temperature of –20°C. Each sample has been ground with sandpaper (grit #1200) and then polished with 0.5µm aluminium powder. Scanning electron micrographs (XL 30 ESEM - FEG) were produced under high vacuum at 20kV with a magnification of 22. The samples have been scanned with an electron beam in order to have a mapping of back scattered electron reflecting the composition of the microstructure. Three types of osteons have been selected through SEM images; white (mineralized osteon), grey (intermediate mineralisation) and dark (low mineralisation) osteons with a total number of osteon of 61, 17, and 39, respectively. Then, nanoindentation tests (Hysitron Inc., Minneapolis, MN, USA) have been performed on three locations of thick lamellae located in the middle of each osteon. This localisation is considered to be representative area of the entire osteon. The nanoindentor 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 [4]:

\[ E_r = \frac{\sqrt{2}}{S} \times \frac{A}{S} \]

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 realized with indents on fused silica, which is characterized as very elastic. ANOVA test has been performed with the software Statgraphics 5.0 (Sigma Plus) in order to study the variation of axial elastic properties (E33) function of its location and the type of osteon.

RESULT: The mapping of the surface of the sample performed with the SEM is represented in Figure 1.

![SEM image](image)

Figure 1. SEM images allowing the selection of the three types of osteon (1: white, 2: grey, 3: black).

The intra lamellar (IL) variation of axial elastic moduli is summarized in Table 1.

<table>
<thead>
<tr>
<th>E33 (GPa)</th>
<th>white osteons (N=61)</th>
<th>grey osteons (N=17)</th>
<th>dark osteons (N=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.19 – 7.76</td>
<td>0.93 – 5.93</td>
<td>0.24 – 8.48</td>
<td></td>
</tr>
<tr>
<td>2.81</td>
<td>2.42</td>
<td>2.12</td>
<td></td>
</tr>
<tr>
<td>2.93 ± 1.83</td>
<td>2.69 ± 1.57</td>
<td>2.45 – 1.70</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Minimum, maximum, median and average with standard deviation values for axial elastic modulus within the lamella.

The reproducibility obtained with three repeated tests executed in the same thick lamellae is about 1.33 GPa. The axial elastic properties of the three types of osteons are summarised in Table 2. Figure 2 shows significant variations between these three groups of osteons. The dark osteons have a lowest axial modulus compared to the other groups (***, P < 0.001 and **, P < 0.05).

![Graph](graph)

Figure 2. Range of values of E33 for different type of osteons

DISCUSSION: The variation of elastic properties within a lamella is approximately 2.6 GPa (with a range of variation from 0.2 to 8 GPa), depending on different types of osteons. This result demonstrates the inhomogeneity of the lamella suggesting that both orientation of collagen fibers and degree of mineralization may vary within the lamella. This result is not in agreement with previous data [5]. The axial elastic properties for the white and grey osteons are within the same range, but significantly higher than the dark osteons. The white osteons have a higher axial moduli compared to the grey ones (about 2GPa) and the dark ones (about 6GPa). These data are consistent with the fact that the grey level of the osteon is related to its degree of mineralization. The more bright the osteon is, the more mineralized it is. Then one would expect that variation of elastic moduli reflect different degree of mineralization. In fact, the dark osteons have a lower degree of mineralization then as expected a lower elastic property. Range of values for the dark osteon is of interest as it is provided for the first time according to our knowledge. Somehow the different range of elastic properties for the white and grey osteons are within the same range, but significantly higher than the dark osteons during its ageing and remodelling process.


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