

# NANO- AND MACRO-SCALE CHARACTERISATION OF THE MECHANICAL PROPERTIES OF BOVINE BONE

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## ABSTRACT

In the present study, nano- and macro-scale characterisations on the mechanical properties of bovine cortical bones have been performed by using nanoindentation and conventional compressive tests. Nanoindentation results showed that the elastic modulus for the osteons and the interstitial lamellae in the longitude direction were  $24.7 \pm 2.5$  GPa and  $30.1 \pm 2.4$  GPa. As it's difficult to distinguish osteons from interstitial lamellae in the transverse direction, the average elastic modulus for cortical bovine bone in the transverse direction was  $19.8 \pm 1.6$  GPa. Significant differences were found in the modulus values between different microstructures of bone tissue and in different testing direction. It was found that the elastic modulus of bone bovine material in nano-level was higher than that in macro-level. The elastic modulus and ultimate stress of large bone samples were  $12.5 \pm 1.9$  GPa and  $195 \pm 19$  MPa respectively from the compression test.

## 1. INTRODUCTION

Understanding the mechanics of living bone continues to be a major scientific challenge, as pointed out by Rho et al [1] and Choi et al [2] in their impressive work. Among the various biomechanical properties of bone, such as creep, fatigue and strength, elastic modulus attracted more research interests, as the elastic moduli are important for characterizing various bone pathologies and guiding the artificial implant design. Also research on determining elastic modulus of bone has been carried out for many years and various methods like mechanical testing [3-8], combinations of microcomputed tomography, finite element modelling [9, 10], ultrasonography [11] and nanoindentation [12-14] have been used. It has been shown that bone has a hierarchical structure and primarily composed of mineralized collagen fibril. The elastic modulus of large tensile cortical specimens has been shown to be in the 14-20 GPa range [3], while that of microbending cortical specimens (single osteon) was 5.4 GPa [6]. Extensive researches have been done on mechanical properties of bone at macro level using tensile, compressive and bending tests [3-8]. However, the mechanical properties of bone at micro- and nano-level remain poorly understood. In present study, elastic moduli of bovine cortical and trabecular bone at the lamellar level have been characterised by using nanoindentation. To compare with the results from nanoindentation, conventional compressive tests also been done on large specimens.

## 2. MATERIALS AND METHODS

### 2.1 Sample Preparation

Tests were conducted on fresh bovine tibia obtained from a local slaughterhouse. Using a band saw, cortical bone from between the tibial metaphysis and diaphysis

were first cut down to 15 mm thick slices, then into precise size (8 x 4 x 4 mm) for compressive test and 3 mm thick slices for nanoindentation with a low-speed diamond saw (Isomet, Buehler Corp., Lake Bluff, IL, USA). The cutting was performed under constant deionized water irrigation to minimize the undesired mineral formation on the surface of the specimens. After cutting, the large specimens were soaked in 0.9% NaCl and stored at -20 °C. Before testing, the specimens were held at a temperature of 4 °C until fully thawed. For nanoindentation test, the 3mm specimens were dehydrated in a series of alcohol baths after cutting and embedded in epoxy resin at room temperature. The embedded samples were metallographically polished to produce the smooth surfaces needed for nanoindentation testing. After being ground with silicon carbide abrasive papers of decreasing grit size (600, 800, and 1200 grit) under deionized water, the specimens were polished on microcloths with successively finer grades of diamond powder slurry, the finest being 1 µm grit. The last polishing step was on plain microcloth under deionized water, and the specimens were cleaned in ultrasonic bath to remove surface debris.

### 2.2 Nanoindentation

In this study, all experiments were performed using the UMISII (CSIRO Division of Telecommunications and Industrial Physics), at room temperature. The system has force and displacement resolutions of 0.1 µN and 0.1 nm respectively. The apparatus is enclosed in an insulated cabinet to provide thermal stability. A sharp Berkovich diamond indenter was used for all the measurements. The microcomponent to be indented was located in the microscope, and then positioned beneath the indenter using the x-y table. (The distance between the indenter and the microscope was set to be constant during the test) Fused silica, which exhibits elastic isotropy and has a relatively low modulus- to -

hardness ratio, was used to calibrate the tip shape function. The elastic modulus of fused silica was calculated to be 73.25 GPa, which is similar to the known value of 72.5 GPa.

Each nanoindentation test was conducted to a maximum load of 20 mN at a constant loading rate of 1 mN/s with 20 incremental points, which produced hardness impressions with depths of about 1  $\mu\text{m}$ . At the beginning, the indenter was slowly driven toward the surface of samples, until surface contact, with a force of 0.05 mN. The impression was held for a period of 10 s at this peak load to eliminate the creep behaviour and then unloaded to 2 % of the peak load. The first 7 points of unloading curves were used for calculation of the elastic properties of the entire unloading curve, which is non-linear. Any indentations close to the mounting resin were removed from the data set to minimize the effects of embedding on the measurements.

The indentation load-displacement data obtained in these tests were analysed to obtain the hardness, H, and the elastic modulus, E, using the method of Oliver and Pharr [15]. The first step is to determine the contact

stiffness, S, representing the resistance of the material to elastic deformation.

$$E_{ind} = \left( \frac{1}{E_r} - \frac{1 - \nu_{tip}^2}{E_{tip}} \right)^{-1} \quad (3)$$

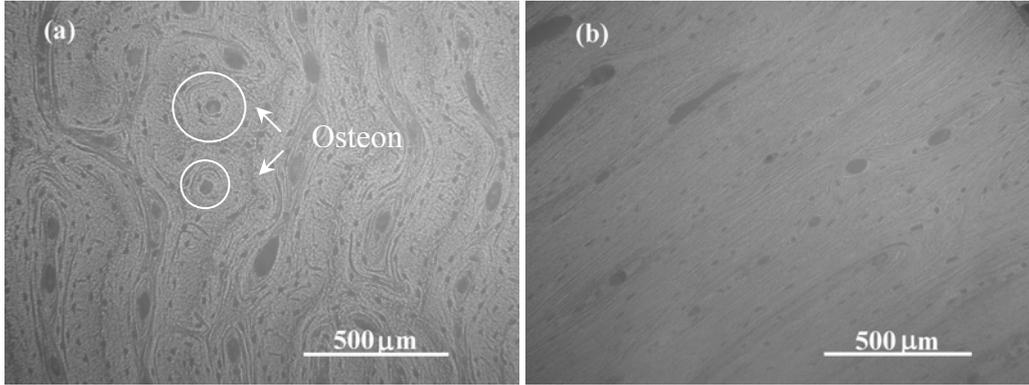
This variable represents:

$$E_{ind} = \frac{E_{specimen}}{1 - \nu_{specimen}^2} \quad (4)$$

which represents a combination of the local Young's modulus, E specimen and the local Poisson's ratio,  $\nu$  specimen, whereby the material is assumed to be isotropic.

The classical hardness property represents the mean pressure under the tip at maximum load P ( $h_{max}$ ):

$$H = \frac{P(h_{max})}{A_c(h_{max})} \quad (5)$$



**Figure 1** Optical micrographs of bone microstructure, (a) Transverse cross section of tibial cortical bone; (b) Longitudinal cross section of tibial cortical bone;

$$S(h_{max}) = \frac{dP}{dh}(h_{max}) = \frac{2}{\sqrt{\pi}} E_r \sqrt{A_c(h_{max})} \quad (1)$$

Where, P represents the applied load. S( $h_{max}$ ) is the derivative of the unloading curve at the point of initial unloading,  $h_{max}$ , which is determined by fitting 40 %-95 % of the unloading curve,  $A_c(h)$  is the contact area over which the indenter and the material are in instantaneous contact. The latter function is determined by a calibration procedure. The reduced modulus,  $E_r$ , depends on the deformation of the material the diamond tip. It consists of the sum of two contributions:

$$\frac{1}{E_r} = \frac{1 - \nu_{specimen}^2}{E_{specimen}} + \frac{1 - \nu_{tip}^2}{E_{tip}} \quad (2)$$

The indentation modulus (equation 3) can be calculated with the reduced modulus and the elastic properties of

the diamond indenter tip,  $\nu_{tip} = 0.07$  and  $E_{tip} = 1140$  GPa.

### 2.3 Compressive Test

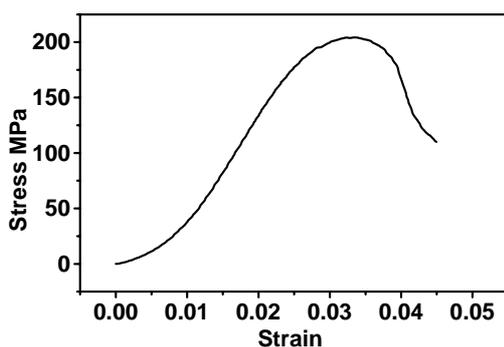
Compressive test were performed in a servo hydraulic materials test machine (MTS insight 100, MTS system corp.). Load was measured by a load cell integrated with the test system. Global strain, determined on the basis of platen-to-platen displacement, was measured by an extensometer attached to the loading platens. The specimens were placed carefully at the centre of the platens. To avoid non-uniform stresses on the end of the specimens, the samples were cut into precise size, with length to width ratio of 2:1.

### 3. RESULTS AND DISCUSSION

Fig.1 shows the microstructure of cortical bone tissue in longitude direction and transverse direction. Cortical bone consists of repeating units called Haversian systems or osteons. Osteons has concentric layers of the mineralized collagen fibers called lamellae [1, 16]. The area between two osteons is called interstitial lamellae. It is believed that Osteon has lower mineral content than interstitial lamellae, because Osteon being newer bone compared to interstitial lamellae. As it's difficult to distinguish osteons from interstitial lamellae in the transverse direction, the elastic modulus in the transverse direction is the mean elastic modulus of osteons and interstitial lamellae.

Fig.2 is a typical compressive stress strain curve for large cortical bone sample. Test results are summarized in Table 1. The mean values of ultimate stress and elastic modulus are  $195 \pm 19$  MPa and  $12.5 \pm 1.9$  GPa respectively.

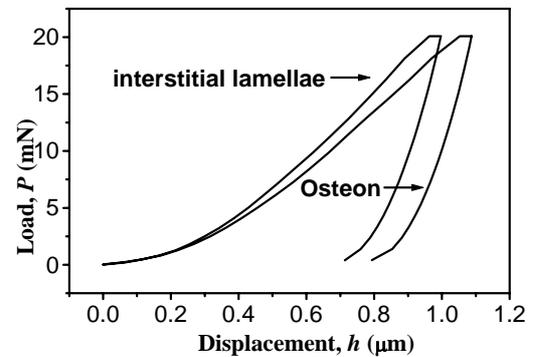
Fig.3 is a typical set of load-displacement data for an osteon and a trabecular bone. A total of 127 indentations were made in 15 microcomponents. A summary of the elastic modulus,  $E$ , and hardness,  $H$ , is presented in Table 1. It was found that the interstitial lamellae has a highest modulus ( $30.1 \pm 2.4$  GPa), which followed by osteonal lamellae ( $24.7 \pm 2.5$  GPa). Average hardness range between 0.647 GPa to 0.892 GPa. Significant differences in the elastic moduli of osteons and interstitial lamellae in the longitude and transverse direction were analysed using one-way ANOVA. It was found that the mean values of the elastic moduli for all of the bone components were statistically different ( $p < 0.05$ ). Results showed that the elastic modulus values of bone bovine material got from nanoindentation were higher than that form compression test.



**Figure 2** Compressive stress strain curve for cortical bone sample

The average elastic modulus for interstitial lamellae measured in this study,  $E = 30.1$  GPa, is significantly higher than that for osteons,  $E = 24.7$  GPa. This possibly result form the osteons being newer bone compared to interstitial lamellae. It is believed that newer bone have a lower mineral content, resulting in a lower elastic modulus [17]. The observation that

interstitial lamellae have a higher modulus than osteons is consistent with the work of other researchers [13, 14, 18]. The moduli of osteons and interstitial lamellae in the longitudinal direction, 24.7 GPa and 30.1 GPa, respectively, are significantly greater than that of transverse modulus (19.8 GPa, an average of osteons and interstitial lamellae) and the anisotropy ratio of the cortical bone here is 1.38.



**Figure 3** Force-displacement curves obtained by the indentation of osteonal and interstitial lamellae

It is believed that drying of bone affects the elastic modulus and hardness, as drying leads to contraction of individual collagen fibrils. Tests were conducted to examine the degree to which elastic property are affected by drying [19]. For wet bovine femora, it was found the elastic moduli of osteons and interstitial lamellae were  $21.1 \pm 2$  GPa and  $25.1 \pm 1.6$  GPa, while in dry bovine femora the elastic moduli of osteons and interstitial lamellae were  $27.5 \pm 1.2$  GPa and  $24.4 \pm 2.2$  GPa. The elastic moduli of interstitial lamellae and osteons are increased by drying by approximately 10 % and 15 %. Assuming this same factor applies to our data, the moduli of interstitial lamellae and osteons would be 27 GPa and 21 GPa.

The elastic modulus and ultimate stress of large bone samples were  $12.5 \pm 1.9$  GPa from the compression test, which is lower than the values from nanoindentation (27 GPa for interstitial lamellae and 21 GPa for osteons). Rho et al [1] assumed the modulus of a macroscopic sample of cortical bone should fall somewhere between that of the osteons and interstitial lamellae. But what we observed in this study is both values of osteons and interstitial lamellae are higher than the modulus of a macroscopic sample. The reason that the elastic moduli measured by nanoindentation technique are larger than those measured in macroscopic scale is not clear and can not be confirmed without systematic study. We should notice that nanoindentation elastic modulus measurement are not as sensitive to the defects and inhomogeneities as other testing techniques, since an indentation is about 1-5  $\mu\text{m}$  in depth and edge length and the defects can be avoided by careful placement of the indentations. Compared with those for nanoindentation, large

compressive specimens contain microstructural defects such as cement lines and voids (Haversian and Volkmann canals, lacuna, osteocytes, canaliculi and so

on), which may reduce the ability of cortical bone to resist deformation, resulting in the lower modulus.

**Table 1** Average elastic moduli and hardness of cortical bovine bone  
\*Standard deviations are shown in parentheses (SD)

	Tested Direction		No.of Samples	Ultimate Stress MPa	Elastic Modulus, (SD) GPa	Hardness, (SD*) GPa
Nanoindentation	longitude	Osteons	5		24.7 (2.5)	0.811 (0.155)
		Interstitial lamellae	5		30.1 (2.4)	0.892 (0.113)
	Transverse		5		19.8 (1.6)	0.647 (0.06)
Compression	longitude		10	195 (19)	12.5 (1.9)	

Although the elastic modulus is considered an intrinsic materials property, a constant value regardless of sample size or testing direction, this assumes that the material is homogeneous. In fact bone is not a homogeneous material; it consists of different constituent materials and possesses structural heterogeneity at every level. The values of elastic moduli of bone tissue at micro- and nano- scale are very different with those measured in macroscopic tests. Therefore, we believe that the direct comparisons between elastic modulus of microstructural components and large cortical bone modulus could introduce a misunderstanding of mechanical properties of bone materials. Both of these two values provide essential information for finite element analyses of bone structure or bone-implant interfaces.

#### 4. CONCLUSIONS

Our study provides detailed data about elastic moduli both at nano- scale and at macro- scale of dry bovine bone material. Results could provide useful data in the development of theoretical micromechanical models, and finite element modelling. It was found that the elastic modulus of bovine bone material in nano-level was higher than that in macro-level.

The elastic modulus for the osteons and the interstitial lamellae in the longitude direction were found to be  $24.7 \pm 2.5$  GPa and  $30.1 \pm 2.4$  GPa. The average elastic modulus for cortical bovine bone in the transverse direction was  $19.8 \pm 1.6$  GPa. And the elastic modulus for trabecular bone in the longitude and transverse direction were  $20 \pm 2$  GPa and  $14.7 \pm 1.9$  GPa respectively. The elastic modulus and ultimate stress of large bone samples were  $12.5 \pm 1.9$  GPa and  $195 \pm 19$  MPa from the compression test.

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