Experimental Measurement of Cortical Bone Properties

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Introduction

Bone properties vary according to size, shape, structural pattern and materials composition; the complexity of tissue arrangement and orientation molecules makes bones highly heterogeneous and anisotropic.

Mechanical property tests by a combination of microcomputed tomography, finite element modeling, ultrasonography and nanoindentation were used to investigate the elastic modulus of bones [1], reported elastic modulus of large tensile cortical bone specimens to be in the range of 14 - 20 GPa [2], while only 5.4 GPa by microbending [1], and 22-25 GPa by nanoindentation as reported by RHO et al. [3, 4] and Hengsberger et al. [1]. Since bone is an elastically anisotropic material so using nanoindentation technique can provide a high resolution (<1µm) and accuracy in tests examining microstructural properties, particularly when the bone possess regional variation in materials compositions and structural patterns.

Therefore, the goal of this study is to use surface nano-indentation technique to measure the mechanical properties of bovine cortical bone in both longitudinal and transverse directions. Results obtained from this study can be deployed in in-depth understanding of the property changes of bones in different directions.

Experimental Study

Sample Preparation

Two specimens of ribs from a fresh frozen bovine bone were obtained and sectioned into 5mm thick pieces along its transverse plane with a low-speed diamond saw (Metkon, resin bonded diamond cut-off wheels) under constant water irrigation. Marrow inside the specimens was removed and dehydrated at room temperature (~23) for 24 hours. The specimens were embedded into epoxy resin to provide support and allowed to cure overnight at room temperature (~23).

Surface of the embedded specimen was polished by the use of seriese of silicon carbide papers (60, 320, 800, 1200 and 2000 grit progressively), again under constant water irrigation, before polishing with 15µm, 6µm and 1µm diamond power. Finally, the specimens were cleaned by distilled water to remove any remaining surface debris. All specimen preparation was examined under the optical microscope to ensure the lamellar microstructure of the bone had to appear as clear as possible (example shown in Fig. 1).

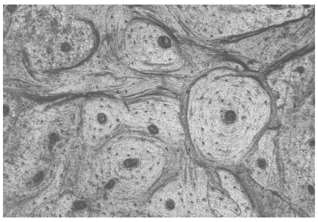

Fig.1. Cross sectional view of the rib cortical bone in the (a) longitudinal and (b) transverse directions

Nano-indentation was conducted by using a scanning nano-indenter (TriboScratch; Hysitron, Inc., Minneapolis, MN) at room temperature (~23), to determine the Young's modulus and hardness of the specimens in both longitudinal and transverse directions. A sharp Berkovich (three-sided pyramid) diamond indenter was used for measurement.

At start of the test, the indenter was slowly driven towards the specimen surface, with surface contact force of 30 mN and a constant loading rate of 2000 µN/s was applied. A hardness impression was held for a period of 5s at the maximum load to eliminate the creep behavior, before unloaded at rate of 2000 µN/s.

The data obtained from indentation load-displacement curves were analyzed for calculation of the Young's modulus, *E*, and the hardness, *H*, using the method developed by Oliver and Pharr, where the indenter area function have been well documented [5]. The relationship between contact stiffness and the elastic properties of the sample is shown as follows:

$$S = \frac{dP}{dh} = \frac{2}{\sqrt{\pi}} E_r \sqrt{A} \qquad (1)$$

Where P is the load and h is depth of penetration into sample; E_r is the reduced modulus, and A is the projected area of the elastic contact. The reduced modulus is related to the elastic modulus, E as:

$$\frac{1}{E_r} = \left(\frac{1-\nu_s^2}{E_s}\right) + \left(\frac{1-\nu_i^2}{E_i}\right) \qquad (2)$$

Here, E_s and ν_s are the elastic modulus and Poisson's ratio for the specimen and E_i and ν_i are the same parameters for the indenter; for a standard diamond indentor probe, E_i is 1140 GPa and ν_i is 0.07. The elastic modulus is derived by measuring the initial unloading stiffness and assuming that the contact area is equal to the optically measured area of the hardness impression. The hardness, H, is calculated as:

$$H = \frac{P_{max}}{Ac} \quad (3)$$

Where, P_{max} is the maximum indentation force, A_C is the projected contact area.

Results and Discussion

The elastic modulus and hardness data of the bovine cortical bone in the longitudinal and transverse directions were obtained from the load-displacement curve. A total of 75 indentations as shown in Fig. 4 were produced in the longitudinal direction, the average elastic modulus in longitudinal was 16.23GPa, and the average hardness was 0.505GPa; 120 indentations as shown in Fig.5 were made in the transverse direction, the average elastic moduli and harness were measured as 10.26GPa and 0.387GPa, respectively. A summary of the elastic modulus, *E*, and hardness, *H*, is presented in Table 1.

Bone type	Direction to be tested	No. of Indentations	Elastic Modulus, average (GPa)	Hardness, average (GPa)
Cortical bone	Longitudinal	75	16.23	0.505
	Transverse	120	10.26	0.387

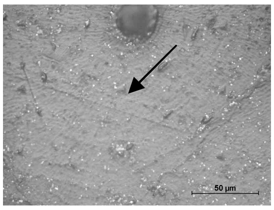

Fig.4. Indentation marks in longitudinal direction

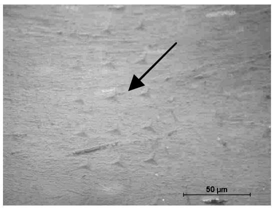

Fig.5. Indentation marks in transverse direction

The results obtained from this study demonstrate that both the elastic modulus and hardness properties of the bone samples are varied, the measured values on longitudinal direction were significantly higher than that of the transverse direction. Although these results are comparatively lower than reported in WEN et al. [1], where the recorded elastic modulus of osteons in the longitudinal and transverse directions were 24.7 GPa, and 19.8GPa, respectively; and hardness were 0.811 GPa, and 0.647 GPa, respectively. RHO et al. [6] also reported that the elastic modulus of osteons in cortical bone under dry condition was 24.4 GPa, and hardness was 0.68 GPa. However, TAI et als [7] recorded a modulus of 12.9 GPa by nanoindentation for dry bovine tibial bone, which is correlated to the presented result.

Two main factors that contributed to the difference between this presented results with other researchers:

i) Different anatomical original of the bones lead to a significant different result.

ii) Other effect on the bone mechanical measurement is due to the collagen orientation and distribution. According to the work by ASCENZI et al. [8], different distribution of osteon types distinguished by predominant collagen fiber orientation, may lead to distinctive average mechanical properties.

Consequently, the elastic modulus of bone tissue is strongly depended on tissue type within, anatomical location, and bone mineral content distribution should also be considered. In addition, Evans et al. [6] have shown that bone hardness can be influenced by varies factors such as its storage, preparation and testing. Moreover, EVANS et al. also found that hardness correlates with Young's modulus, specifically, hardness increases with modulus.

Conclusion

This study provides data about elastic modulus and hardness of cortical part of bovine rib bones, using surface nanoindentation technique. Properties measured by nanoindentation could offer useful data in the development of theoretical micromechanical models, and in finite element modeling [1,3]. The average elastic moduli in the longitudinal and transverse directions were 16.23 GPa, and 10.26 GPa, respectively. The average hardness was 0.505 GPa, and 0.387 GPa, respectively. Since, this is only a preliminary work, and due to the complexity of the bone structures, therefore, in order to gain more insight on the mechanical properties, it is necessary to investigate more in depth into the relationship between bone structure and the mineral and organic material distribution.

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