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Measurement of Bovine Bone Properties through Surface Indentation Technique

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Abstract

The mechanical and micro-structural properties of cortical bovine bone have been extensively studied due to the fact that its micro-structural components would significantly reflect the global mechanical behavior of the bone. The objective of this paper is to study the elastic modulus and hardness of a bovine cortical bone, in both longitudinal and transverse directions by using nano-indentation technique. The moduli in the longitudinal and transverse directions were recorded as 16.23 GPa and 10.26 GPa, respectively. While the hardness readings in the longitudinal and transverse directions were 0.505 GPa and 0.387 GPa, respectively. According to the result shown, it is suggested that heterogeneous properties of the bone is assumed and the measurements of their properties are highly dependent on tissue types, anatomical location and bone mineral content distribution.

Keywords: Tissue engineering; Nano-indentation; Bone Properties.

Introduction

Bone property

Bone properties vary in size, shape, structural pattern and materials composition in order to perform distinct mechanical, biological and chemical functions; such as skeleton supports against the pull of gravity, protection of organs, and storage of healing cells and excess calcium. Due to the complexity of the irregular arrangement and orientation of the composition of bone, it makes the material of bone heterogeneous and anisotropic. The hierarchical structure of the bone comprises in five levels, as shown in Fig. 1, they are: (1) the macrostructure: cancellous and cortical bone; (2) the microstructure (from 10 to 500 μ m): Haversian systems, osteons, single trabeculae; (3) the sub-microstructure (1–10 μ m): lamellae; (4) the nanostructure (from a few hundred nanometers to 1 μ m): fibrillar collagen

and embedded mineral; and (5) the subnanostructure (below a few hundred nanometers): molecular structure of constituent elements, such as mineral, collagen, and non-collagenous organic proteins [1,2].

Fig.1. The hierarchical structure of bone from [1,2]

Due to the complexity of bone structure, it is a need to clearly understand its mechanical behaviour in relation to other parameters because they reflect the problems of the structure and function, such as bone fracture, bone remodeling, and the design of bone-implant systems. The mechanical properties of cortical and trabecular bones have been well studied experimentally and shown that their mechanical properties diverse in various structural levels. Mechanical property tests and combinations of microcomputed tomography, finite element modeling, ultrasonography and nanoindentation were used to investigate the elastic modulus of bone [3]. For examples, the elastic modulus of large tensile cortical bone specimens was reported in the range between 14 - 20 GPa [4], while that of microbending cortical specimens was only 5.4 GPa [3]. RHO et al. [5,6] and Hengsberger et al. [3] have reported that the elastic modulus of cortical bone in microstructure level were in range of 22-25 GPa using nanoindentation technique.

Since bone is a elastically anisotropic material, so using nanoindentation technique, often allows to obtain a high resolution ($<1\mu\text{m}$) and has the potential to provide more accurate results about its microstructure properties, particularly its properties vary much with its localized materials compositions and structural patterns. Therefore, the goal of this study is to

measure the mechanical properties in both longitudinal and transverse directions, of a bovine cortical bone through surface nano-indentation technique. The results obtained from this study are crucial to have an in-depth understanding of the property change of the bone in different directions.

Indentation Technique

Nanoindentation, which evolves from the conventional Vickers microhardness testing, is commonly used to quantify the mechanical properties of microstructural components of bone tissue down to the lamellar level [7,8]. Through the analysis of the indentation load-displacement behavior, it is able to obtain the values of the Young's modulus (E), and hardness (Hv) of materials [3,5,7,9].

Nanoindentation is an indirect measurement of the contact area, that is, when a desired load is applied to an indenter and in contact with the specimen, the depth of penetration of the indenter to the specimen surface is measured in nanometers (10^{-9} m); together with the known geometry of indenter, the area of contact at its full load can further be determined [10]. The hardness is determined by dividing the load by the area of contact; whereas, the elastic modulus is examined by the shape of the unloading curve.

Experimental Study

Sample preparation

Two specimens of rib from a fresh frozen bovine bone were obtained. Bone specimens were sectioned into 50mm thick along its transverse plane with a low-speed diamond saw (Metkon, resin bonded diamond cut-off wheels) under constant water irrigation. The marrow inside the specimens was removed by using a soft water jet followed by an ultrasonic bath to remove the surface debris. All specimens were dehydrated at room temperature (~23) for 24 hours and embedded in epoxy resin to provide support and allowed to cure overnight at room temperature (~23).

The embedded specimen surface was polished to produce a smooth surface which is required for nano-indentation. Progressive grades of silicon carbide paper (60, 320, 800, 1200 and 2000 grit) were used to grind the specimens under constant water irrigation and polished manually by 15 μ m, 6 μ m and 1 μ m diamond power. Finally, the specimens were cleaned by distilled water to remove the surface debris. All specimens preparation was controlled under the optical microscope to ensure the lamellar microstructure of the bone had to appear as clear as in Fig.2.

Fig.2. Cross sectional view of the rib cortical bone in the (a) longitudinal and (b) transverse directions.

Nanoindentations

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. The specimens were adhered on a stainless steel stage. A sharp Berkovich (three-sided pyramid) diamond indenter was used to conduct the measurements. The microstructure to be indented was positioned under the indenter and optics using the x-y plane and z height. (The distance between the indenter and specimen was remained constant during the test.)

At the test started, the indenter was slowly driven towards the specimen surface, the surface contact with a force of 30 mN at a constant loading rate of 2000 $\mu\text{N/s}$ was applied. A hardness impression was held for a period of 5s at the maximum load to eliminate the creep behavior, and then unloaded at rate of 2000 $\mu\text{N/s}$.

The data obtained from indentation load-displacement curves were analyzed to calculate the Young's modulus, E , and the hardness, H , using the method of Oliver and Pharr, in which the indenter area function have been well documented [11]. The contact stiffness S was determined from the measurement of the upper portion of the unloading data as:

$$S = \frac{dP}{dh} \quad (1)$$

The relationship between contact stiffness and the elastic properties of the sample is shown as follows:

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

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

$$\frac{1}{E_r} = \left(\frac{1 - \nu_s^2}{E_s} \right) + \left(\frac{1 - \nu_i^2}{E_i} \right) \quad (3)$$

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 indenter 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:

$$H = \frac{P_{\max}}{A_c} \quad (4)$$

Where, $P_{\max}$ is the maximum indentation force, A_c is the projected contact area.

Results and Discussion

Cortical bone consists of repeating units named Haversian systems or osteons; osteons is composed of regular, cylindrically layers of mineralized collagen fibers called lamellae [1,3]. The elastic modulus and hardness data of the bovine cortical bone in the longitudinal and transverse directions were obtained from the load-displacement curves as shown in Fig.3, indicating that the response of osteons in the longitudinal and transverse directions were different. A total of 75 indentations as shown in Fig. 4 were produced in the longitudinal direction, the average elastic modulus in longitudinal direction was 16.23 GPa, and the average hardness was 0.505 GPa. 120 indentations as shown in Fig.5 were made in the transverse direction. While in the transverse direction, the average elastic moduli and hardness were measured as 10.26 GPa and 0.387 GPa, 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

Table 1 Average elastic modulus and hardness of cortical bovine in two different directions bone

Fig.3. Load-displacement curves obtained by indentation in longitudinal and transverse direction

Fig.4. Indentation marks in longitudinal direction

Fig.5. Indentation marks in transverse direction

Several advantages could be gained from the nano-indentation technique when compared with the conventional micro-hardness measurement, included Vicker and Knoop techniques. One of the major advantages is the accurate positioning capabilities (better than 1 μm) and the high resolution load and depth sensing capabilities which allows relatively small area of material to be tested and measured. Furthermore, unlike conventional micro-hardness technique, the area of nano-indentation is not necessary to be optically measured in order for the hardness or elastic modulus to be determined, hence, indentations smaller than the limit of optical resolution can be used in the testing, and very small features can be explored [5].

The results obtained from this study demonstrate that both the elastic modulus and hardness

properties of the bone varies, the measured value from its longitudinal direction was significantly higher than that of the measurement in the transverse direction. Although these results comparatively lower as WEN et al. [3], which recorded the elastic modulus of osteons in cortical bone in the longitudinal and transverse direction is 24.7 GPa, and 19.8GPa, respectively; and hardness were 0.811 GPa, and 0.647 GPa, respectively. RHO et al. [7] 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 al [12] 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:

1. Different anatomical locations lead to a significant result measured. Since most of the researchers were used femur bone to measure the elastic modulus and hardness, in which femur is used to support the body weight, therefore, the mineral content is much higher than the rib that was used in this study.
2. Other effect on the bone mechanical measurement is due to the collagen orientation distribution. According to the work by ASCENZI et al. [13], different distribution of osteon types distinguished by predominant collagen fiber orientation, may lead to distinct average mechanical properties.

Consequently, the elastic modulus of bone tissue is strongly depended on tissue type, anatomical location, and bone mineral content distribution should also be considered. In addition, Evans et al. [7] 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 rib of bovine cortical bone using surface nanoindentation technique. Undoubtedly, nanoindentation provides a measure of elastic properties of bone at the microstructural scale ($<1\mu\text{m}$), hence, allows a more accurate position to obtain result. Properties measured by nanoindentation could offer useful data in the development of theoretical micromechanical models, and in finite element modeling [3,5].

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 about the relation between the bone structure and the mineral distribution.

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