Assessing Heat-treatment Effects on Bovine Cortical Bones by Nanoindentation

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Abstract

Among different sterilization methods, heat-treatment of bone is recognized as one of the simple and practical methods to lower the human immunodeficiency virus (HIV) infection and overcome the risks of rejection and disease transfer from allograft and xenograft during bone transplantation. In order to best characterize the micro-structural mechanical property of bone after heat treatment, the nanoindentation technique was applied in this study to measure the localized elastic modulus and hardness for interstitial lamellae and osteons lamellae of bovine cortical bones at temperature 23°C (room temperature- pristine specimen), 37°C, 90°C, 120°C and 160°C, respectively. The elastic modulus (E) and hardness (H) of interstitial lamellae obtained higher values as compared with osteons lamellae which show that interstitial lamellae are more stiff and mineralized than osteons. Moreover, as a specimen pre-heat treated at 90°C, the E and H values of interstitial lamellae and osteons were closed to a pristine specimen. For a specimen pre-heat treated at 120°C, both interstitial lamellae and osteons obtained an increase in E and H values. As a specimen pre-heat treated at 160°C, the interstitial lamellae and osteons obtained a slight decrease in E and H values. These findings are correlated to results reported by other researchers [1, 2] that calcified collagen molecules starts to degenerate at about 120°C and complete at 160°C. Interestingly, when a specimen was pre-heat treated at 37°C, both interstitial lamellae and osteons obtained significant decreases in E values of 57% and 40%, respectively as compared to the pristine specimen; while in H values, there was a decrease of 27.4% and 15%, respectively. Thus, this paper will investigate the mechanical properties of bovine cortical bones under various temperature ranges by nanoindentation technique.

Introduction

Heat-treated cortical bone has been proposed as an excellent alternatives material substitute for bone grafts and synthetic bone due to the advantages obtained in biological and mechanical properties. Todoh et al. [3] have reported that, the Young's modulus of cortical bone along the bone axis, after degeneration at 200^{o}C, to be about 12 GPa (using the four-point bending test), whereas that of an intact specimen was 20 GPa. The tensile strength of the degenerated specimen was 38 MPa as compared to 178 MPa for the intact specimen. However, a contradictory result was obtained by Catanese III et al. [4]: the elastic modulus (mean ± SD) remained similar to that of an intact cortical bone along the bone axis, being 16.3 GPa for compression and 16.3 GPa for tension when the cortical bone was heated up to 350^{o}C. In addition, the cortical bone was found to have maintained 63% of its intact strength in compression after being heated up to 350^{o}C, this makes it well suited for compressive load-bearing applications as mentioned by Catanese III et al. [4].

Nanoindentation is one of the promising novel techniques recently used to quantify the nano- and microscale mechanical properties in tissues and other biomaterials [5]. The nanoindentation is well suited to examine the microstructual features of material surface to provide a spatial resolution

which is less than 1μm [3, 6-8]. By analyzing the indentation load-displacement behaviour, it is possible to obtain the measurements of Young's modulus, E, and hardness, H, of the bone [4, 6, 9, 10]. The present study is to employ the nanoindentation technique to determine the mechanical properties of pristine and pre-heat treated single osteons and interstitial lamellae at various temperature ranges of femur bovine cortical bone.

Materials and Methods

Sample Preparation

A fresh frozen bovine cortical bone of femur was machined into 5mm thick perpendicular to its longitudinal axis by a low-speed diamond saw (Metkon, resin bonded diamond cut-off wheels). Totally, 15 specimens were produced and randomly divided into 5 groups to carry out the nanoindentation test. A soft water jet was used to remove marrow inside the specimens. All the specimens were then placed into an ultrasonic bath to remove surface debris for 5 minutes. Bone specimens in Group 1 were pristine sample, the specimens were dehydrated at room temperature (~23°C) as a control group; whereas Group 2 to 5 were pre-heat treated in an oven for 1 hour at four distinct pre-set temperature ranges, they were 37°C, 90°C, 120°C, and 160°C. The bone specimens were embedded in the resin block without being vacuumed into epoxy resin to provide support and allow them to cure for 24 hours at room temperature (23°C). Araldite GY251 epoxy resin (with hardener HY956 mixture ration of 5:1) was used to provide support for the bone specimens. The surfaces of all embedded specimens were finely grounded by different grades of silicon carbide papers (60, 320, 800, 1200 and 2000 grits) under a soft water jet and then further polished manually by soft synthetic flock polishing cloths with different grades of diamond powder (15μm, 6μm and 1μm). After grinding and polishing, the specimens were placed into the ultrasonic bath to remove the surface debris for 10 minutes. All indentations in this study were conducted away from the bone edge and resin boundary to enhance the accuracy of data.

Nanoindentation test

TriboScratch (Hysitron, Inc., Minneapolis, USA) was used to carry out the experiments at room temperature (~23°C) throughout the study. A sharp Berkovich (three-sided pyramid) diamond indenter tip was embedded in the transducer to measure the nanoindentation modulus and hardness. The Berkovich indenter tip was slowly driven towards the specimen surface at the test start in a constant displacement rate and a permanent hardness impression was made after the surface contact. A maximum load of 30mN at a loading and unloading rate of 0.6mN/s produce a surface contact depth and the hardness impression was held for a period of 5s at the maximum load to eliminate any creep behaviour. The data obtained from indentation load-displacement test was analyzed to calculate the elastic modulus, *E*, and the hardness, *H*, using the method of Oliver and Pharr, where the indenter area function have been well documented [11]. This method is based on the measurement of the contact stiffness *S*, from the upper portion of the unloading data to determine the relationship between contact stiffness and the elastic properties of the specimen.

The relationship between contact stiffness and the elastic properties of the specimen is defined as

$$S = \frac{dP}{dh} = \frac{2}{\sqrt{\pi}} E_r \sqrt{A} \tag{1}$$

Where P is the load and h is the depth of penetration into the 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:

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

Here, E_s and ν_s are the elastic modulus and Poisson's ratio for the specimen, respectively 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. Bone has been assumed to be isotropic, elasto-plastic material; thus, the Poisson's ratio ν_s may be taken as 0.3. As mentioned by Rho et al. [6], the change of measured

value of E_s should not excess 8% while ν_s ranged from 0.2 to 0.4. 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:

$$H = \frac{P_{\max}}{Ac} \tag{3}$$

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

Results and Discussion

Five groups of elastic moduli (E) and nanoindentation hardness (H) of osteonal and interstitial lamellae bones in the longitudinal direction were obtained through load-displacement curves as presented in Table 1.

Table 1 Average elastic moduli and hardness of cortical bovine bone in various temperatures (Standard deviations are shown)

			Osteons		Interstitial lamellae	
Temperature range (°C)	**No. of Specimen**	**No. of Indentation**	**Elastic modulus, GPa (SD)**	**Hardness, GPa (SD)**	**Elastic modulus, GPa (SD)**	**Hardness, GPa (SD)**
Pristine	3	40	26.13±1.61	0.96±0.06	28.89±2.11	1.07±0.09
37	3	40	18.59±1.28	0.83±0.08	18.38±1.53	0.84±0.06
90	3	40	26.32±2.46	1.06±0.08	28.50±1.69	1.17±0.06
120	3	40	28.77±2.33	1.11±0.11	30.54±2.57	1.24±0.11
160	3	40	28.65 ±2.07	1.25±0.11	29.14±1.70	1.27±0.10

This table indicates that the average E and H response of osteons lamellae and interstitial lamellae vary with temperatures and regions. In total, 1200 indentations were produced in the study, among them, 600 indentations were produced in osteons lamellae along the longitudinal direction and another 600 indentations were made in intersitial lamellae region as shown in Figures 1 and 2.

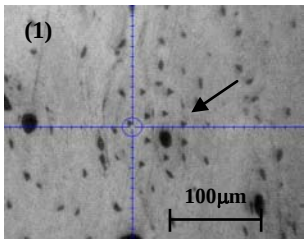

Fig.1 A total of 600 indentations were produced within 15 specimens in 5 randomly chosen osteons lamellae along longitudinal direction of the bovine cortical bone.

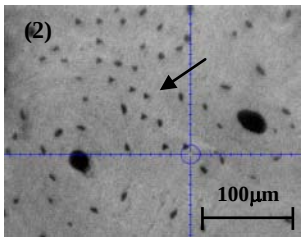

Fig.2 A total of 600 indentations were produced within 15 specimens in 5 randomly chosen interstitial lamellae along longitudinal direction of the bovine cortical bone.

Eight indentations were produced in five different single osteons, which were randomly choosen in each specimen and indentation take place around the Harvesian canel. Also eight indentations within five different interstitial lamellae regions were randomly choosen in each specimen. For pristine bone specimens, the average elastic modulus (E) is 28.89 GPa and hardness is 1.07 GPa in intersitiial lamellae. While, the average values of E and H in osteons lamellae are 26.13 GPa and 0.96 GPa, respectively. However, the E and H values in pre-heat treated bones at 37°C shows significant decrease to 18.38 GPa and 0.84 GPa, respectively in interstitial lamellae. In addition, the E and H values in osteons also show a decrease to 18.59 GPa and 0.83 GPa in E and H values, respectively. Nevertheless, bone specimens which heat-treated at 90°C, 120°C and 160°C showed an increase of E and H values in both osteons and interstitial lamellae. According to Rho et al. [12], they reported that interstitial bone is made from primary bone tissue while osteons are made of remnants of old osteons, thus, osteons are less stiff and less mineralized than interstitial bone.

Specimens heated at 90^0C show a slight increase of 0.7% and 10% in E and H values respectively in osteons. However, the E values in interstitial lamellae demonstrate a slight decrease of 1.3% and H values increase of 9.3%. As specimen pre-heat treated at 120^0C, both E and H values in osteons have a moderate increase of 10% and 15.6% in osteons as compare to the pristine specimen. In interstitial lamellae, there also demonstrate a moderate increase of 5.7% and 15.8% in E and H values. When specimens pre-heat treated at 160^0C, the E values in osteons also demonstrated a moderate increase of 9.6% in E value, but there only was a slight increase of 0.85% in interstitial

lamellae as compared to the pristine specimen. However, the H values in both osteons and interstitial lamellae demonstrate a significant increase of 30.2% and 18.7%, respectively. This finding showed that heat treatment in bone is not only a simple and practical way to reduce the chance of disease transmission, indeed, it also obtains advantages in biological and mechanical properties. Thus, it is believed that pre-heat treated bones can be excellent alternative materials for bone grafting.

As reported by Wang et al. [1, 2], non-calcified collagen molecules in bone degenerate at about 43^0C and calcified collagen molecules degenerate at about 150^0C. It is interesting to note that when temperature is at 37^0C, both E values in osteons and interstitial lamellae have significant decrease of 40% and 57%, respectively. The H values lies with the same pattern as E values that a decrease of 15.6% and 27.4%, respectively. According to Leikina et al. [13], the triple helices of type 1 collagen is unstable and easily to melt just several degrees above the body temperature. Nevertheless, the calcified collagen molecules, which involving pyridinoline or pyrroles are formed as hydroxypyridinium bonds are very stable. Wang et al. [1] also mentioned that collagen molecules degenerated at about 120^0C and completes at 190^0C. Although the collagen molecules were not measured in this study, however, the elastic modulus and hardness started to increase at about 120^0C and decrease at 160^0C as compared to the pristine bones.

This paper has presented the experimental results on localized mechanical properties of bovine cortical bones treated at different temperatures, in their longitudinal direction. In the nano-indentation test, it was shown that the osteons and interstitial lamellae of 120°C and 160° pre-heat bones exhibited higher Young's moduli and hardness than that of a pristine specmens. Based on the result obtained, it was demonstrated that pre-heated bone can be an excellent substitute material for bone transplantation without inducing any risks of rejection and disease transfer.

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