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Study of Microstructure and Mechanical Properties of Human Cortical Bone

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Abstract— The microstructure along with the micro-mechanical properties of bone have been extensively observed in this study. It has also been attempted to correlate the mechanical properties with the microstructural aspects. The load deformation pattern of human cortical bone (both male and female) under uniaxial compressive and tensile loading was studied. The physical properties like density and porosity of the whole femur as well as different parts of it was studied to get a mapping of the femur. Microstructure of bone, including the osteon structure, its distribution and its deformation under different stress fields have been thoroughly studied with the help of Scanning electron microscopy and reported here.

Index Terms—bone porosity, fractography, hardness anisotropy, load-deformation curve, Osteon deformation.

1 Introduction

Bone is a highly hierarchical structure and its mechanical properties are dependent on its structure and composition. Previous studies have focused on the macroscopic properties and their changes with respect to age and diseased condition. One such study showed with increasing tissue age, mineralization increases at certain portions and these hypermineralised portions become stiffer to some extent [1]. In another study about the post yield behavior it was found that middle-aged bone specimens demonstrated higher yield strain and yield stress than those from the elderly ones [2]. Recent studies are focusing on the microstructural effect [3], [4]. Due to the presence of haversian canals as an integral part of its structure, bone is quite porous in nature. Studied have been done to find out the effect of porosity on mechanical properties [5]. A significant negative correlation was found between the elastic anisotropy and porosity of cortical bone [6]. Density also varies from person to person and also with age. Osteoporosis has become a serious issue all over the world and efforts have been made to predict the risk of fracture through different techniques [7], [8]. Nanoindentation studies have also become popular for determining the hardness and elastic modulus of bone [9]. Variation of properties at specific sites like lamella under different physiological conditions was also studied by few people [10]. Fracture toughness was another area of interest for many researchers who tried to correlate it with age and microdamage accumulation [11], [12]. In one of the very recent studies with the help of finite element models and wavelet transforms, complete mapping of the energy descipation in bone after nanoindentation was shown. [13]. Laser engineered net shaping (LENS) is a rapid prototyping technique in which the machine gets instructions from CT Scan data and can produce or mimic a part of bone with powdered materials and without using any other tools. Very recent studies indicate that the in vivo life of implants depends on the porosity and mechanical properties of the material which should match with that of bone [14]. In this study we have tried to map the apparent density and the porosities of different parts of femur both in males and in females. The load-deformation curve was also studied in details for both male and female under compression and tension to get the idea of its mechanical properties and the unde deformation of osteons under stress field was observed. Hardness was measured in both males and females throughout the femur shaft and its anisotropy was observed.

2 Methodology

Femurs from both male and female human cadavers were collected, pretreated and cleaned. Density and porosity of the whole femur (shown in Fig-1a) were calculated by applying Archimedean principle. Then after drying they were sectioned into different parts as shown in Fig-1b.

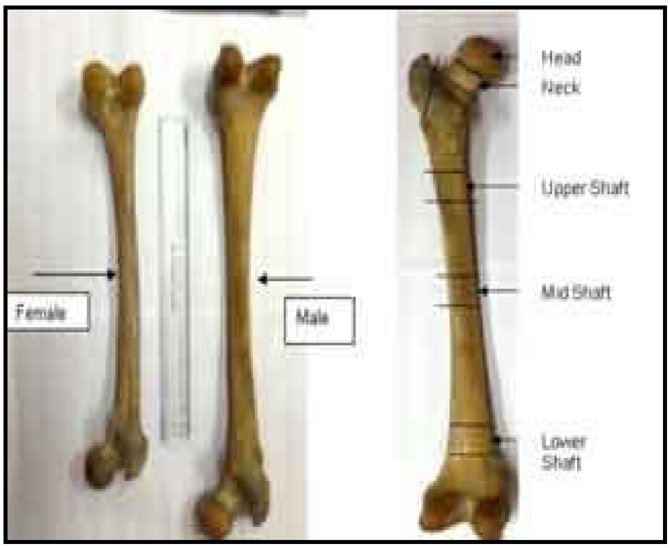

Fig. 1. Human Femur and the different parts in which it was sectioned for measurements.

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Then density and porosity of individual parts were also measured in the same way. A variation between the parts was very distinct. For observing the mechanical properties, samples of different sizes were cut and prepared following the ASTM standard (ASTM F 451 RE).The load-deformation curve of the cortical parts was obtained for both tension and compression from Universal Testing Machine (UTM: INSTRON 4204, U.K.). Young's modulus and work of fracture was calculated from the load-deformation curve itself. The loading was done till fracture for few samples, while in others it was stopped at various intermediate points, e.g. in the linear zone, in the plastic flow zone and then at the point of fracture to observe the deformation of the osteons at different stages of loading. This was mainly performed under compression as preparation of samples for tensile testing was little bit difficult due to some machine constraints. Experiments were also performed at different crosshead speeds of UTM i.e., with varying strain rate to determine its effect on stiffness of the bone. The cross section of the samples was polished in the polishing machine (LECO Spectrum System 1000, USA) with different grits until the value for the average centerline reached around 0.2534 micro meters.

Hardness was measured in both longitudinal and transverse direction to check the anisotropy using Vicker's Hardness testing machine (LV 700AT, LECO, USA). The structures of osteons were observed from the cross sectional images of the cortical bone (of both male and female) by a Reflecting inverted optical microscope (Olympus GX51, Japan). Using image analysis software (Analysis five, Olympus, Japan) the aspect ratio, lamellar thickness, diameter of haversian canals were calculated.

3 Results And Discussions

The structure of bone was thoroughly observed under the optical microscope under different magnifications. Osteons were clearly visible in the polished cross sections of the cortical part. Osteonal density was slightly different in different regions of the cross section. The average number of osteons per mm^2 was nearly 19 (1 .9 x 10^{-5} per sq. micron) in the inner part (towards the bone cavity) to about 14 (1.3 x 10^{-5} per sq. micron) in the outer part (Fig-2). With increasing magnification, the lamella, which are the concentric layers of tissue around the Haversian canal, were clearly visible. The thickness of the lamella ranged from 6.25 micron to 10.55 micron from inward to outward with a scatter of ±10%. Almost all the osteons had a combination of thin and thick lamella. The diameter of the Havesian canal had an average value of 78.18 micron towards the periphery as compared to 54.66 micron in the inner part with a large scatter in the values all over the region. Towards the inner side, the osteons were found to be more closely spaced which may be indicative of comparatively higher rate of bone formation in that region. Pictures taken from the scanning electron microscope revealed even minute structures called the lacune which are the sites for the bone cells (osteocytes).

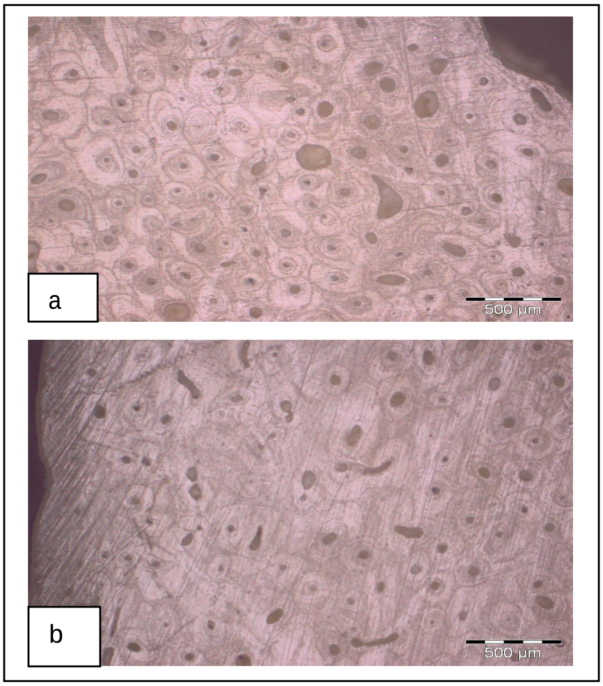

Fig. 2. Cross section of cortical bone (a) inner part (b) outer part man

The lacunae were visible along the layers of lamella. Interconnections between them are established by the canaliculi. Thus the structure reveals that such discontinuities are integral part of the bone structure. It will be shown later how they contribute to the fracture of bone by promoting the coalescence of cracks under different types of loading. Therefore concentration of porosity is to be taken into consideration when properties of bone are concerned. We measured the porosity of the whole femur and found it different when compared to the porosity of different parts of the same femur considered separately. Fig-3 shows the porosity distribution of in two male and two female samples. The cortical part is quite less porous than the head or the trabecular part in general. The density in females was found to be slightly lower in males as shown in Fig-4.

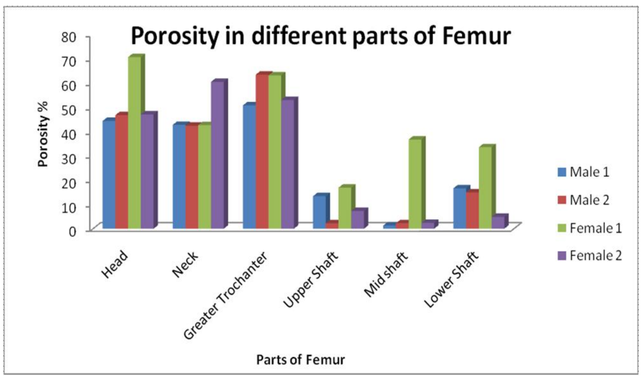

Fig. 3. Porosity distribution in Femur

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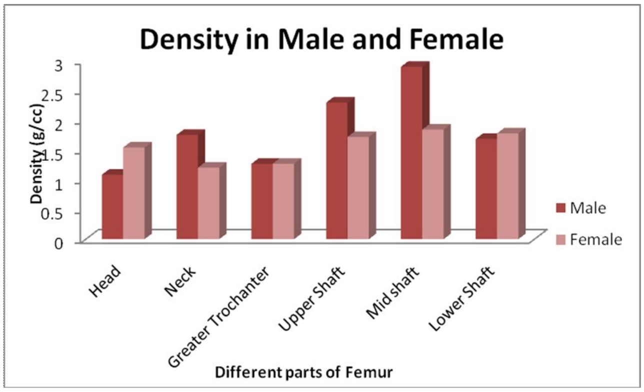
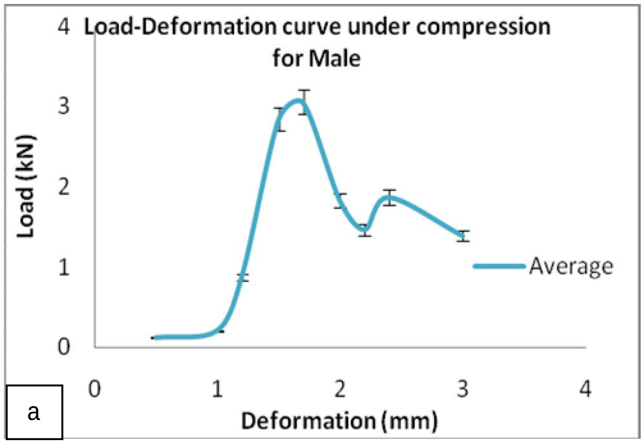


Fig. 4. Density distribution in male and female femur

But the results of a students t-test performed on both the sets of data showed that the difference in males and females were not significant at 95% confidence level. In our samples, porosity of one female femur was extremely high than others. This may be due to increased susceptibility to osteoporosis at higher age.

The load-deformation curves were obtained from compressive and tensile loading to study the mechanical properties. The initial part showed a stunning similarity for all the samples both in male and female. The load-deformation curve could be divided into two zones i.e., the elastic zone which was linear in all cases, and a nonlinear zone. Under compression, after reaching the peak load the curve was more flat in females than in males. The nonlinear zone revealed number of kinks representing initiation of fracture, progressive fracture and finally complete breakage occurred with a comparatively sharp drop of load. However during experiment, at these intermediate points no major splitting was observed, there were some primary signs of cracking. The parts of load-deformation curve corresponding to fracture zone were not truly identical in all the cases but the general pattern was comparable. Figure-5 shows a representative curve for the load deformation curves in both male and female with error bars showing 5-10% scatter at each data point.



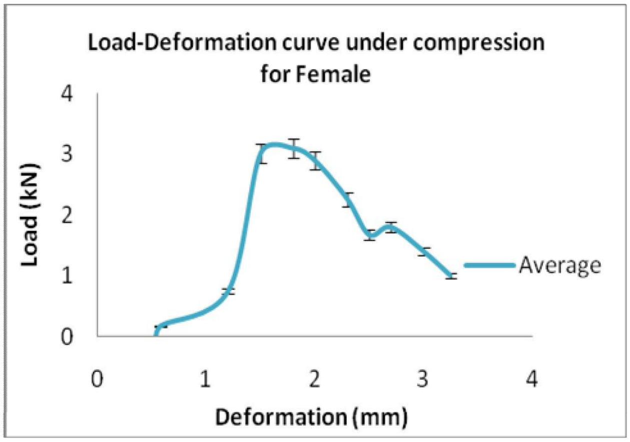


Fig. 5. Representative Load-Deformation curves a) For Male samples b) For Female samples of Femur

The equations fitting the individual curves of these two zones are given in the Table1. In the elastic zone, they are all fitted into linear equations with $R^2>0.95$ indicating a good amount of fitting, although slopes may have been different. For the non-linear part, most of the curves fitted with a 2nd degree polynomial and few with 3rd degree, all having R^2 values greater than 0.9. The ultimate strength was in the order of 158.66 MPa with standard deviation of 5.9 in females and 150.76 MPa with standard deviation of 11.9 in males. The elastic modulus was also calculated. The stiffness value determined in our study ranged from 10 to 15 GPa which was also verified by the nanoindentation technique. Work of fracture, which is the energy absorbed by the bone before complete fracture was obtained separately from the area under each curve and was slightly more in females in most of the cases than in males.

TABLE 1
EQUATIONS DESCRIBING THE LINEAR AND NON-LINEAR PART OF THE LOAD-DEFORMATION CURVES

Sample	Linear part	R^2 Value	Non-Linear Part	R^2 Value
Male 1	y=3.320x-32.39	0.97	$y=0.183x^3-10.4x^2+192x-1144$	0.957
Male 2	y=3.135x-31.5	0.94	$y=0.113x^3-6.622x^2+126.2x-774.9$	0.908
Male 3	y=2.611x-27.09	0.947	$y=0.021x^2-1.595x+35.62$	0.952
Male 4	y=6.2635x-6.7718	0.976	$y=1.5574x^3-11.456x^2+25.687x-14.891$	0.962
Female 1	y=3.196x-34.25	0.967	$y=-0.038x^2+1.286x+5.264$	0.96
Female 2	y=3.627x-38.50	0.962	$y=-0.094x^2+3.682x-20.67$	0.965
Female 3	y=5.919x-6.243	0.962	$y=11.85x^3+65x^2-121.8x+78.02$	0.98
Female 4	y=6.8574x+7.6309	0.946	$y=-2.6055x^2+8.0409x-2.667$	0.955

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The difference in the average values of work of fracture has been around 10% which is well within possible experimental scatter. Rather we should say the values were quite comparable. This strongly contradicts the prevailing hypothesis of higher vulnerability of female bone due to increased porosity particularly at higher age. The samples taken were rectangular in shape and in case of both tensile (Fig-6a) and compressive (Fig-6b) fracture it was observed that the fractured surface made an inclination with the vertical axis. The crack propagated making almost a 30^{o} angle with the vertical axis of the bone.

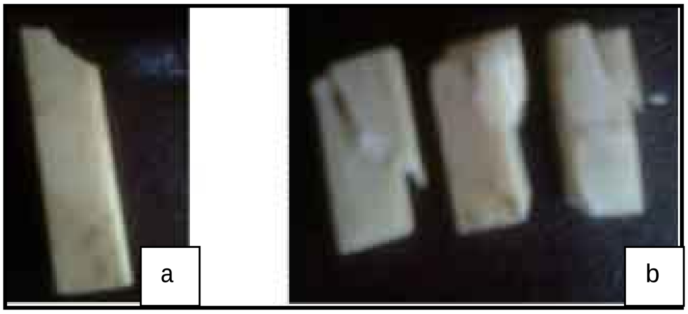
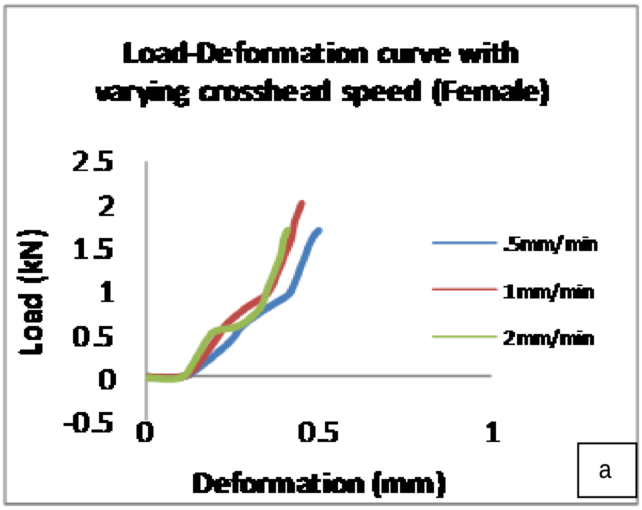


Fig. 6. Fracture plane making an angle with the vertical axis (a) under tension (b) under compression

In few cases of compressive loading for female bone samples, the samples experienced a crushing pattern where instead of forming asharp edge it got smashed from the top up to the bottom surface. The slope of the load-deformation curve changed as the crosshead speed was varied from 0.5mm / min to 4 mm / min. It was thus in accordance with the previous findings that stiffness of the bone increases with increasing loading rate or strain rate in case of tensile loading [15].Fig-7 shows the variation of stiffness with varying crosshead speed, or in other words varying strain rate. Thus from this figure it can be observed that not only under tension,



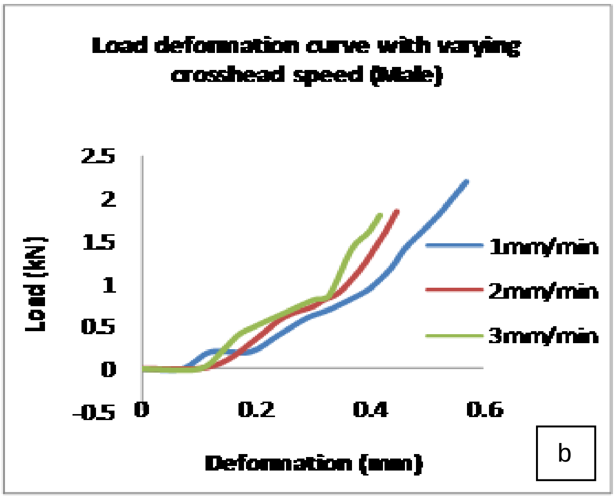


Fig. 7. Load-Deformation curve with varying cross head speed (a) for Female (b) for Male

effect of varying strain rate on the slope of the load-deformation curve was prominent in case of compressive loading also.

SEM images (Fig-8) of the fractured surface both under compression and tension showed the deformation of osteons very clearly. In case of compressive fracture, the osteons deformed to such an extent that the aspect ratio of the osteons reached about 1:2. But in case of tensile fracture, osteons were seen to be protruding from the surface like tubular structures without much change in the aspect ratio. Again, fractography showed the fracture in the cementing zone appeared to be comparatively smooth or cleavage in nature in contrast with fibrous fracture in lamellar zones. As observed in case of porosities, for other mechanical properties also, there is a high chance that their values will differ when the whole bone is considered than when different parts are considered separately.

Hardness in the cortical part along the femur length did not vary significantly. But values of the indentations over the lamella were little higher (around 0.38 GPa) than those in the cementing zone (0.33GPa). The hardness of the cortical part of female bones was found to be lower as compared to males. Not much difference could be observed in longitudinal and transverse values. More or less it was observed that the microstructural features like lamella and interstitial zone had some effect on the hardness and also on crack propagation. Also the typicality in fracture and deformation of osteons under various types of loading had been identified quite clearly but the quantitative estimate of their (collagenous lamella or mineralised interstitial zone) individual contribution in controlling load-deformation as well as minute fracture events is yet to be understood in a comprehensive manner. It needs controlled set of experimentation, that are being done at present.

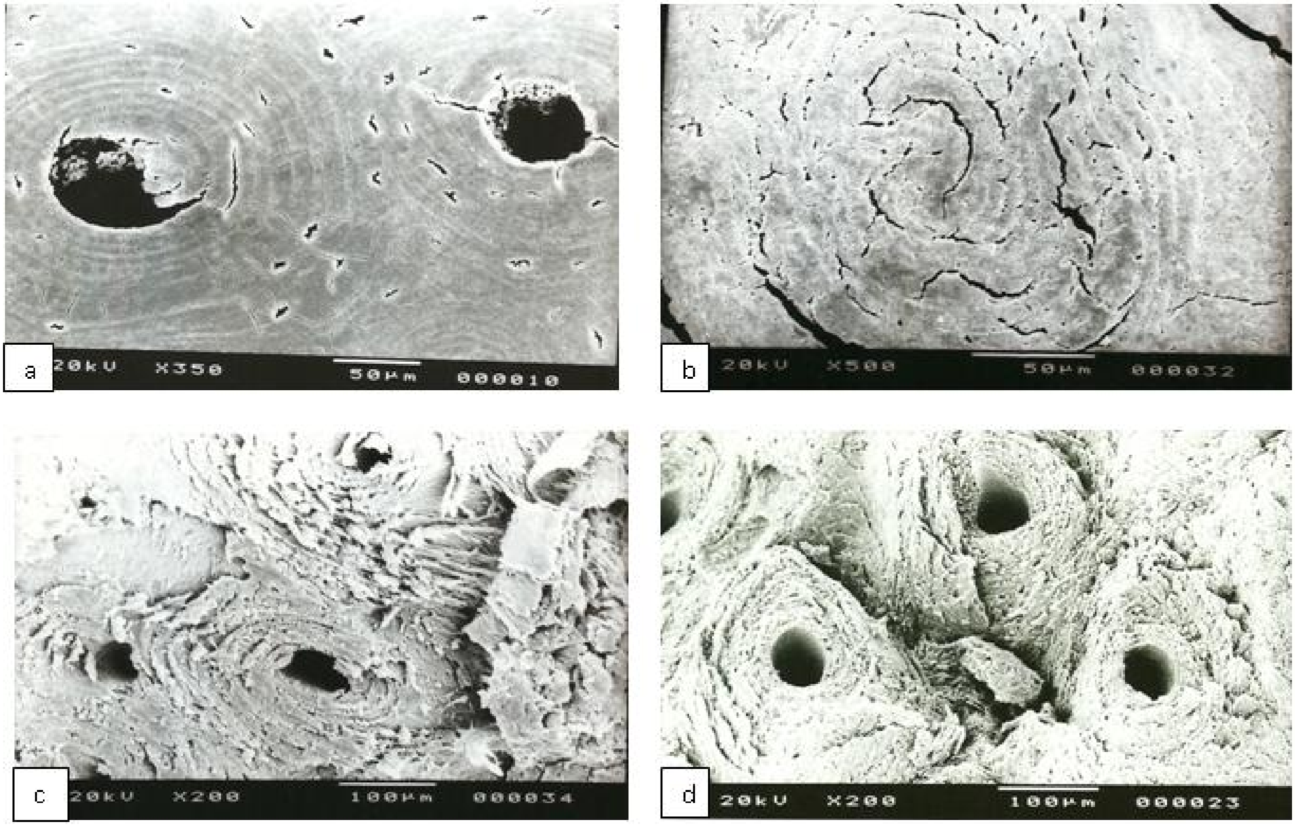


Fig. 6. SEM images of (a) undeformed osteons (b) Cracks developed along the lamellae well as across it.(c) Surface under compressive fracture (d) Surface under tensile fracture

4 Conclusion

From the study done so far, it has been observed specifically that the osteons suffer different types of damage and fracture in tensile and compressive loading. More deformation of microstructure was found in compression while in tension, there were more signs of shearing and tearing in-between the osteons. From the load-deformation curves it was evident that both male and female bones behaved similarly before yield point but post yield characteristics were different. It may be attributed to more energy absorption capacity of female bones. Although the lamellar structure is known to provide ductility to bone, cracks propagating across the lamella under high stress may be indicative of some inherent brittleness of bone. Finally it may be noted that mechanical behavior of only the mid-shaft cortical part is considered here for detailed study, the other portions are also to be studied before we make any generalization about the micro-structure-property relationship of bone.

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