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NANOINDENTATION MEASUREMENT ON MECHANICAL PROPERTIES OF OSTEOGENESIS IMPERFECTA (OI): A REVIEW

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ABSTRACT

Nanoindentation was commonly used to examine the intrinsic (microstructure level) mechanical properties of OI human bone tissue. The findings that available will describe bone material properties with this disorder. This review presents an overview of nanoindentation measurement on Osteogenesis Imperfecta (OI) across OI type, patient age, structure of study and anatomy site. Nanoindentation was capable of probing the mechanical properties of volumes of tissue as small as lamellae. In this technique, an indentation test was performed with a depth sensing indenter tip to measurement. The force displacement results were analyzed to obtain the modulus and hardness. The results of this study indicate that severity of OI type and microstructure level (interstitial and osteonal) had a significant effect on modulus and hardness. While, the age of patient, structure of study, and anatomy site shows non-significant findings were observed in all the measurement for both cortical and trabecular bones.

Keywords: nanoindentation, osteogenesis imperfecta, bone moduli, bone hardness.

1. INTRODUCTION

Bone has a different arrangement of material structures at different scales. The composition of bone enables it to perform unique mechanical, protective, and homeostatic functions. As shown in Figure-1, the hierarchical structure of bone was usually divided into five levels: (1) the macrosturcture: cortical and cancellous bone; (2) the microstructure (from 10 to 500 µm): Haversian systems, osteons; (3) the sub-microstructure (1-10 µm): lamellae; (4) the nanostructure (from a few hundred nanometers to 1 µm): 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]-[3]. Based on the differences in the composition of human bones, the study of mechanical properties different according to the level of the study in the bones as shown in Figure-1 [4]. To obtain a more accurate bone mechanical properties, the study was conducted at an intrinsic level (microstructure level). Using nanoindentation methods, nano-level studies can be carried out as conducted by previous studies under a few hundred nanometer to 1µm (nanostructure) [5] [6].

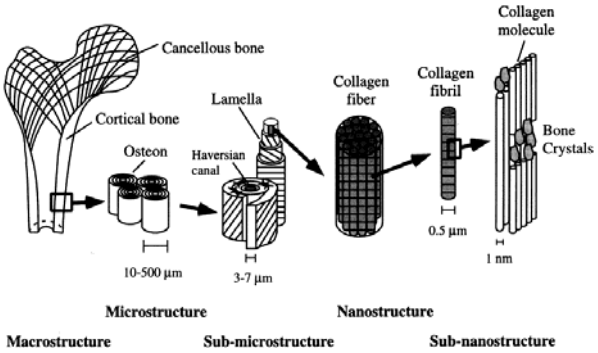

Figure-1. Hierarchical structural organization of bone from [1].

In the nanostructure level, the main constituents of bone are collagen type I and a mineral phase best defined as crystalline apatite. The mineral has been suggested to provide mechanical rigidity and load bearing strength to the bone composite. The organic matrix, predominantly type I collagen, provides strength and flexibility to bone and also determines its structural organization. The mechanical properties of bone are dependent upon the properties of these constituents. Osteogenesis imperfecta (OI) is predominantly caused by various mutations in type I collagen genes (COL1A1 and COL1A2). Both of the production and collagen assembly are affected. The consequence is either a decrease in collagen content or production of an abnormal collagen. OI was frequently classified through Sillence's classification into four major types [4], [7]. The classification is shown in Table-1.

Table-1. OI classification [4], [7].

Type	Form	Inheritance	Typical clinical features
I	Mild	AD*	Blue sclerae, mild pre-pubertal fracture, little or non-deforming, mild short stature
II	Perinatal lethal	AD*	Perinatal - lethal, extreme bone fragility, marked long bone deformity
III	Progressive deforming	AD*, AR*	Severe bone fragility, progressively deforming bones, very short stature, normal sclerae
IV	Moderately severe	AD*	Mild to severe bone fragility, moderately deforming, variable short stature, normal sclerae

*Note: AD: autosomal dominant; AR: autosomal recessive.

For the past decades, researcher provided evidence that the abnormalities occur in both mineral content and the organic matrix [8], [9]. Like other composite materials, bone mechanical properties are dependent on the components and their arrangement. The histological and morphological alterations observed in OI bone correlate well with clinical severity [8], [9]. OI type I and type IV, the mild types, showed the least abnormalities in bone ultrastructure. OI type II and type III show many varied abnormalities such as increased numbers of osteoclasts and osteocytes, thin osteoid with thin collagen fibrils, and patchy mineralization. These observations suggest that the mechanical properties of OI bone are degraded, since both collagen network and mineral are abnormal. Although OI bone has been known for its poor quality, the knowledge of intrinsic mechanical properties has been limited due to the lack of appropriate testing techniques. It is unclear that the intrinsic (microstructural level) mechanical properties of OI bone have been degraded.

Throughout this preview, nanoindentation technique was used to measure the intrinsic mechanical properties of OI bone. Nanoindentation is a technique used to probe a sample's intrinsic mechanical properties with 1 μm localization resolution and nanolevel load resolution [10], [11] [12]. This capability makes it possible to obtain intrinsic mechanical properties at the lamellar level without structural influences, therefore, nanoindentation measurements are representing usually considered as the intrinsic mechanical properties of a material.

2. MATERIALS AND METHOD

This review presents an overview of the nanoindentation method to assess bone intrinsic mechanical properties. The method starts from specimen category, specimen preparation and procesure.

A. Specimen category

There are several types to identify the specimen category as listed in Table-2; which are (1) OI type, (2) patient age, (3) structure study and (4) anatomy site. Normally, bone specimens were collected from young individual with OI and the donor were paediatrics patient. From Table-2, the study was concentrated on OI type III [13]-[16] and compared with type I [13] and IV [16]. Unless Imbert *et al*. [17] did not mention the type of study conducted.

The study is different when meeting different specimen category of patient age. The age range was started with 1.9 year to 18 years [13]-[17]. Albert *et al*. [13] conducts studies from 5 to 8 years of age. In contrast to Zaifeng *et al*. [14], it was conducted at the age of 3.2 to 12.4 years. Imbert *et al*. [17] also tests the age of OI patients from 4 to 16 years. The youngest age is Fan *et al*. [15], [16] that study the age between 1.9 to 13.2 years old.

The study also carried out different factors involving structural of study. Albert *et al*. [13] studies only the lamellar structure where it involves interstitial and osteonal. For cortical and trabecular, Zaifeng *et al.* [14] and Fan *et al*. [15] were examined on the same structures. Only Imbert *et al*. [17] was investigated on cortical structure. Fan *et al.* [16] conducted studies on lamellar and trabecular.

For anatomy site, studies were focused on femur, tibia and Illiac crest. The study of femur was investigated by Imbert *et al*. [17] to obtain the bone mechanical strength. The related studies were conducted on femur and tibia of anatomy location by Albert *et al.* [13] and Zaifeng *et al*. [14]. Numerous location of anatomy were examined by Fan *et al*. [15], [16] on femur, tibia and Illiac crest. The anatomy site is one important factor in the study due to mechanical strength.

Table-2. Specimen category.

Authors	OI type	Patient age (year)	Structure of study	Anatomy site
Albert *et al*. [13]	I & III	5 - 18	Lamellar (Interstitial & osteonal)	Tibia, Femur
G.F.H *et al*. [14]	III	3.2 - 12.4	Cortical and trabecular	Tibia, Femur
Fan *et al.* [15]	III and IV	2.1 - 9.8 (type III) 1.9 - 13.2 (type IV)	Cortical & trabecular	Femur/Tibia, Illiac Crest
Fan *et al*. [16]	III and IV	2.1 - 9.8 (type III) 1.9 - 13.2 (type IV)	Lamellae and trabecular	Femur/Tibia, Illiac Crest
Imbert *et al.* [17]	Not mentioned	4 - 16	Cortical	Femur

B. Specimen preparation

Table-3 shows the process of preparation of specimens that include the way of preparation that is divided into the number of specimens, dehydration and specimen surface treatment. To begin this process, the specimens bone sample were cut into cross-sectional area by using a diamond saw under constant water irrigation accord-ing to the required size. The number of specimens varies considerably from previous studies. Albert *et al.* [13] reports that used 6 specimens of type I and 5 specimen type III. However, Imbert *et al*. [17] and Zaifeng *et al.* [14] used 8 number of specimen to the same type. In addition, Fan *et al*. [15], [16] used 4 specimens of Type III and 6 specimens of type IV.

After cutting process, the specimens were fixed and dehydrated in graded ethanol solutions. Albert *et al*. [5] was used the ethanol to dehydration the specimen with different time immersion (range 2h to 24h) and percentage level (70%, 80%, 95% and 100%). Other researcher, however, used a dehydration method for specimen but not mention the detail graded ethanol solution [14]-[16] .

On completion of dehydration, the specimens were air-dried using air vacuum un-der room temperature and polymerization. After polymerization, this specimen was through surface treatment. According to some studies, surface treatment is important to produce smooth surface before conducting nanoindentation test [13]–[17]. Albert *et al*. [13] using grit size (400, 600, 800 and 1200), rough polishing using 3 µm aluminum oxide coated disc and completion with fine polishing 0.05 µm alumina suspension. Zaifeng *et al*. [14] used a grit size (600, 800 and 1200) and completed with fine polish-ing 0.05 µm alumina suspension. Imbert *et al.* [17] used only grit size 2400 and rough polishing 1µm diamond powder. Fan *et al.* [15], [16] only mention about completion surface treatment using 0.1 µm alumina suspension. After the surface treatment process is completed, nanoindentation test may be performed on specimens.

Table-3. Bone preparation for nanoindentation.

Author	No. of specimen	Dehydration	Surface treatment		
			Grit size	Rough polishing	Fine polishing
Albert *et al*. [13]	6 Type I 5 Type III	70 - 100% of ethanol in 2 -24 hrs	400,600,800 & 1200	3µm aluminium oxide coated disc	0.05 µm alumina suspension
Zaifeng *et al.* [14]	8	Yes	600,800 & 1200		0.05 µm alumina suspension
Fan *et al.* [15]	4 Type III 6 Type IV	Yes	Not mention	-	0.1µm aluminium suspension
Fan *et al*. [16]	4 Type III 6 Type IV	Yes	Not mention	-	0.1µm aluminium suspension
Imbert *et al.* [17]	8	Not mention	2400	1µm diamond powder	-

C. Procedure

Once the specimen preparation were completed, the nanoindentation test was per-formed using a Berkovich diamond indenter tip to measure elastic modulus, E and hardness, H. The measurements were obtained using Continuous Stiffness Measure-ment (CSM) algorithm method at low magnitude [13] and Oliver-Pharr method [14]-[17]. According to Albert *et al*. [13], frequency and amplitude were set at 45 Hz and 2 nm with a strain rate and depth limit of 0.05 s-1 and 2000 nm, respectively (refer Table-4). While Imbert *et al.* [17] was indicated similar parameter in strain rate and depth limit. However, Zaifeng *et al*. [14] and Fan *et al*. [15], [16] did not mention about the parameter of nanoindenter. As in previous studies, Poisson's ratio was assumed to be 0.3 for the bone specimen [13]–[17]. The number of indent should be greater than or equal to 16. This is due to get the range of indentation depth with approximately constant [13]-[17].

Table-4. Parameter of nanoindentation.

Author	Indenter tip	Number of indent per specimen	Nanoindenter parameter
Albert *et al*. [13]	Berkovich	20	45 Hz freq, amplitude 2 nm, strain rate 0.05 s-1, depth limit 2000 nm
Zaifeng *et al*. [14]	Berkovich	16	Not mention
Fan *et al*. [15]	Berkovich	16	Not mention
Fan *et al*. [16]	Berkovich	16	Not mention
Imbert *et al.* [17]	Berkovich	25	Strain rate 0.05 S-1, depth limit 2000 nm

3. RESULTS AND DISCUSSIONS

This review summarized the results of young modulus and hardness as listed in Table-5. The results of the nanoindentation method described here provide the mechanical properties of OI bone based on different: (1) OI type, (2) patient age, (3) structure study and (4) anatomy site. Albert *et al.* [13] reports that the severity of type I (17.5GPa) and type III (16.3GPa) had a significant effect on modulus (P= 0.024). For the hardness, OI severity had a significant effect on bone tissue hardness (P= 0.003) where OI type III having lower hardness than OI type I. However, Imbert *et al*. [17] only finding young's modulus were significant (P = 0.024) between OI patient and control specimen (normal).

For the factor patient age, Fan *et al* [16] indicated that the P value 0.8423 for cortical modulus of age (1.9 to 13.2 years). This finding show not significant factor in explaining the differences in the measurement. The non-significant findings were observed in all the measurement for both cortical and trabecular bones.

For an investigation based on differences structure, Fan *et el*. [15] study for both cortical and trabecular bone. Modulus and hardness do not show any significant difference between OI type III and type IV using a T-test (0.05 significance level). However, Albert *et al*. [13] investigate in microstructure in interstitial and osteonal lamellar bone had a significamt effect (P<0.001), with osteonal bone having lower modulus than interstitial lamellar bone.

For both cortical and trabecular bone anatomy site, modulus and hardness do not show any significant difference between OI type III and type IV reported by Fan *et al*. [16]. The modulus of P value for cortical and trabecular were 0.7291 and 0.4113, respectively. While the hardness P values were 0.5929 (cortical) and 0.1215 (trabecular). However, the ratio of E/H shows marginally significant decrease for type III cortical bone (near significant P = 0.067) and a significant decrease for trabecular bone (significant P = 0.026).

In addition, Zaifeng *et al*. [14] is more concerned with orientation between longitudinal and transverse directions. The P value for modulus and hardness of 0.102 and 0.6622, respectively. The modulus and hardness were observed in an analysis of the cortical and trabecular samples. It indicated that no significant difference found for each sample between the two directions.

Of all the studies conducted, there is a significant part and non-significant part shown in the outcome. However, studies conducted vary depending on the variable: (1) OI type, (2) patient age, (3) structure study and (4) anatomy site. This shows that the findings of the previous study are very limited and can be disputed. This is because the results obtained from research studied vary even though their value does not show a significant difference. The results of previous studies can be used as benchmarks for future studies. Studied can choose the important effects of the results obtained from previous studies such as OI type. It is obviously an important effect in the study by giving mechanical properties to the bones.

Table-5. The results young modulus and hardness of OI bone.

Author	OI Type	Factor	Parameter measured		Remarkable findings
			Young's Modulus, E (GPa)	Hardness, H (GPa)	
Albert *et al*. [13]	I and III	Severity	Type I = 17.5, Type III = 16.3	Type I = 0.656, Type III = 0.602	Modulus: Severity, $E_I > E_{III}$ (P <0.05) Microstructure, (P < 0.001) Anatomic site, (P < 0.05) Hardness: Severity, $H_I > H_{III}$ (P < 0.05) Microstructure, (P < 0.001)
		Microstructure level	Interstitial = 17.5, Osteon = 15.32	Interstitial = 0.656, Osteon = -0.079.	
		Anatomic site	Femur = 17.53, Tibia = 18.93.		
Fan *et al*. [14]	III and IV	Microstructure level	Cortical; Type III = 19.67, Type IV = 19.19	Cortical; Type III = 0.70, Type IV = 0.66	Modulus; Cortical, $E_{III} > E_{IV}$ (P > 0.05) Trabecular, $E_{III} > E_{IV}$ (P > 0.05) Hardness; Cortical, $H_{III} > H_{IV}$ (P > 0.05) Trabecular, $H_{III} > H_{IV}$ (P > 0.05)
			Trabecular; Type III = 19.23, Type IV = 18.27	Trabecular; Type III = 0.65, Type IV = 0.62	
Zaifeng *et al*. [15]	III	Microstructure level	Cortical: 15.22 ± 1.94 (L) , 13.92 ± 2.76 (T)	Cortical: 0.42±0.04 (L), 0.43±0.05 (T)	Cortical vs. Trabecular; Modulus, (P > 0.05), Hardness, (P > 0.05) Longitudinal (L) vs. Transverse (T); Modulus, (P > 0.05)
			Trabecular: 13.60 ± 3.38	Trabecular: 0.42±0.06	
Fan *et al*. [16]	III and IV	Microstructure level	Cortical; Type III = 19.19, Type IV = 19.24	Cortical; Type III = 0.67, Type IV = 0.66	Cortical; Modulus, (P > 0.05) Hardness, (P > 0.05) Ratio E/H, (P < 0.05) Trabecular; Modulus, (P > 0.05) Hardness, (P > 0.05) Ratio E/H, (P < 0.05)
			Trabecular; Type III = 18.56, Type IV = 18.27	Trabecular : Type III = 0.68, Type IV = 0.62	
Imbert *et al*.[17]	Not mention	Severity	OI = 17.6 Normal = 20.5	OI = 0.65 Normal = 0.68	Modulus, (P < 0.05)

4. CONCLUDING REMARKS

For this review paper, it is about the use of nanoindentation as a way of gaining modulus and hardness on human bones. The scope of this framework review paper is using sample from patient disorder Osteogenesis Imperfecta from human bone. Previously, most of studies are more likely to perform against animals such as mice. This is because the sample of human bones is limited compared to animal bones. However, studies using human bones are very important to get the best results in the study.

This review addressed the experimental method of nanoindentation for assessment of bone young modulus and hardness. The results were analysis for characterization of bone mechanical properties between; (1) OI type, (2) patient age, (3) structure study and (4) anatomy site.

The results of previous study indicate that severity of OI type and microstructure of study (interstitial and osteonal) have a significant effect on modulus and hardness. While, the age of patient, structure of study, and anatomy locations show non-significant findings were observed in all the measurement for both cortical and trabecular bones. In addition, the orientation between longitudinal and transverse direction also not significant difference.

From these finding, the use of nanoindentation can give a more precise result based on modulus and hardness. However, in order to obtain a more accurate result, the specimen preparation and procedure should be carried out according to experimental method of nanoindentation. This is because the level of the study is smaller (microstructure level) and very sensitive. Taken together, these review also findings that the surface specimens and the use of chemicals against different

density specimens was influences the result of modulus and hardness.

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