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Nanoindentation as a means for distinguishing clinical type of osteogenesis imperfecta

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Abstract

Due to abnormalities in basic bone building components (collagen and mineral), the intrinsic material properties of Osteogenesis Imperfecta (OI) bone were hypothesized to be degraded and correlated to clinical types. Nanoindentation techniques were used to compare intrinsic mechanical properties of OI types III and IV bone. Young's modulus (E) and hardness (H) were measured using the Oliver–Pharr method. Analysis showed that no significant difference existed in Young's modulus and hardness measurements. However, the ratio of E/H exhibited a significant decrease for OI type III bone tissue. Since the ratio is proportional to fracture toughness, the decreased E/H indicates less fracture resistance for OI type III, which is consistent to the clinical observation. The results of this study suggest that nanoindentation may serve as a means to distinguish clinical types of OI.

Keywords: B. Elasticity; B. Hardness; C. Micro-mechanics; Osteogenesis imperfecta

1. Introduction

Bone has a varied arrangement of material structures at different scales. The composition of bone enables it to perform unique mechanical, protective, and homeostatic functions. As shown in Fig. 1, the hierarchical structure of bone is 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 submicrostructure (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].

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 [2,3]. Osteogenesis imperfecta (OI) is predominantly caused by various mutations in type I collagen genes (COL1A1 and COL1A2). Both the production and assembly of collagen are affected. The consequence is either a decrease in collagen content or production of an abnormal collagen. OI is frequently classified through Sillence's classification into four major types [4]. The classification is shown in Table 1.

The abnormal collagen network and mineral content have been reported in the literature. Many studies have provided evidence that the abnormalities occur in both mineral content and the organic matrix [5–10]. Like other composite materials, bone mechanical properties are

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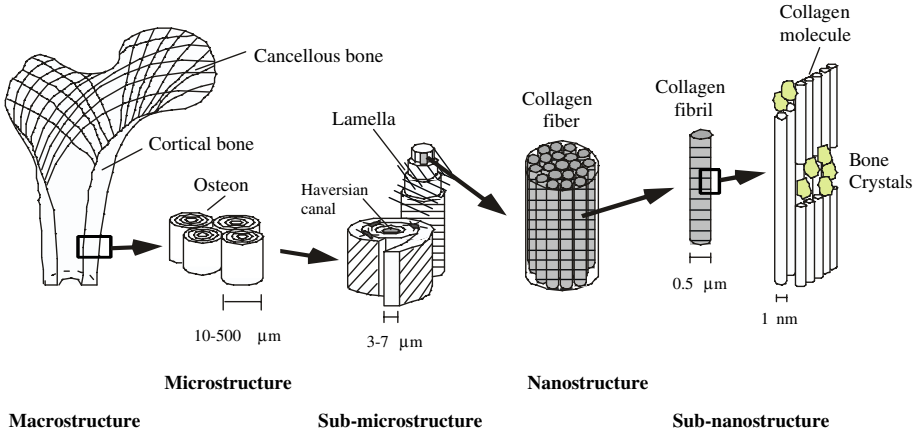

Fig. 1. The hierarchical structure of bone from [1].

Table 1
OI classification

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(rare)	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

AD: autosomal dominant; AR: autosomal recessive.

dependent on the components and their arrangement. The histological and morphological alterations observed in OI bone correlate well with clinical severity [5–10]. 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: (1) if the intrinsic (microstructural level) mechanical properties of OI bone have been degraded, and (2) how these mechanical properties correlate to the clinical types.

In this study, we hypothesize that the impaired collagen network and abnormal mineralization of OI bone degrade the intrinsic mechanical properties and correlate with clinical severity. To test his hypothesis, nanoindentation techniques are used to measure the intrinsic mechanical properties of OI type III and type IV bone. Nanoindentation is a technique used to probe a sample's intrinsic mechanical properties with 1 μm localization resolution and nanolevel load resolution. 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. Method

OI bone samples were harvested from ten (10) subjects (aged 1.9–13.2 years old) from Shiners Hospital, Montreal. Among the ten subjects, four (4) of them were OI type III (aged 2.1–9.8 years) and six (6) of them were OI type IV (aged 1.9–13.2 years). An iliac crest biopsy was obtained from each subject using the standard procedure. Biopsy specimens were fixed in 10% phosphate-buffered formalin (pH 7.1) and kept at room temperature for 48–72 h. They were then dehydrated in increasing concentrations of ethanol, cleared with xylene, and embedded in methylmethacrylate. After polymerization, the blocks were trimmed with an Isomet diamond saw (Buehler, Lake Bluff, IL) to remove excess plastic. After further polishing with a microcloth (using a 0.1 μm aluminum suspension) to produce a smooth surface for nanoindentation measurement, a Nanoindenter XP (MTS, Oak Ridge, TN) was used to conduct the indentation tests.

The Oliver–Pharr method was used to determine the elastic modulus and hardness [11]. During nanoindentation testing, the force required to press a sharp diamond indenter into the bone was measured as a function of indentation depth with nanometer resolution. The quasi static nanoindentation measurements were used to determine the bone's modulus, or stiffness, and its hardness. Measurements of load and displacement were used to determine the contact stiffness. The reduced modulus E_r was determined from the contact stiffness. The equations used to calculate the hardness (H) and the reduced modulus (E_r) are: