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STORAGE AND LOSS MODULI OF BONE IN OSTEOGENESIS IMPERFECTA (OI)

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INTRODUCTION

Osteogenesis imperfecta (OI), or brittle bone disease, is a genetic disorder of bone fragility affecting between 20,000 and 50,000 people in the United States [1]. Severity varies widely between individuals with OI. While the mildest form, type I OI, generally presents with few fractures, the most severe form in children surviving the perinatal period, type III OI, leads to multiple fractures and progressive skeletal deformities [2].

Bone fragility in OI results in part from a bone mass deficiency. Histomorphometric analysis of iliac crest biopsies has shown that children with OI have lower trabecular bone volume and thinner cortices than age-matched controls [3]. Impaired collagen network and abnormal mineralization have also been observed in OI bone [4], suggesting that compromised material properties may further contribute to bone fragility. Few studies have examined bone material properties in OI. Using nanoindentation, it was found that elastic bone modulus and hardness are higher in type III OI than in age-matched controls [5], while no significant differences were found between moderately severe (type IV) and severe OI (type III) [6]. These studies reported elastic moduli between 11 and 24 GPa for type III OI, and the reason for this wide range of values has not been explained. Finally, no studies have yet characterized bone material properties for the most common form of OI, type I.

The objectives of this study were to compare the storage (elastic) modulus, E', and loss (viscous) modulus, E", of cortical bone between individuals with type I and type III OI, and to examine how these properties vary between osteonal and interstitial lamellar bone regions.

METHODS

Under an IRB approved protocol (HR-2167), osteotomy specimens were collected from the femur or tibia of ten individuals with OI during routine surgical procedures. The donors were between ages 7 and 16 years. Five had type I OI, and the other five type III. The bone specimens were stored in a freezer (-70°C) prior to testing.

The specimens were cross-sectioned with a diamond saw (IsoMet, Buehler, Lake Bluff, IL) with the cross-section surfaces being approximately perpendicular to the long bone axis. The specimens were then dehydrated in ethanol and embedded in resin (EpoThin, Buehler, Lake Bluff, IL). The cross-sections were polished with a grinder-polisher (Metaserv® 3000; Buehler, Lake Bluff, IL), and the polished cross-sections were indented using a nanoindenter (Nanoindenter XP, MTS, Eden Prairie, MN). A continuous stiffness measurement method was used, in which a low magnitude oscillating force was superimposed onto a quasistatic force ramp, at a frequency of 45 Hz, amplitude of 2 nm, and a strain rate of 0.05 s^{-1}. The measured moduli, E' and E", were averaged between indentation depths of 800 and 1600 nm, a range over which these measurements were found to be approximately constant.

Twenty indentations were performed in each specimen. Using a reflectance microscope, lamellar microstructure was observed at each indent site and classified as being located in either osteonal or interstitial lamellar bone regions (Figure 1). Indents that were in contact with voids or that were not easily identified as osteonal or interstitial were excluded from the study.

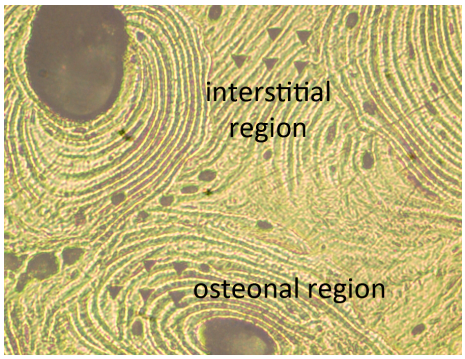

Figure 1: Typical nanoindentation sites (triangles): interstitial lamellar bone (top) and osteonal bone (bottom). This specimen was obtained from the femur of a 12 year-old female with type III OI.

A linear mixed effects model was used to assess the effects of OI severity (types I/III) and lamellar microstructure (osteonal/interstitial) on the moduli. Four covariates were explored: age, gender, site (femur/tibia), and history of bisphosphonate treatment (yes/no). Only covariates and interactions that were found to be significant ($p<0.05$) were included in the final statistical model.

RESULTS AND DISCUSSION

Anatomic site had a significant effect on E' only, therefore this covariate was included only in the final statistical model for E'. Other covariates and all interactions did not have significant effects and these were not included in either model. Results of the linear mixed models for E' and E" are shown in Tables 1 and 2.

Table 1: Linear mixed model results for E'.

	Coefficient (GPa)	SE	P value
(Intercept)	17.5	0.5	<0.001
Severity = OI type III	-1.5	0.6	0.01
Microstructure = Osteonal	-2.1	0.3	<0.001
Anatomic site = Tibia	1.2	0.6	0.04

Table 2: Linear mixed model results for E".

	Coefficient (GPa)	SE	P value
(Intercept)	0.82	0.02	<0.001
Severity = OI type III	-0.03	0.02	0.11
Microstructure = Osteonal	-0.05	0.01	<0.001

OI severity, microstructure and anatomic site had statistically significant effects on E' ($p<0.05$, Table 1). Only microstructure, however, had a significant effect on E" (Table 2). Mean E' was lower in individuals with type III OI than in those with type I by 1.5 GPa (9%). Mean E' and E" were lower in osteonal than in interstitial regions by 2.1 GPa (12%) and 0.05 GPa (6%), respectively. Finally, E' was higher in the tibia than the femur by 7%.

The results of the current study indicate that OI severity affects the ability of bone tissue to store energy under load, as denoted by E'. The ability of bone material to dissipate energy through viscous mechanisms, as denoted by E", however, does not appear to be affected by OI severity. The results also demonstrate that significant heterogeneity in material properties is present between regions of different lamellar structure, with osteonal regions having lower moduli than interstitial regions. This heterogeneity is likely attributed to variations in local degrees of mineralization between these regions.

Bisphosphonate treatment has become common in children with OI. In this study, patient history of such treatment did not have a significant effect on the moduli.

A limitation of this study is that nanoindentation does not provide fracture-related properties. Future research is therefore needed to determine how strength and toughness of bone tissue are affected in OI, and whether these properties are compromised by bisphosphonate treatment.

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