GROWTH-RELATED CHANGES IN BONE TISSUE MECHANICAL PROPERTIES

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Introduction

We recently demonstrated that fracture toughness, i.e. bone resistance to crack propagation, increases with skeletal growth [1]. When testing 4 and 14 week old (w.o.) mouse bones from healthy (WT) and brittle bone disease (osteogenesis imperfecta (OI)) we observed that initiation toughness increases with skeletal growth and that bone exhibits different toughening mechanisms during crack growth. Notably, for the first time in brittle bones, we observed crack growth toughness in very young (4 w.o. [2]) OI bone, being 2/3 of the one of WT counterparts at the same age, and that disappears in almost mature bones (8 w.o. [3] and 14 w.o. [2]). Disorganized collagen networks have often been suggested as contributing factors to the diminished fracture toughness in OI bone. When analyzing these bones with second harmonic generation we observed the presence of lamellar (L) and microlamellar (mL) regions in the WT bones at any age, and in OI bones at 14 w.o. [4]. In the 4 w.o. OI bones we observed the presence of only amorphous non-lamellar (NL) bone [4]. To date, little is known about the mechanical integrity of these different bone compartments and their changes during skeletal growth and in diseases. Here, we employ nanoindentation to determine the mechanical properties of these regions during bone growth. We focus on analyzing the medial and lateral sides of the femoral mid-shaft because crack grew in these sites in our fracture toughness experiments [1-2]. To note, according to the cortical drift, the medial and lateral side have different "bone growth".

Methods

Femora from 4 and 14 w.o. *oim/oim* (B6C3Fe-a/a-Col1a2$^{oim/oim}$) and their WT counterparts (N=6/group) were dissected, cut at the midshaft, embedded in PMMA, and the mid-shaft surfaces finely polished. Nanoindentation tests were performed on the specimens at room temperature in dry condition using a TI 950 TriboIndenter (Hysitron) equipped with a Berkovich tip. A loading profile with a maximum load of 8mN achieved in 10 s (loading/unloading rate 0.8mN/s) with a 30s holding period was used to obtain 9 indentations (10 µm distance) in the L, mL and NL compartments found in both the medial and lateral quadrants of each bone. The plane strain modulus (E') and hardness (H) were calculated from the unloading portion of the load-displacement curve using the Oliver-Pharr method.
Statistical significance between and within strains at different ages were assessed as well as differences between bone quadrants and compartments (L, mL and NL) within ages and strains were assessed.

Results

Results show a similar modulus in WT and *oim* at 4 w.o. bone, that increases with growth only in healthy bone at 14 w.o (Fig. 1). No differences were found in the modulus E' between medial and lateral quadrants between the groups. Between bone compartments, we found a reduced E' in the *oim* bone (18.7 ± 2.5 GPa) compared to WT (21.4 ± 2.7 GPa) at 14 w.o. ($p<0.05$).
Values for hardness show an increase in *oim* bone at any age, and with growth in both strains (Fig. 1). No differences were found in the hardness between medial and lateral quadrants in the bone groups. However, in 4 w.o. groups only, hardness increases in the non-lamellar bone of *oim* bone vs. lamellar bone of WT bone, and in the WT medial quadrant, mL region showed higher hardness in comparison to the adjacent L zones.

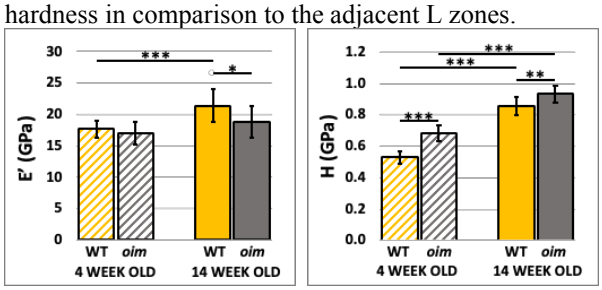

*Fig. 1: Means and standard deviations of plain strain modulus E' and hardness H for young (stripes) and almost mature (full) WT (orange) and oim (gray) bones. * indicates $p<0.05$, ** $p<0.01$, *** $p<0.001$.*

Discussion

This study reveals discernible differences in tissue mechanical properties of WT and *oim* bone during skeletal growth. Particularly, hardness is always higher in *oim* bone, and also increases with growth, indicating that there is less bone resistance to plastic deformations due to penetration in OI and almost mature bone, in agreement with mineralization and crosslinks changes we observed in these tissues [5]. The cortical drift and bone compartments have an effect on H in healthy and brittle young bone. These results are critical for the development of successful age-specific targeted treatments for OI bone fragility.

References

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