SITE-SPECIFIC CHANGES IN COLLAGEN ORIENTATION IN OSTEOGENESIS IMPERFECTA MOUSE BONE

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Introduction

In osteogenesis imperfecta (OI) mutations in collagen type I alter the whole bone hierarchical structure and composition, increasing its vulnerability to spontaneous fractures and fragility [1]. Bone fracture toughness of the OI murine (*oim*) model is significantly smaller than its healthy counterpart [1, 2]. We also recently showed that fracture toughness increases with skeletal growth in both healthy and *oim* mice, and that toughening mechanisms to resist crack propagation are different in young and older *oim* mice [3]. Disorganized collagen fibers have often been suggested the reason for the low fracture toughness of OI bone. However, to date it is not known if collagen orientation is site-specific in OI bone. To better understand the brittle behaviour of these bones, we here investigate the structural changes in collagen orientation in *oim* and healthy mouse bone with respect to anatomical directions using second harmonic generation (SHG) microscopy.

Methods

Tibiae from 14 week old *oim/oim* (B6C3fe-a/acolla2$^{oim/oim}$) mice model of OI and their wild type (WT) counterparts (N=5-6/group) were dissected, removed from soft tissue and cross-sections of 3 mm height were generated at 37% of tibial length using a slow speed saw machine with a diamond blade. Bone cross-sections were then glued on microscope slides and maintained hydrated with PBS.

SHG images of the bone cross-sections at their anterior, posterior, medial and lateral sides were collected at a fundamental excitation wavelength of 920 nm using a 40X 0.8 N.A. water immersion objective using a Prairie Tech. Ultima IV Multiphoton Microscope (Bruker; Madison, WI). Objective was focused on each tibia to achieve the maximum birefringence before capturing an image Collagen fibers degree of orientation (weighted-average), and distribution of orientation (second-order susceptibility ratio) were determined (OrientationJ-ImageJ (NIH)) as descriptors of the hierarchical organization of collagen.

Results

The orientation color surveys and histograms for representative sample of WT and *oim/oim* tibiae cross-sections at their anterior, posterior, medial, and lateral sites are shown in Figure 1. Color plots of the collagen fibers orientation show that in WT bone at each site the preferred orientation of collagen fibers are mostly parallel to the bone surface, while in the *oim/oim* bone there are smaller concentrations of fibers following the bone contour, mostly in the medial and lateral sites at the periosteum. Well organized WT collagen fibers along the tibia cross-section are also evidenced by the tall and narrow peaks of the red histograms. The *oim/oim* tibia generally has not well organized collagen fibers, as shown by the small and wide peaks of the green histograms, except in the medial side (showing a tall and narrow peak in the green histogram).

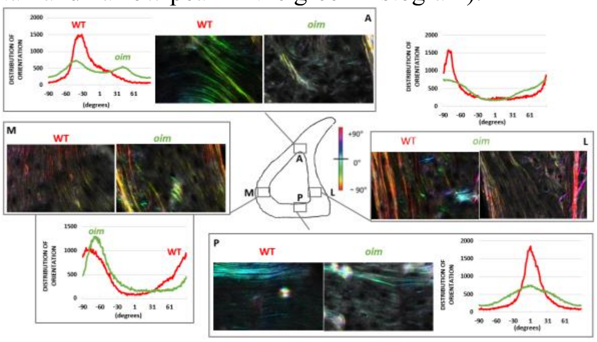

Figure 1: Degree and distribution of collagen fiber orientation for the anterior (A), posterior (P), medial (M) and lateral (L) regions of WT and *oim/oim* tibiae. Black box indicates region of interest for each site. Peaks of histograms for WT bone are higher and narrower than those of oim bone. FWHM values for *oim/oim* and WT are in A (79° vs. 34°), M (40° vs. 43°) and P (97° vs. 28°) sites for these representative tibiae.

Discussion

This work reveals the presence of site-specific orientation of the collagen fibers in OI bone. The presence of concentrations of well aligned collagen fibers in the medial and lateral sides of the *oim/oim* bone is important when considering the low bone strength and toughness of these bones. Because of the curved shape of the tibia, when loaded, its medial side is in tension, while its lateral side is in compression [5]. Thus, it seems that the *oim/oim* bone has optimized its collagen structure in an attempt to resist deformations and fractures at these sites of high tensile/compressive strains by having a concentration of well-organized fibers. Our future studies will further explore the relation between site-specific collagen fibers orientation and fracture toughness in *oim/oim* bone during skeletal growth.

References

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