Site-specific collagen orientation and organization in osteogenesis imperfecta mouse bone

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Introduction: Osteogenesis imperfecta (OI or *brittle bone disease*) is characterized by extreme bone fragility and spontaneous fractures [1]. In OI, mutations in genes encoding collagen type I or involved in osteoblast function and differentiation alter the whole bone hierarchical structure and composition resulting in brittle bone. We have shown that bone fracture toughness is significantly smaller in the OI murine (*oim*) model compared to its healthy counterpart [1,2]. Furthermore, toughening mechanisms to resist crack propagation are present in healthy bone but completely absent in the *oim* mouse model at 12-14 weeks of age [3]. Low fracture toughness of OI bone has often been reasoned by collagen fibers being disorganized in the matrix. In our previous work we have shown that degree and distribution of orientation as calculated with OrientationJ (plugin of ImageJ) in second harmonic generation (SHG) images are not good descriptors of collagen organization. Osteogenesis follows angiogenesis and even in healthy bones, the collagen fibers well-organize around the canals following their porosity to then reconnect to their endosteal and periosteal surfaces. In other cases, medial side of the bone shows a two-phase orientation most probably as a consequence of the cortical drift during growth [4]. In this study we have applied a new methodology developed with MATLAB in our lab [5] and quantified the site-specific collagen fiber orientation and collagen organization surface in the *oim* mouse bone.

Methods: Tibiae from 14 week old *oim/oim* (B6C3fe-a/acolla2$^{oim/oim}$) mouse model and their healthy (WT) counterparts (N=5-6/group) were dissected, cleaned of soft tissue and cross-sections cut at approximately 37% mid-diaphyseal tibial length with a 3 mm thickness using a slow speed saw machine with a diamond blade. Tibiae transverse cross-sections were then glued on microscope slides and polished to a final finish with 0.5 μm diamond suspension solution, and imaged using a Prairie Tech. Ultima IV Multiphoton Microscope (Bruker; Madison, WI) while being in PBS. Backward SHG images were attained through a titanium sapphire two-photon laser at 920 nm excitation wavelength for each of the four quadrants (anterior, posterior, medial and lateral) with a 40X 0.8 N.A. water immersion objective lens. For the quantification of collagen fiber organization, a custom image processing code was developed using MATLAB [5]. This program calculates the orientation of each pixel within the figure over 180 deg (Fig. 1 color maps). Then it decomposes the entire bone surface in facets of 32.83 μm^2 surface area and for each of them it calculates its mean orientation. Subsequently the connectivity of the collagen orientation of each facets with its eight neighboring quadrants is established within +/- 20 deg to determine the collagen orientation continuity over a surface. Finally, only surface regions with continuous well-organized collagen fibers that accounted for more than 1/6 of the total bone surface area are retained to quantify the overall collagen organization in bone [5].

Results: The collagen orientation maps and the bone surface of well-organized collagen surface of a representative WT and *oim/oim* tibiae cross-section at their anterior, posterior, medial, and lateral sites are shown in Figure 1. The collagen orientation maps show that in WT bone collagen fibers are oriented with each other and follow either the endosteal surface or the canal surfaces that are present within the cortical bone. In *oim* bone is not well organized and mostly follows the endocortical surface. When plotting bone surfaces with well-organized collagen, we found that anterior quadrants are more organized than posterior and that medial quadrants are slightly better organized than lateral. In WT we have an average organization of 73% vs. the *oim/oim* bone surface organization of 56%.

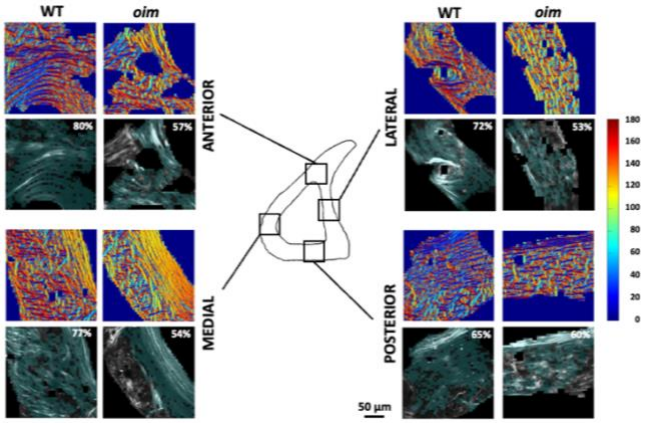

Figure 1. Site-specific collagen orientation in the color maps (0-180°), and the bone surface of well-organized collagen in blue color in the picture below the orientation map of a representative WT and *oim/oim* tibia.

Conclusions: A reduction in collagen organization in the *oim/oim* bones by a fourth can help explain their low fracture resistance and absence of crack-growth toughness in these bones at 14 weeks of age. The discontinuity and disruption in the collagen fibers organization in the patchy regions of the *oim* bone that are not highlighted in blue, may facilitate the crack propagation though the tissue with no resistance from the brittle bone.

References:

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