Age Effect On Bone Toughness In Osteogenesis Imperfecta

Anxhela Docaj[1], Minsup S. Jeong[1], Elizabeth A. Zimmermann[2], Robert O. Ritchie[3,4], Alessandra Carriero[1]
[1]The City College of New York, NY, [2]Shriners Hospital Montreal, QC [3]Lawrence Berkeley National Laboratory, CA [4]University of California Berkeley, CA
adocaj000@citymail.cuny.edu, acarriero@ccny.cuny.edu

Disclosures: All authors state that they have no conflicts of interests.

INTRODUCTION: Osteogenesis imperfecta (OI or *brittle bone disease*) is a heritable bone disease caused by mutations in the quality and quantity of collagen, which result in extreme bone fragility, skeletal deformities and spontaneous fractures [1,2]. Bone fracture toughness of the osteogenesis imperfecta murine (*oim*) mouse model of OI measured at 8 weeks of age is very small compared to healthy counterparts [1]. The *oim* bones have reduced resistance to crack initiation and propagation, as they are lacking toughening mechanisms, such as crack deflections and twistings, observed instead in healthy bone [1]. These deficient mechanical properties are due to alterations in the *oim* intracortical porosity architecture, and in the *oim* multiscale structure and composition [1]. The knowledge we currently have on the OI bone fracture toughness and its toughening mechanisms is, however, very limited. To date, it is still unknown how fracture toughness in OI varies during growth and development. This study therefore analyzes the effect of age on bone fracture toughness and its toughening mechanisms in the *oim* mouse model of severe OI (*oim/oim*) between 4 and 12 week old (w.o.) – i.e. 0.5 and 25 year old in humans.

METHODS: Bones from the *oim/oim* mouse model of OI (B6C3fe-a/acolla2$^{oim/oim}$) at 4 and 12 w.o. and their WT counterparts (N=7-11/group) are considered to characterize the toughness and crack propagation of OI bone during growth. Prior to testing, femora were dissected from fresh frozen euthanized mice and wrapped in physiological solution (PBS) soaked gauze. For the fracture toughness analysis, right femurs were tested in notched 3-point bending at a displacement rate of 1 µm/sec in 37°C PBS bath. The toughness was calculated with the instability method to account for the contribution of the crack extension to the toughness [1]. To identify the dominant sources of toughness and their contributions to the bone's resistance to crack propagation in the *oim* mouse bone during growth, left femurs were polished on their periosteal surface and tested in notched 3-point bending using an in situ loading stage (Gatan, UK) within an environmental scanning electron microscope (Hitachi S-4300SE/N) used in backscattering mode at 25kV with a pressure of 35 Pa [1, 3]. Fracture toughness crack-resistance curves (R-curves) were determined in terms of the stress-intensity factor, K, characterizing the toughness as a function of crack extension. Results for the 4 and 12 w.o. mice were compared to the data from 8 w.o. mice presented in our previous study [1]. Levene's and the Shapiro-Wilk's tests were used to analyze variables' homogeneity of variance and their normal distribution, respectively (SPSS, NY). *Oim/oim* bone toughness was compared to WT using analysis of variance (one-way independent ANOVA). Variables were tested within the *oim/oim* and WT groups during growth using post-hoc procedures for multiple comparisons. All tests were two-tailed and p-values<0.05 were considered to be significant.

RESULTS: *Oim/oim* bone at all ages showes a dramatic decrease in toughness compared to their WT counterparts. Specifically, a 67% and 50% decrease in toughness is observed in the 4 w.o. and 12 w.o. mice, respectively (Fig. 1A). Similar results were found in our previous study on 8 w.o. mice [1]. During growth and development, *oim/oim* bone showes similar toughness at 4 and 8 w.o., which significantly increases being more than double at 12 w.o.. Healthy bone also increases its toughness during growth, with the toughness significantly increasing by 35% and 54% from 4 to 8 and 12 w.o., respectively. R-curves for the growing stable cracks in 4, 8 and 12 w.o. mice from both OI and WT samples presented in Fig. 1B (in terms of stress-intensity factor, K, as a function of the stable crack extension Δa) reveal a loss in stable crack extension, crack initiation and crack-growth toughness in the *oim/oim* bone that improves with age.

DISCUSSION: The increase in fracture toughness in both *oim/oim* and WT bones with the age is in agreement with the reduced fracture rate observed in adults with OI [4] and suggests that changes in the intracortical porosity and/or tissue structure and composition are naturally occurring during growth in bone, both in healthy and disease. Our future studies will examine the high-resolution real-time ESEM images of the crack propagation during mechanical testing to compare changes in the crack path at different ages in relation to changes in the R-curves. Further multiscale studies will also help explain differences in bone toughness with age in OI and/vs. WT.

SIGNIFICANCE/CLINICAL RELEVANCE: Knowing how bone toughness changes in OI during growth will allow to develop targeted clinical therapies that effectively restore their mechanical resistance at all ages. Examining healthy counterparts will inform on how bone toughness naturally changes during growth providing targeting values for bone therapies. Our findings will have implications for developing effective clinical therapies targeting bone fragility at different ages, not just in OI.

REFERENCES: [1] A. Carriero, et al., J Bone Miner Res 29(6) (2014) 1392-401
[2] A. Carriero, et al., Bone 61 (2014) 116-24
[3] A. Carriero, et al., J Mech Behav Biomed Mater 39 (2014) 38-47
[4] L. Folkestad, et al., J Bone Miner Res 32(1) (2017) 125-134

ACKNOWLEDGEMENTS: The authors thank the National Science Foundation (Award 1829310) for sponsoring this study.

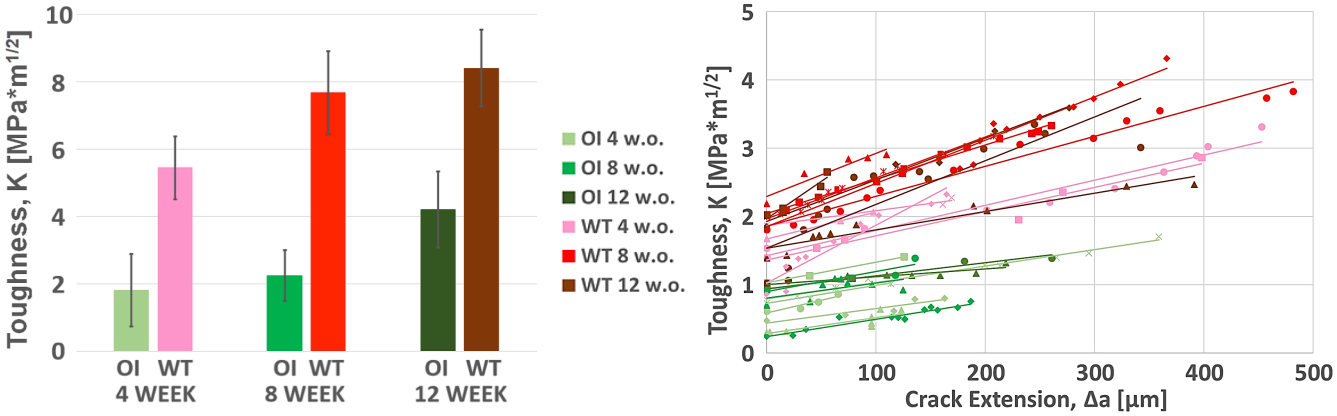

Figure 1 *Bone toughness* ***(A)*** *and crack-resistance curves* ***(B)*** *for healthy (red tonalities.WT) and severe OI (green tonalities.oim/oim). Fracture toughness R-curve properties at different ages show a reduction in the stable crack growth and initiaton toughness (at Δa =0) in oim/oim bones vs. WT, which increase with the age. Crack-growth toughness (slope of R-curve) is high in WT and almost nonexistent in oim bones, regardless of their age.*