Multiscale characterization of bone mechanics: what is the role of TIMP3?

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ABSTRACT

Bone's strength and toughness originate form its organised hierarchical structure and composition. Every element constituting the bone may therefore dictate its mechanical and material properties. Recent studies have emphasized the importance of TIMP3 gene for the proper formation of mechanically competent cartilage and bone. TIMP3 is an inhibitor of several matrix metalloproteinases that regulate the generation and remodelling of bone and cartilage. Mice models of TIMP3 deficient (KO) develop spontaneous osteoarthritis, collagen degradation and abnormal bone growth with a delayed formation of secondary ossification centres in long bones, resulting in shorted bones [1]. Nevertheless these studies, it is still unclear the influence of TIMP3 on the mechanical and material properties of the bone. This multiscale study aims to reveal the role of TIMP3 on the mechanical integrity of the bone.

At the whole bone level, mechanical strength of the TIMP3 KO and control (WT) bone was characterised by loading humeri and L5 vertebrae in 3-point bending and compression, respectively (Instron). Toughness of the bone was examined by loading in 3point-bending mice femora, previously notched on their posterior surface at the mid-diaphysis [2]. At the nanoscale length, collagen fibril and mineral mechanics of the bone were obtained by tensile loading the ulnae with simultaneous SAXS/WAXD data collection (Beamline I22, Diamond Light Source, Oxford, UK) [2]. Bone microstructure properties were defined by imaging cross-sectional surface area into an environmental scanning electron microscope (ESEM – JOEL).

TIMP3 KO bones exhibited reduced mechanical properties and reduced elastic energy and yield stress. They also exhibited a more brittle behaviour with the coefficient of toughness *k* of TIMP3 KO bone being significantly lower than for WT bone. At the nanoscale, a reduction of the collagen fibril/tissue strain of TIMP3 KO bones was observed in the elastic region.
High-resolution ESEM images of bone cross-sectional areas showed that TIMP3 KO bones are smaller compared to WT bone. We believe that also bone composition is altered in the TIMP3 KO bones and our future studies will examine it.

This multiscale study reveals for the first time the functional importance of TIMP3 gene in determining and maintaining the mechanical integrity of bone. Examining the structure, composition and mechanics of bone at its multiscale length is fundamental to understand the role of single genes in determining bone strength and toughness.

REFERENCES

[1] S. Sahebjam, R. Khokha, J.S. Mort. *Arthritis Rheum*, Vol. **56**, pp. 905-9, (2007)

[2] A. Carriero, E.A. Zimmermann, A. Paluszny, S.Y. Tang, H. Bale, B. Busse, T. Alliston, G. Kazakia, R.O. Ritchie, S.J. Shefelbine J Bone Miner Res. Vol. **29**, pp.1392-401 (2014)