ANALYSIS OF THE APATITE CRYSTAL STRUCTURE OF BRITTLE AND DUCTILE BONES

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Introduction

The structural integrity of bone and its mechanical response are conditioned by the bone quantity and quality. Bone quality depends on the hierarchical structure and mechanical, structural and compositional properties at different length scales. In order to explore how compositional and structural differences at the collagen/apatite level influence whole bone mechanics, we examined the apatite crystal structure of mice models exhibiting tough and brittle behaviour using a novel combination of X-Ray Diffraction (XRD) and Thermogravimetric Analysis (TGA) techniques.

Methods

Osteogenesis Imperfecta Murine (OIM) mice have very brittle bones [Camacho, 1995; Vanleene, 2012]; Phospho Knock-Outs have ductile bones [Huesa, 2011]. The right femurs of six OIM/OIM and Wild Type (WT) mice and the right and left femurs of three Phospho KO and Phospho WT mice of 7 weeks were defatted, dehydrated in series of increasing concentrations of ethanol and ground in acetone. XRD was used to assess the average crystal size [Boskey, 2003]. TGA to 800°C measured the mineral to matrix ratio, calculated as the ratio between the percentages of mass remaining after heating to 600°C and the organic mass loss between 200°C and 600°C [Peters, 2000]. A second XRD analysis was done in order to evaluate the thermal decomposition of the bone mineral after TGA [Rogers, 2002].

Results

XRD measurements of the bones indicated that the diseased bones had smaller crystal sizes than their controls (about 29% smaller for OIM/OIM mice and 7% for Phospho KO). The mineral to matrix ratio of OIM bones was smaller than their controls' (1.7 ± 0.2 for OIM/OIM and 2.7 ± 0.1 for OIM WT), whereas no difference was found for the Phospho bones. The initial XRD patterns did not capture differences between strains. However, XRD analysis after heat treatment at 800°C revealed that controls evolve to crystalline hydroxyapatite; while in the case of diseased bones, the mineral decomposes in HA and B-tricalcium phosphate [Fig. 1].

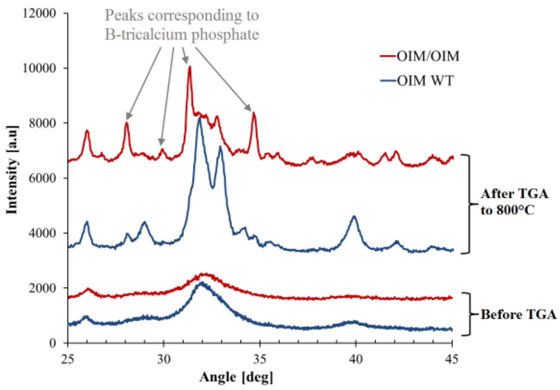

Figure 1: XRD patterns of OIM/OIM and OIM WT tibiae before and after the heat treatment to 800°C.

Discussion

Bones of OIM/OIM and Phospho KO mice have two extreme mechanical behaviours; however they show some similarities at the mineral level. The results indicate systematic differences in thermal decomposition between diseased and healthy bone, due to chemical deviations from the stoichiometric. This is in agreement with reports in literature that claim that healthy bone mineral tends to be more stoichiometric [Rogers, 2002]. XRD analyses of untreated bones do not provide information about the similarities or differences among the mice strains. This study demonstrates that the thermal decomposition of the mineral is significantly different for each strain, thus revealing subtle changes in mineral size and composition in healthy and brittle or ductile bone.

References

Boskey, Osteoporosis Int., 14:16–21, 2003.
Camacho *et al*, JBMR, 14(2):264–272, 1999.
Huesa *et al*, Bone, 48(5):1066–1074, 2011.
Peters *et al*, Thermochimica Acta, 361(1–2):131–138, 2000.
Rogers *et al*, Biomaterials, 23(12):2577–2585, 2002.
Vanleene *et al*, Bone 50(6):1317-1323, 2012.