Characterization of cortical bone demineralization by X-ray-based techniques: A micro and nano scale study.

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Bone is a composite material with hierarchical structures at different size scales and performs mechanical, biological, physiological and chemical functions [1]. Like all composite materials, each bone structure has a different but intimately related contribution to the overall quality of the tissue. Techniques that examine bone on the micro and nano scales can reveal information about individual lamellae and trabeculae as well as the architecture of mineral crystals and collagen fibers [2]. The structural properties of individual collagen fibrils and the single mineralized crystallites are key determinants of bone strength and altered tissue mineralization might be a transient characteristic of fragility [3]. Therefore, understanding the detailed micro and nanostructure of bone can assist in understanding why bone becomes fragile, can provide ways to assess the aged and diseased bones, and also in the development of next generation bio-inspired materials [1].

The aim of this study was to track the distribution of minerals in bovine cortical bone with different stages of in-vitro demineralization from the micro (10-500 μm) to the nano scale level (below 1 μm), and for this purpose Synchrotron radiation micro X-ray fluorescence spectroscopy [4] (SR-μXRF) and X-ray diffraction [1] (XRD) were used.

In total, 40 fresh cortical bone samples of different ages from 2 diaphyseal femurs were selected. They were obtained at several stages of a 4 days decalcification experiment with 2 concentrations of EDTA acid (0.1 M pH 10, and 0.5 M pH 7.4, at 25°C, without agitation). EDTA is a slow agent and permits better tissue preservation of bone specimens. Before and after demineralization, samples were embedded in epoxy resin and sectioned to about 200 μm thickness for SR-μXRF and XRD analysis. Results can help to determine what parameters contribute to decreased bone quality in diseased tissue.

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