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Evaluating Structural Differences in Cortical Bone Tissue After Demineralization and Calcination

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Although the best results in bone grafting have been achieved with autogeneuos bone tissue, allografts and xenografts have been widely used either in mineralized, demineralized, or calcined forms. Demineralized bone has been proven to stimulate new bone formation by exposing, proteins and growth factors necessary for osteoinduction. On the other hand, calcined bone offers a natural architectural mineralized matrix, not present in synthetic apatite materials, as well as an excellent source of calcium. Despite the extensive use and importance of these materials, systematic works regarding their characterization are relatively scarce.

The present work aims to investigate some of the characteristics of three types of cortical bone grafts: natural bone matrix (without any treatment, thus taken as reference), demineralized bone matrix (with two different demineralization degrees, 80% and 57% residual Calcium, resulting from immersion in HCl 0.6 M and 2.4 M for 24 hours) and bone matrix submitted to calcination at 700 ºC. All samples were from a human femoral diaphysis supplied by the Bone Bank of Coimbra University Hospital.

Morphological alterations were analysed by light microscopy and SEM-EDS. Chemical composition (FTIR), phase identification (XRD) and porosity (mercury intrusion) were also determined.

FTIR spectra (Fig 1 a)) clearly denote the differences in the samples chemical composition: as expected, the demineralized bone (organic matrix) exhibits a spectrum practically coincident with that of the Type I Collagen; also the peaks of calcined bone (mineral matrix) spectrum are identical to those of synthetic Hydroxyapatite; however a more detailed examination reveals that some bands of the natural bone spectrum are slightly shifted or have different relative intensities, resulting from intra and intermolecular interactions between Collagen and Hydroxyapatite.

As for the XDR results (Fig 1 b)), the diffractogram of the control sample reflects the low crystallinity of cortical bone as it presents typical peaks of Hydroxiapatite overlapped with a broad peak from Collagen. This Collagen pattern was found in demineralized samples and calcined bone was identified as Hydroxiapatite.

The plots of the volume of mercury intruded (corresponding to pore volume) versus pore diameter (Fig.1 c)) indicate that the control sample exhibits very low porosity, as expected, since it is a compact bone. The demineralization process considerably increases sample porosity (e.g. for HCL 2.4 M the increase in porosity is about 80 %) but it is the calcination process that originates more pores, specially in the smaller pore region, corresponding to an increase of nearly 400%.

Finally, SEM-EDS allowed the determination of the composition (chemical elements) of selected points of the sample enabling, for instance, to confirm the absence of Calcium in the demineralized bone (Fig 1 (d)).

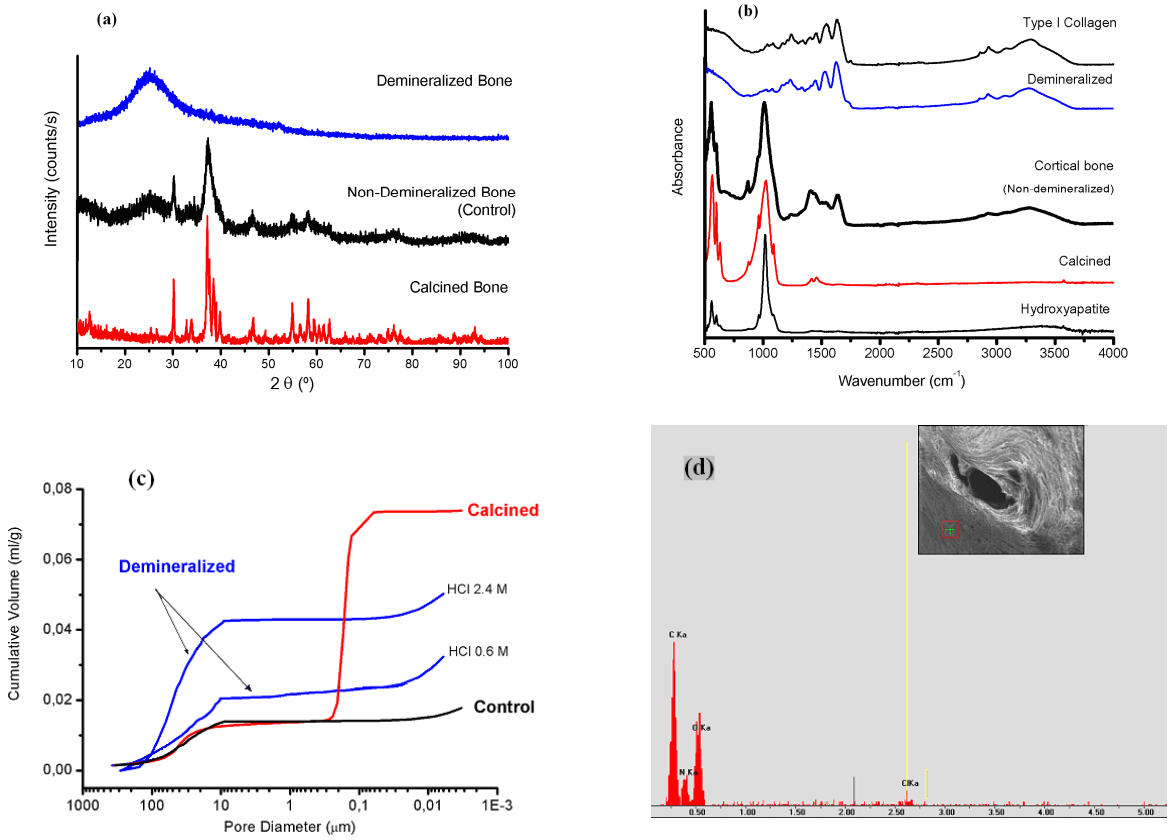

Figure 1 – Results from XDR (a), FTIR (b) and porosimetry (c) of the tested samples; SEM –EDS of demineralized bone (d).

The microscopical observations reflect the effects of the different treatments to which samples were submitted. Although the basic micro-structure of cortical bone matrix (osteons) was preserved in all samples (Fig 2), in demineralized bone most Haversian systems present a structural deformation with irregular and increased channel diameter. On the other hand, calcined samples exhibit numerous micro-cracks and an accentuated surface roughness.

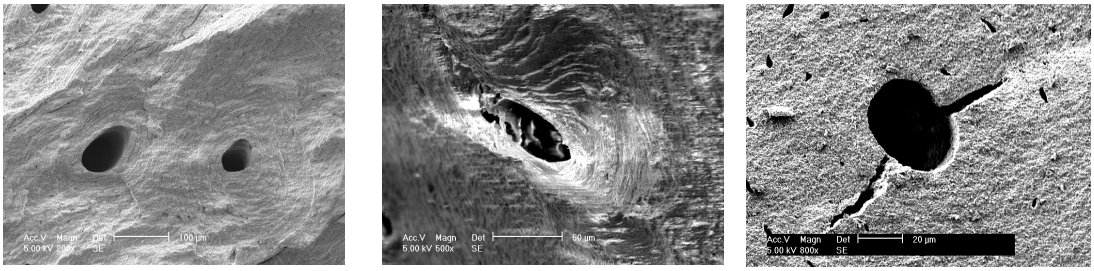

Figure 2 –From left to right: SEM of the control, demineralized and calcined cortical bone matrices.

It is believed that the combined use of microscopical techniques and mercury porosimetry will provide most useful information about bone structure in both qualitative and quantitative terms.