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TEM investigations on the pathological biominerals of the valvular tissues of the human heart

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The growth of pathological mineral deposits in human heart valves, better known as calcifications, is an important problem for the human health because it represents the leading cause of failure of natural and bioprosthetic heart valves [1]. From a mineralogical point of view this pathological deposit is a bio-mineralization constituted by calcium phosphate biominerals. The knowledge of such deposit is still limited and the formation mechanism is poorly understood. Electron Microscopy was used to investigate, from the micrometer up to the nanometer-scale, the inorganic phase grown in different types of calcified human cardiac valves collected as waste from different patients undergoing valvular replacement. Previous SEM-EDS analyses at micrometer-scale [2,3] revealed the presence of deposits constituted by unusual spherical particles with variable size (1-3μm in diameter) and different agglomeration degree.

Morphology at nano-scale, crystallinity and elongated direction of the grown crystals were investigated by TEM using a Jeol JEM 2010 (point-to-point resolution of 1.9 Å) operating at 200KV. Powdered samples, previously treated with trypsin to remove the organic component were ultrasonically dispersed in ethanol. Subsequently, few small drops of the slurry were deposited onto a 3 mm diameter Ni-Cu grid coated by a holey carbon film. The complete removal of the collagen fibrils from the samples was impossible, thus the Anti-Contamination Device (ACD) was used to prevent the contamination of the column due to decomposition of the residual collagen and to minimize the effect of irradiation. Bright Field (BF) and lattice fringe images, Selected Area Electron Diffraction (SAED) and Nano Beam Diffraction (NBD) patterns of nano-crystals were recorded on Kodak films. EDS spectra were also collected.

BF images reveal a dense packing of organic matrix and nano-hydroxylapatite crystals that form randomly distributed aggregates occasionally arranged in a fan-shaped configuration. In several cases, the thick bundles were difficult to separate into individual crystals and appear much darker. The detected nano-crystals appear to have a platy morphology elongated mainly along the c axis. Significant differences about the crystal size of the samples can be noted although the shortage of individual nano-crystals. The crystal size, determined for a tricuspid aortic valve, covers the range from 10 to 30 nm in length while the values determined on the basal section {0001} ranging from 50 to 60 nm. The crystal size range determined for a bicuspid aortic valve appears to be wider, from 40 to 300 nm in length; the bundles of nano-crystals show a lower density and several needle-like isolated crystals can be observed. The mitral valve showed a thicker inter-growth between inorganic-organic matter, thus none individual crystal was observed.

Finally, NBD patterns and lattice fringe spacings of crystals were indexed by comparing their d spacings and interplanar angles with calculated values of hydroxylapatite, confirming an hexagonal unit cell (space group P63/m). Precursors of CaOH or additional phases were not found as well as spherical amorphous forms.

[1] RF Weska et al. (2010). Artif Organs 34: 311-318; [2] A Maras et al. (2010) Acta Mineral. Petrogr. 6: 374. [3]A Maras et al. (2010). Plinius. 36: 513;