

ANALYTICAL AND CRYSTALLOGRAPHIC INFORMATION FROM THE TRANSMISSION ELECTRON MICROSCOPE FOR BONE MINERAL PHASES AND IMPLANT CHARACTERISATION.

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INTRODUCTION: Bone, implants and bone interfaces with soft tissue or implants constitute heterogeneous, sometimes highly porous, media. Mineral phases are most often below the micrometer range. Transmission electron microscopy (TEM) associated with electron diffraction and sometimes chemical microanalysis by X-ray energy dispersive spectrometry (EDS) or electron energy loss spectrometry (EELS) is the only route to identify unambiguously their nature.

METHODS: X-ray EDS spectrometry in a SEM is the most direct way and widespread method to chemically identify the nature of particulate matter. However, it is too often forgotten that quantification programs assume that electron and X-rays propagate in a bulky and single-phase material. Thus, a trade is required between an accelerating voltage high enough to ionise all the elements and still low enough to keep the interaction volume smaller than the object size. There is most often no solution to this contradictory constraint in biomaterials and quantitative analysis is not possible. For instance, hydroxyapatite in bone, blood vessel or scaffolds is constituted of thin platelets a few nm or tens of nm thick. Excitation of the Ca K-lines requires about 7 kV and detected P K-lines comes from depths down to 0.5 μm (fig. 1).

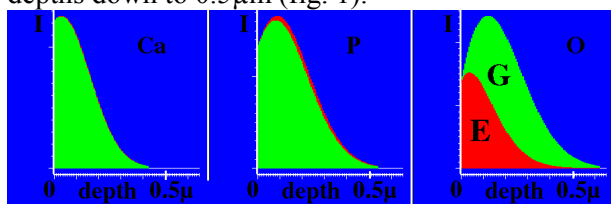


Fig.1: Distribution of generation G and emission E (after absorption) of Ca, P and O K-lines vs. penetration depth.

In TEM, the electrons leave the thin sample before multiple scattering occurs and the volume of interaction is bounded by cylinder whose diameter and length are the probe diameter (1-100nm) and sample thickness (10-200nm). The signal intensity decreases with volume and long measurement times are required together with very bright probes that lead to irradiation damage and element losses.

Spurious signals from electrons backscattered on remote areas and channelling effects also reduce accuracy. Distinction between octacalcium-, tricalcium-phosphate and hydroxyapatite with Ca/P atomic ratio 1.33, 1.5, 1.67 respectively, is unreliable. Diffraction lead to more reliable phase identification. Selected area electron diffraction on 0.1 μm sample surface diameters or microdiffraction with probes of a few nm in diameter supersede XRD (individual particle, link to direct images of the object, less background). High resolution (HRTEM) imaging in the TEM is another way to record crystallographic information. The unscattered transmitted beam and the diffracted beams are allowed to reach the microscope screen by using a large objective lens aperture and to interfere in an image that mimics the atomic crystal structure when optimum condition are found (sample thickness and objective lens defocus). If not, information is retrieved by comparison between micrographs and image simulations from models of the crystal and the microscope optics. Fourier transform of the HRTEM image gives patterns similar to diffraction patterns down to nm-sized areas [1]. The information in diffraction patterns and HRTEM images is complementary (amplitude and phase of the diffracted beams) and lead to unambiguous identification of the crystalline phases, but assessment of stoichiometry requires an accurate measurement of diffracted intensities that still remains a challenge.

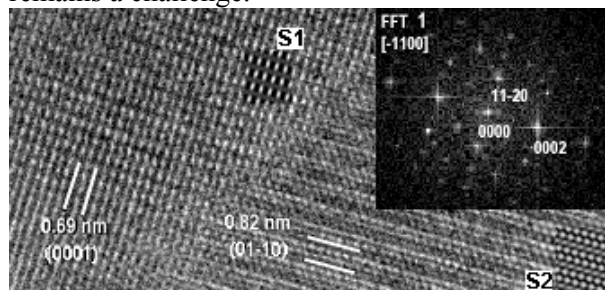


Fig. 2: HRTEM image, simulated images S1, S2, and Fourier transform identify these nanoparticles as crystalline hydroxyapatite, at contrary of XRD which concluded to an amorphous phase.

REFERENCES: ¹ E.I. Suvorova and P.A. Buffat (1999) J. Microsc. **196**:46-58