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Comparative tem study of dental tissue hydroxyapatite with chemically obtained apatite

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CONSTANTA STEFANOV[1], AURELIANA CARAIANE[1], V. CIUPINA[1], G. PRODAN[1], STELA ZAMFIRESCU[1], MADALINA PRODAN[1], IULIANA STANESCU[1], E. VASILE[2]
[1] Ovidius University Of Constanta, Mamaia Avenue No.124, 900527, Romania
[2] Metav Cd, Bucharest, C.A. Rosetti No. 31, 020011, Romania

Abstract

Development of biocompatible materials research is an evolving process, driven by increasing number of accidents and the average lifetime. Prosthetic devices is a replica that replaces a poorly functioning body part or through one sound, taken from another subject, either through an artificial device that would provide the same functions and to be made from a biocompatible materials. In general, biocompatible materials are substances other than food and medicines, which come into contact with tissue or other body fluids present. Placing material in the body determines interaction implant - tissue that can generate genuine conflicts. They can be toxic, mechanical, biological and electrochemical. It can be reached even in serious injuries such as bone and metal assembly used. Deposition of hydroxylapatite nanostructured on metals, will allow better osteointegration in addition to optimizing the wear resistance and dissolution rate. Hydroxylapatite (HA) is a bioactive ceramic with a crystalline structure similar to natural bone and tooth minerals. Material of great interest in dental research for implantable biological response because it results in a positive and adheres well to surrounding tissues. However applications are limited at present HA to powders, deposition, porous bodies and implants are not subjected to heavy loads, and because of difficulties obtaining the weak mechanical characteristics of conventional HA. Nanostructuring process applied to HA base materials is used to obtain the desired mechanical properties and improve responsiveness multifunctional implants used to replace the different hard tissues. Nanostructured HA can be obtained at low sintering temperature, which leads to a chemical homogeneity and a uniform structure throughout the mass of material, with a significant reduction of internal cracks.

In the paper we present a comparative study conducted between HA present in the test tooth and apatite produced by chemical methods. For the study we used a Philips CM120ST microscope techniques for material characterization, using TEM, SAED, PED, HRTEM. The study was complemented by EDX spectra. To investigate dental tissue, collected samples were included in Epon812 after dehydration and sectioned with Leica ultramicrotomului UltracutR using a diamond knife. For apatite nanopowders dispersion was done in absolute alcohol which was filed on grids coated with formvar support after an ultrasonication for 30 minutes.

Morphology observed in TEM images show the osteocytes location in dental tissue surrounded by hard tissue consisting of hydroxyl or fluorapatite. Compared apatite obtained chemically in the form of nanoparticles are evenly distributed around 15 nm. Electron diffraction has specific issues in both cases of polycrystalline materials. The structure of HA are relatively close to that of fluorapatite, so the EDX study was needed to identify the phases of the samples studied. High resolution images obtained are specific hydroxilapatite interference fringes.

Keywords: dental tissue, hydroxylapatite, TEM, electron diffraction, precession

Introduction

Biocompatible materials research development process is evolving, driven by increased number of accidents and the average lifetime. Prosthetic devices is a replica to replace a poorly functioning body part or through one sound, taken from another subject, or by an artificial device that would provide the same functions to be made from biocompatible material (DINH, [1]. In general, biocompatible materials are substances other than food and

medicines, which come into contact with tissue or other body fluids present. Placing material in the body determines interaction implant - tissue that can generate genuine conflicts (RIORDAN, [2], (BAWDEN [3]. They can be toxic, mechanical, electrochemical and bio can be reached even serious bone injury and used metal mounting (SCHMALZ & ARENHOLT-BINDSLEV [4]. Adverse reactions to metal implants are presented in the literature, either as comments on the incident osteosynthesis or arthroplasty, is that research on animals experience posted on the dispersion of metal ions by corrosion.

The main purpose of preimplant augmentation is alveolar ridge regeneration, aesthetics and functionality dentures (MOUNT & HUME [5]. This is possible through a correct relationship between crown and gum, so the augmenting direction and size not only going to position and length of implants, which are to be made, but to achieve an optimal aesthetic. For this purpose different surgical techniques have been developed in order to increase bone volume of alveolar ridge to make possible implant-prosthetic therapy.

Materials and methods

The study was performed on whole human teeth, extracted for orthodontic purposes, keep in distilled water. Diamond cutters were used with conventional speed sampling. Samples were collected using glass plates and slides, including the milled material was transported to the electron microscopy laboratory for analysis. Electron microscopy is a powerful tool used in nanoscience, which in recent years has a strong development (CIUPINA & al [6]. At the same, developing methods of obtaining the materials is an important factor which requires electronic microscopy evolve to succeed as accurately characterize new materials and close to reality, so that the physical parameters determined by examination with microscopy studies on these materials to precede . Regarding the use of electron microscopy instrumentation is already classical, it can be helped with the experimental data processing software in an objective

Sample 1 is enamel powder collected by milling from the vestibular face of a tooth after spray drying with air, using a diamond cutter at conventional speeds. Therefore, a sample is a clean sample on which the enamel was not interfered in any way.

Sample 2 was harvested from four teeth were cut with diamond disc into two symmetrical longitudinal fragments, A and B. For sample 2, in turn, held in forceps fragments were air-dried and demineralized gel was applied to vestibular face for 2 minutes. We used phosphoric acid with concentration 37%. Milled material was collected from fragments A

Section sample is a cross section through the tooth, made empirically, with the high speed diamond cutter, after demineralization. It should provide an image of demineralized enamel is ideal to observe the depth to its product but also in dentin demineralization on which obviously has not occurred.

Sample 3 Demineralized fragment B was maintained 24 hours in natural saliva simple.

Sample 4 and 5 are demineralized fragments B were maintained for 24 hours in saliva solution: Fluorostom 1:1 (sample 4) and Fluorostom (sample 5).

HRTEM (High Resolution Transmission Electron Microscope), nanoprobe, diffraction methods of investigation are solids. The most spectacular remains HRTEM which can penetrate the material and structure can make direct measurements of the lattice constant or even photograph the atoms. Difficulties involved in HRTEM study are conditions which are to be met by studied sample. Thickness must be sufficiently small to have a strong enough beam forming the image, to obtain sufficient contrast to highlight details (REY & al,[7], (EICHERT & al [8]. Crystal orientation must be the direction of crystalline planes.

The probability of finding crystals is much higher in polycrystalline films case. Diffraction pattern obtained in this case is a superposition of images obtained by beam scattering on small crystals. Diffraction rings are obtained as corresponding planes on which diffraction occurred. Depending on the orientation and size of crystallites and eventually the fact that crystal belongs to a space group are obtained contrasted rings or less contrasted. This is because the chemical composition of the primitive cell of the crystal base.

Supplementing the data obtained by diffraction to study crystalline structure is necessary. Thus, the first argument indicating this would be the quality of information. A second argument, direct imaging study of the structure is a goal long pursued. Thus, since 1669 (NIKOLAI STENON,[9] established law of constancy of angles of crystals, based on observations of natural polyhedral crystals. Using HRTEM images we can observe the crystal structure directly. One observation remains to be done: lines appearing in the picture are a direct consequence of electron diffraction by crystal planes.

Nanopowders are prepared from dilution in alcohol, after ultrasonication, by depositing a drop of dilution on formvar or carbon. Another method is encapsulation in epoxy resin of samples and used furture to obtain sections by ultramicrotomy. In the laboratory we use an alternate Epon reactive, namely Embed 812. Dehydration is first step in case if material not requires fixation. There are a variety of protocols for conducting dehydration, depending on material type and quantity of water to be extracted. For cutting included samples we use Leica UltracutR ultramicrotome and diamond knife angle 45 °.

TEM examinations were performed with Philips CM120ST microscope at an operating voltage of 100kV. Were used to investigate the conventional modes of transmission (TEM), electron diffraction (SAED) and high resolution (HRTEM).

Results and discution

Sample 1 is vestibular face millied enamel, on which was not involved (Figure 1).

Of image diffraction were identified d_{012} = 0.337nm, d_{300} = 0.269nm, d_{412} = 0.167, d_{341} = 0.180, d_{312} = 0.201nm lines, indexed using the monoclinic structure of hydroxilapatite (76-0694) space group P21/ c, with lattice parameters a = 0.9421 nm, b =1.8843 nm, c = 0.6881nm.

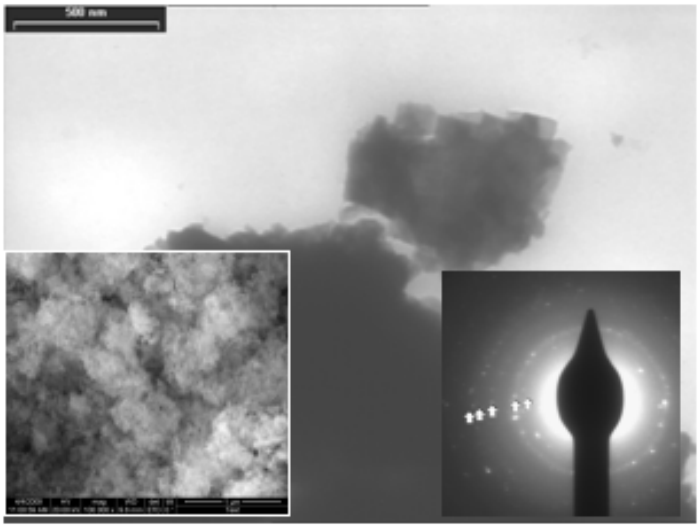

Figure 1. Enamel obtained by milling with diamond-image TEM and SEM respectively (left inset) and electron diffraction (inset right)

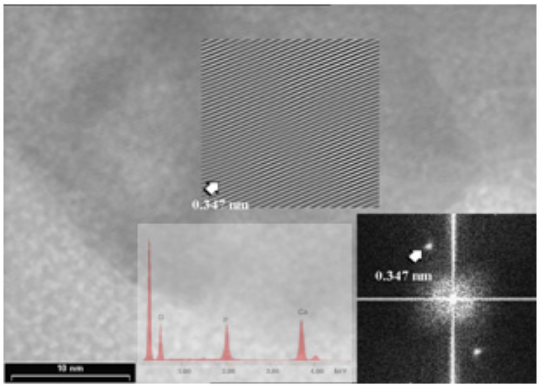

Figure 2. Filtered HRTEM image is observed interference fringes corresponding to planes (002) d_{002}^{calc} = 0.344 nm. In the bottom right corner of the area is filtered Fourier representation. EDX spectrum obtained on a sample which are present elements O, P, As hydroxiapatite specific. F is near a very weak but its intensity is comparable to background noise.

Agglomerations of crystals stands hydroxylapatite, according to calculations obtained from the spectrum of diffraction images and HRTEM image and EDX spectra (Figure 2). The crystals are fragments of different sizes of prisms of enamel (enamel prism size is 4μ).

Sample 2 is Demineralized enamel-two minutes with 37% H3PO4 (Figure 3)

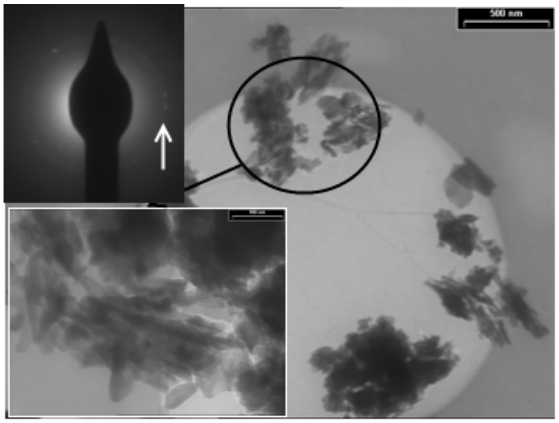

Figure 3. TEM image of nanoparticles of hydroxyapatite with smooth edges, following acid treatment. Diffraction and image detail that shows the 0.270 nm line specfic to hydroxiapatite

Crystals have smooth edges, specific treatment with acid. Easily evaluated, channels created by acid treatment. The crystals are all of the OH-apatite (Figure 4).

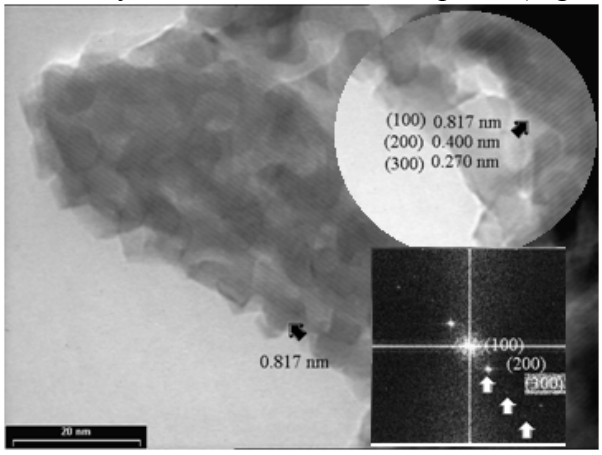

Figure 4. HRTEM image that shows the structure nanocristalelor after treatment with acid. Note that a visible crystal planes (100) d_{100} = 0.817 nm and inset with the same crystal detailed at high magnification are observed (200) and (300) lines.

Section sample on transversely demineralized enamel and dentin was included in the epon without prior processing (fixation, staining specific biological samples).

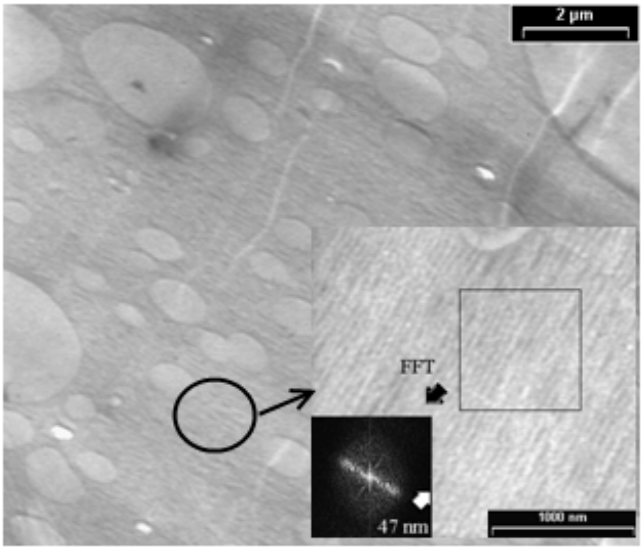

Figure 5. TEM image of the whole tooth tissue where location of osteocyte cells are observed, and filamentary structure is observed around osteocyte cells. To measure distance between filament FFT technique was used, and Fourier representation is shown in the lower left corner of the image

The sample did not sufficiently fine cutting, therefore the depth of enamel demineralization could not be visualized. It could see an amorphous structure area immediately identified with dentin, in which they can easily distinguish dentinal tubules various sizes and densities. Dentinal tubules are 3-4 µ in size on pulp and 1 µ to enamel side. Their density is 75000/mm^2 on pulp side and 15000/mm^2 on enamel side.

Sample 3 demineralized enamel maintained in natural saliva 24 hours (Figure 6)

Crystal images are specific both F- and OH-apatite. Both structures are present.

Sample 4 are demineralized enamel 1:1 solution maintained 24 hours in saliva: fluorostom (Figure 7). It highlights specific fluorapatite diffraction ring at 0.281 nm. Fluorapatite crystal structure gives a specific geometric form, nanorods like.

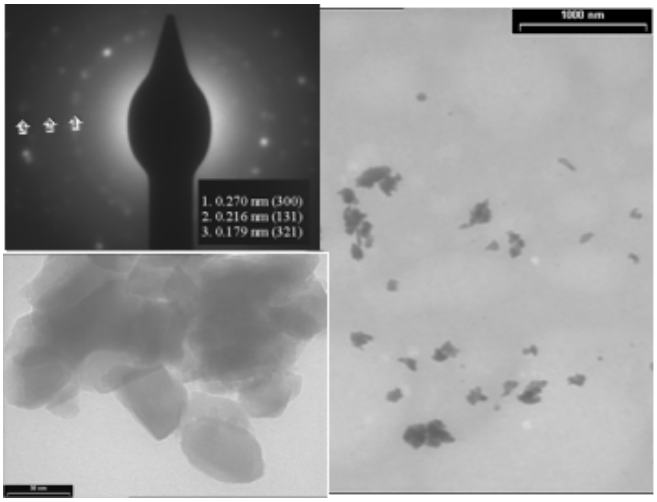

Figure 6. TEM images showing particle agglomerations and details on these particles at higher magnifications, having an irregular shape specific to dental fluorapatite or hydroxilapatite. Electron diffraction pattern obtained in nanoparticles area, demonstrate presence of the large crystallite due to which the spots diffraction pattern occurs.

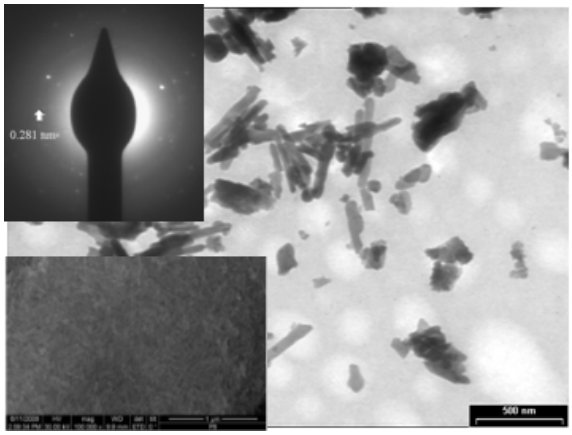

Figure 7. Overview of fluorapatite nanoparticles, electrons with diffraction lines for the fluorapatite to 0281 nm and the magnification SEM image 100kx.

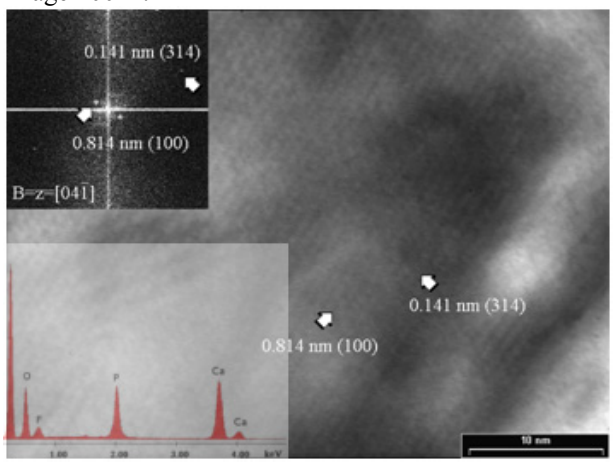

Figure 8. HRTEM image on Nanocrystal-oriented direction [041] and EDX spectrum obtained on sample 4. Fluorapatite existence is evidenced by this spectrum

Sample 5 demineralized enamel maintained 24 hours Fluorostom (Figure 9)

As the sample 4, following calculations based on information obtained from the diffraction spectrum, it highlights the specific physical properties to identify fluorapatite (LOULOUDIADIS, [10]. As a particularity, the empirically observed, the crystals are highly agglutinated, compared with sample 4. We can not verify the current state of the study whether this is coincidence or is due to double concentration of fluorine, which has maintained contact with the sample 5.

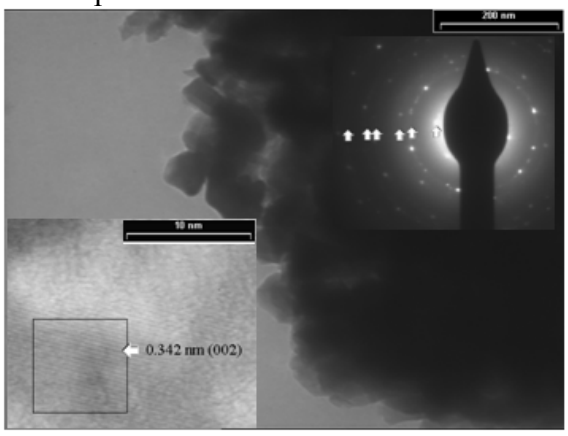

Figure 9. TEM image that shows the agglomeration of fluorapatite nanoparticles with 50 nm size, corresponding diffraction image and high resolution image are highlighted and measured using the FFT technique. The interference fringes corresponding planes (002) are marked on image.

Is shown in figure 10, TEM image of a hydroxyapatite nanopowders obtained by chemical route (D.BIGGEMANN & al [11].

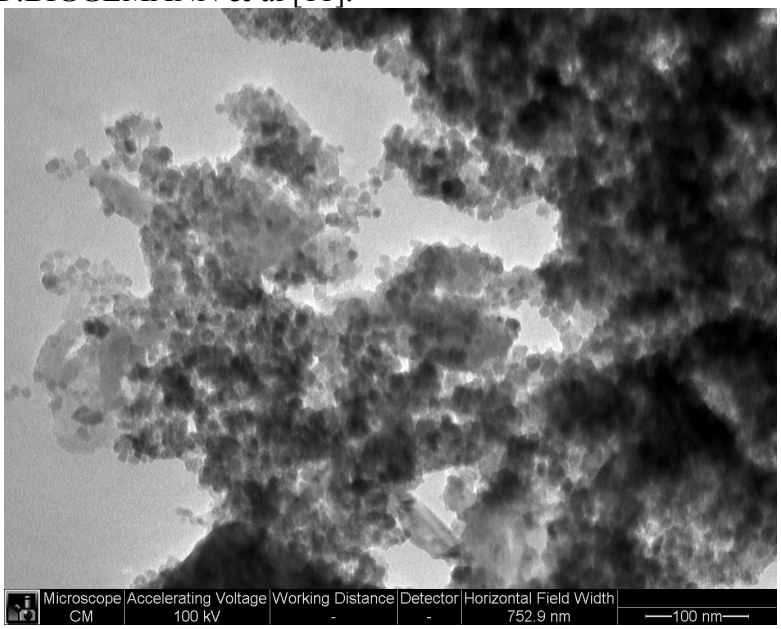

Figure 10. TEM image of HA nanoparticles

Electron diffraction was performed in two ways to compare the results of investigations. In the first case was made standard configuration for electron diffraction, and second precession of electron diffraction was used to obtain electron diffraction pattern. In figures 11 and 12 are those patterns and related RDF.

Diffraction patterns show the presence of polycrystalline materials. Precession of electron diffraction improved intensities of difracted beam which be close to kinematical theory.

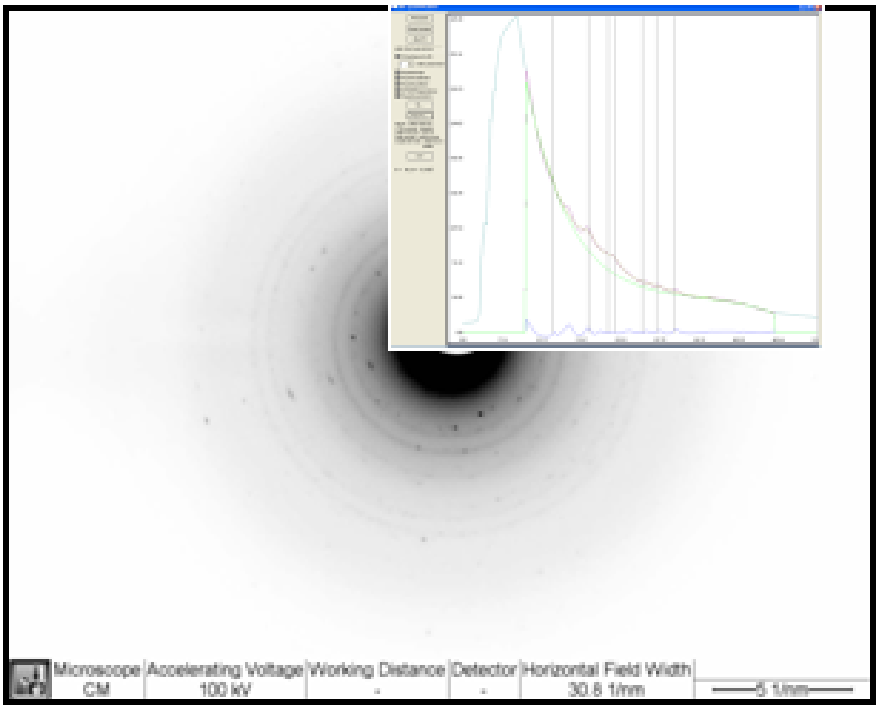

Figure 11. Diffraction pattern and nanopowders HA RDF obtained for standard mode

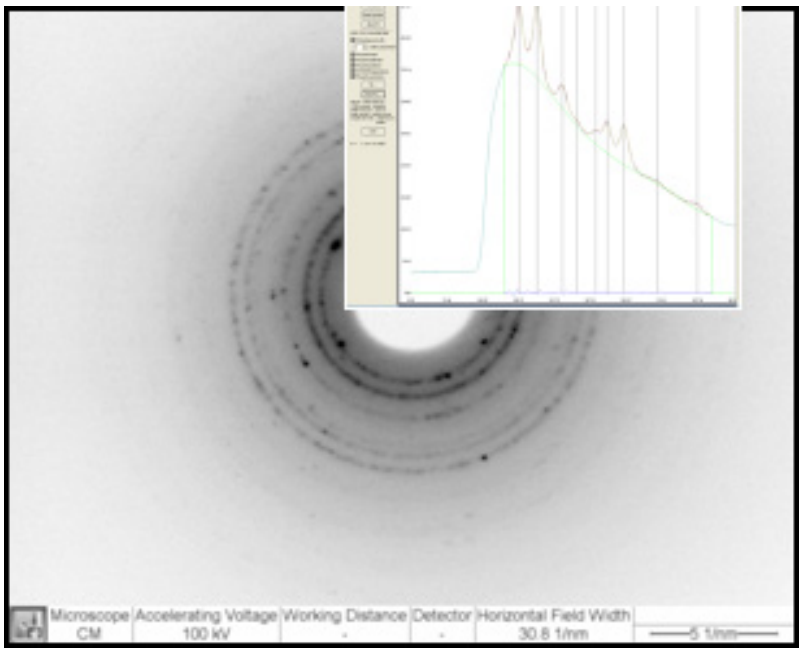

Figure 12. Figure diffraction and RDF of HA nanopowders obtained when using precession

Conclusions

Using electron microscopy techniques tooth tissue morphology was followed without treatment and with the treatment that affects the demineralization/ remineralization. Thus, the TEM micrographs show the osteocyte cell arrangement and size in the dental tissue. Electron diffraction was used to identify crystalline material of dental tissue, especially hidroxylapatita or fluoroapatita. HRTEM images show additional information regarding the structural characterization. They managed to identify suitable crystal size to create the conditions for obtaining high-resolution images.

Nanostructures HA are example of a new generation of ceramic material that was created to interact with cells and tissues. They find application in many medical devices such as cardiac stents, prostheses for joints, biosensors and other implantable medical devices.

HA nanostructures can be used in gene transfer due to their recognized biocompatibility and ease of handling due.

Nanocrystalline ceramics must occupy a prominent place in biomedical research because it may be easier sintered and mechanical characteristics have been improved with the reduced size of internal cracks. Nanocrystalline ceramics have high ductility and elasticity, because a large volume of material nears the edges of grains.

All these nanostructured ceramics are characterized by increasing osteoblasts adhesion, property important for the integration of implants into surrounding tissue.

Many properties are still unknown. In this stage were identified mechanical properties, advantages and disadvantages of nanostructured materials used to manufacture dental implants, and national and international regulations relating to implantable medical devices, biocompatible materials based on the selection, establishment of criteria for design, preclinical testing and clinical evaluation, manufacturing, sterilization, packaging, labeling and delivery.

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