

Synthesis and characterization of hydroxyapatite formed precipitation process

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Keywords

Hydroxyapatite.
Precipitation.
Dental implants.
Bone remodeling.

Abstract

The use of bone substitutes for recovery of lost function represents a constant search within the field of Dentistry, more specifically in contemporary Implantology. For this reason, biomaterials have received a great deal of attention from the international scientific community. The clinical use of hydroxyapatite, mainly in the maxillary sinus, for bone height gain and placement of dental implants has demonstrated very satisfactory results; either when used by itself or in conjunction with other materials, such as PRP (platelet rich plasma), autogenous bone, or other grafting materials. In this study, hydroxyapatite powder was prepared using the precipitation method. Scanning electron microscopy, infrared spectroscopy and x-ray diffraction were used to characterize the obtained material. Clinical cases were undertaken with the objective of verifying the promotion of bone tissue growth for implant placement. Results showed that the obtained powder is composed exclusively of hydroxyapatite, and that phase precipitation of calcium phosphate system did not take place. Histological studies of the grafted areas and x-rays demonstrated that the receptor site displayed adequate bone tissue growth, making possible the placement of the implants and subsequent support of masticatory loads.

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INTRODUCTION

Hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, represents 30 to 70% of the mass of bones and teeth and it is main mineral constituent of these structures. For being synthetic and having biocompatibility properties, the production of hydroxyapatite is being studied by different groups of researchers to obtain a product where different phases do not coexist based on the Ca-P system. The synthesis of calcium phosphate by chemical precipitation has advantages, when compared to the traditional methods of hydroxyapatite production, due to its low cost and simplicity, however, majority of the synthetic procedures include the formation of non-stoichiometric products and mixture of phases. The precipitation processes consist of the addition of phosphate groups to suspensions that contain calcium ions, which may come from different reagents. Depending on the manufacturing process, the properties of hydroxyapatite have different characteristics. When synthesized at high temperatures, they present good crystallinity and large crystals. When synthesized at low temperatures, they present low crystallinity and large crystals. Currently the wet precipitation methods are preferred because the product produced has characteristics similar to that of bone and dental tissue.

LITERATURE REVIEW

The potential of ceramics as biomaterials is based on their compatibility with the physiological medium. This compatibility results from the fact that these materials can contain ions normally

found in the physiological medium (calcium, potassium, magnesium, sodium, etc) besides ions with limited toxicity to body tissues (aluminum or titanium). Therefore, bioceramics are defined as ceramic materials designated to act in a specific physiological medium, and used as construction material of apparatuses, protection or artificial internal organs². Among the bioceramics, hydroxyapatite ($\text{HA} = \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) and β -tricalcium phosphate ($\beta\text{-TCP} = \beta\text{-Ca}_3(\text{PO}_4)_2$) are the most commonly used materials because, besides the chemical composition similar to the bone mineral phase, it provides differential and satisfactory responses, and also, it is known that these materials enable bone growth and facilitate the integration of the implant^{2,7}.

Depending on the methods used, reagents used and the variables adopted to obtain calcium phosphate based materials, it is possible to obtain several phases, besides a wide variety of behaviors resulting from other variations, such as: crystalline defects, superficial areas, affinity for organic materials found on the physiological medium, etc.

CHEMICAL PRECIPITATION OF HYDROXYAPATITE

The synthesis of calcium phosphate by chemical precipitation is advantageous because of its low cost and simplicity; however, majority of the synthetic procedures have the formation of non-stoichiometric products and mixture of phases, which leads to the presence of ion vacancies and substitutions in the network, such as carbonates,

hydrogen phosphates, potassium, sodium, nitrate and chloride³.

The precipitation processes consist of the addition of phosphate groups to suspensions that contain calcium ions, which may come from different reagents. The neutralization reaction that uses orthophosphoric acid and calcium hydroxide shows greater potential for the production of hydroxyapatite (HA) once water is the only sub-product of the reaction.

The precipitation methods show variables like pH, obtainment temperature, molar concentration of the reagents, addition rate of reagents, agitation time, aging time and calcination temperature. The aging time and kinetic reaction are critical variables for purity and crystallographic characteristics of the obtained material.

The composition of the reactions is the hardness of the material, which may or may not have unexpected ions in the network, besides the differences in the morphological and crystallographic characteristics. The rate at which the reagents are added, that is, the drip time, influences the nucleation rate of the crystals³. The drip speed is directly related to the kinetics of the reaction, the slow addition of phosphate ions provide a low nucleation rate and high growth rate, which implies in the obtainment of larger particles; on the contrary, high addition rates of reagents allow the formation of higher numbers of nucleus, but without enough time for grain growth.

The formation of a solid involves precipitation from a solution and crystallization, these two processes

occur simultaneously if the precipitate is crystalline, on the other hand, if the solid obtained is not crystalline, the rate at which these stages occur determines the crystallinity of the material. This rate can be controlled by varying the saturation of the solution and through the average crystallization time, which has temperature and drip rate as parameters¹⁰.

The temperature at which precipitation occurs is of great importance in the phase obtained and in the conversion from one to another. The particle size and morphology are also influenced by temperature. Higher temperatures allow the obtainment of more crystalline powders.

During aging, the crystals formed are subjected to a dissolution and re-crystallization process, in which the smaller crystals disappear in detriment to the larger ones that grow more rapidly; consequently, the total number of crystals reduce, as well as the specific surface area³. The growth of particles during aging proves that precipitation continues even after dripping of the entire volume of acid⁹.

Calcination of the powder obtained can alter the present solid phase because each of the calcium phosphate phases have different thermal stabilities and physical properties. A small variation in the Ca/P ratio of the synthesized powder results in a huge variation of the proportions of the phases formed after calcination⁸.

The formation of HA by precipitation can occur through intermediate precursor phases that show transitory and ephemeral existence during the

obtainment stage³; depending on the conditions used, these phases can be detected when the material undergoes thermal treatments and influence the quality of the final product.

The biological performance of synthetic materials for biomedical use depends on the fundamental parameters like chemical composition, morphology and biodegradability. The analysis of these properties can be conducted through various characterization techniques.

Calcium deficient hydroxyapatite (CDHA) can be prepared by precipitation under basic conditions for subsequent thermal treatment, with temperatures close to 1000°C. Pure HA has a Ca/P ratio of 1.67, network parameter: a-axis = 0.9422nm and c-axis = 0.688nm. The powder obtained showed absorption bands of OH⁻ and HPO₄²⁻ in the infrared spectrum and formation of the mixture of β-TCP and HA in the calcined powders above 700°C, the ratio between these two phases increased with the reduction of the Ca/P ratio. Amorphous calcium phosphate can be prepared at room temperature by precipitation through dripping of calcium nitrate solution over a basic phosphate solution under agitation, and subsequent filtration and drying of the obtained solid; the analysis by x-ray diffraction of this material showed a broad band, due to the fact that it is amorphous, and the transmission electron microscopy showed spherical particles. However, this phase is unstable and rapidly converts to OCP and then to HA. The DCPD phase with ratio of Ca/P of 1.0 and flat crystalline particles were also obtained from dripping calcium ion

solutions over solutions containing phosphate groups at room temperature and initial pH of 6. The DCPA phase was prepared in the same way as the DCPD phase, but with the precipitation temperature increased to 60°C, and the OCP phase took place when the pH was reduced to 4 and the temperature increased to 80°C.

According to Saeri et al.⁹, HA was obtained through the addition of a solution of phosphoric acid (0.3M) in a suspension of calcium hydroxide (0.5M) at a rate of two drips per second at 40°C in pH=7.5, kept constant through the addition of ammonium hydroxide. Next, the material obtained was washed with distilled water and 1mmol/l of phosphoric acid was added to it, and this solution was aged for one night. The synthesized powder was calcined at 850°C and at 1200°C. Saeri ET al.⁹ characterized hydroxyapatite powder by microscopy and observed that the morphology and particle size were altered in each stage of the obtainment process, significantly affecting the synthesization properties. The particles showed flat and elongated morphology, with nanometric scale, but the samples with higher aging time showed larger particles. Furthermore, the nanoparticles tended to form agglomerates. It was also observed that the aging time and precipitation kinetics were determining factors in the purity of the material and its crystallographic properties. Also, the higher the calcination temperature the more crystals were observed, which was shown by the intensity difference of the peaks in the x-ray diffractogram.

It was also reported that the specific surface area of the stoichiometric samples had higher values than the non-stoichiometric samples.

Landi et al.⁵ obtained carbonated hydroxyapatite through the neutralization reaction of calcium hydroxide ($\text{Ca}(\text{OH})_2$) and phosphoric acid (H_3PO_4). A suspension of $\text{Ca}(\text{OH})_2$ was heated at 40°C and carbon dioxide (CO_2) was bubbled over this suspension, concomitantly with the dripping of a solution of H_3PO_4 , for a period of 4 hours. Subsequently, the system was agitated for 2 hours, aged for one day at room temperature, washed and deagglomerated. The obtainment process had variables like the flow of CO_2 , addition rate of H_3PO_4 , concentration of the solution and synthesis temperature. The increased flow of carbon dioxide led to the formation of carbonated hydroxyapatite with substitution of the OH groups; the increased addition rate of phosphoric acid caused the lowest incorporation of carbonate in the HA structure, especially on the phosphate groups. On the other hand, the reduction of this rate resulted in the substitution of phosphate groups by HPO_4^{2-} groups, characteristics of calcium deficient hydroxyapatite. Furthermore, there was an increase in the crystallinity and particle size with increased synthesis temperature, in detriment to the quantity of carbonate present in the sample. Through x-ray diffractograms, it was observed that the crystallinity of the carbonated hydroxyapatite is smaller than that of pure HA. In the infrared spectra, bands relative to the carbonate and phosphate groups were observed.

Afshar et al.¹ prepared hydroxyapatite from the suspension of 0.5M calcium hydroxide, which was heated for 1 hour at 40°C and agitated constantly, and 0.3M phosphoric acid was added to it at a rate of 2 drops/second. The pH was controlled through the addition of NH_4OH . The precipitate was aged for a period of one day in the mother solution, in which it decanted. The study of the obtained material showed, as results, particles with rod shape of nanometric scale through the analysis of the micrographs. The quantity of calcium ions present in the material reduced with the reduction of the pH during precipitation, which was concluded through calculations involving x-ray diffractions.

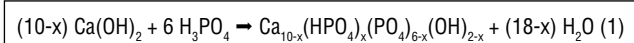
Kumar et al.⁴ added a solution of 0.06M phosphoric acid at a rate of 4ml/min to a solution of 0.1M calcium hydroxide to obtain calcium phosphates. Small inclusions of calcium carbonate were made to obtain carbonated apatite. The effect of the precipitation temperature was studied using 40°C , 80°C and 100°C . During the addition of reagents, the pH was kept constant at 7.4. The suspension was agitated for 2 hours and aged for another 15 hours. Through x-ray diffraction, the formation of HA as the majority phase and small quantity of β -TCP was observed in all the precipitation temperatures. With the increase in reaction temperature, there was an increase in the crystallinity of HA and a reduction in the substitution of carbonated groups in the apatite structure. Through transmission electron

OBJECTIVES

To produce, characterize and study clinical cases using hydroxyapatite obtained by the precipitation method.

MATERIAL AND METHODS

The hydroxyapatite powder was obtained in a clean room (ISO 5) by chemical precipitation, using $\text{Ca}(\text{OH})_2$ (Labsynth, Brazil) and H_3PO_4 (Labsynth, Brazil) as reagents, all of analytical grade according to equation 1 (Produced by Bionnovation Produtos Biomédicos S/A):



Initially, $\text{Ca}(\text{OH})_2$ is mixed with distilled and deionized H_2O in a round bottom flask that was heated and kept agitated at constant temperature. Subsequently, the volume of H_3PO_4 solution with a rate of 5ml/min, keeping the system with at constant agitation and temperature for the aging stage. After this period, the suspension was filtered, dried in an oven at $110^\circ\text{C}/24\text{h}$, the material was deagglomerated in

an agate mortar, passed through a mesh and thermally treated.

The morphology of the powder was analyzed using a scanning electron microscope operating at 25KeV and the structure was analyzed using infrared spectroscopy and x-ray diffraction. Clinical cases using hydroxyapatite to increase bone volume in the maxillary sinus for subsequent placement of dental implants was performed on patients with the purpose of promoting ideal conditions for the placement of implants.

RESULTS AND DISCUSSIONS

Characterization of the material

Figure 1 shows the DRX spectrum for hydroxyapatite powder. It was observed that all the peaks are associated to the HA phase, without the formation of other compounds based on the Ca-P system.

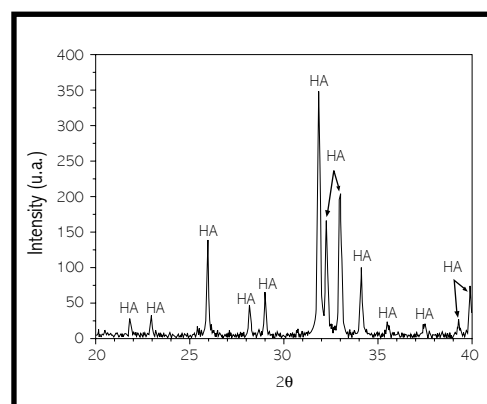


Figure 1 – X-ray diffraction of hydroxyapatite powder.

Absorption infrared spectrum (Figure 2) are present as bands that characterize the HA phase; at 491cm^{-1} , 563cm^{-1} , 603cm^{-1} , 1043cm^{-1} and 1088cm^{-1} there are peaks referring to the PO_4^{3-} cluster, 636cm^{-1} and 3574cm^{-1} referring to the OH- cluster. The band at 871cm^{-1} represents molecular vibrations of the CO_3^{2-} group, which indicates the presence of this group in the phase, therefore being carbonated hydroxyapatite due to the ion substitution of the carbonate in the HA structure.

The microstructure of the powder obtained by this process was examined by scanning electron microscopy (Figure 3). We observed that the powder is composed of small particles, forming various agglomerates.

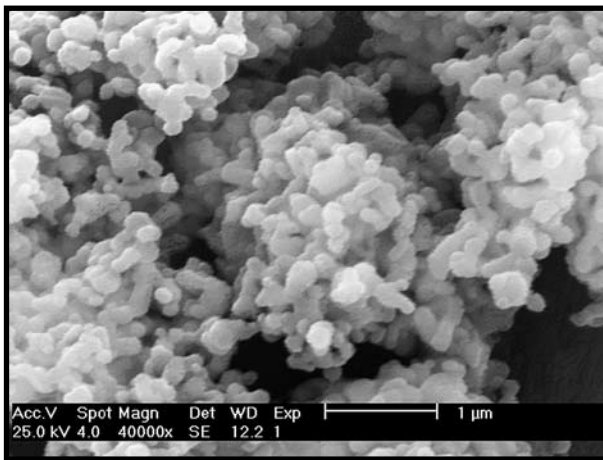


Figure 3 — Micrograph of hydroxyapatite powder by scanning electron microscopy.

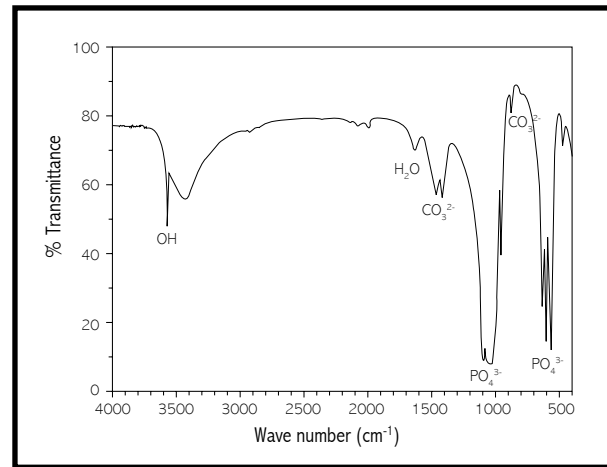


Figure 2 — Infrared spectroscopy of hydroxyapatite powder.

CLINICAL CASE

In these clinical cases, hydroxyapatite of the Bionnovation brand (ANVISA REGISTRATION NUMBER 10392710010) was used to increase the volume in the maxillary sinus for subsequent placement of dental implants.

Figure 4 shows an x-ray of the region where a large pneumatization of the maxillary sinus can be observed, hindering the placement of implants.

Figure 5 shows the filling moment of the maxillary sinus with hydroxyapatite to increase the bone tissue.

Figures 6 and 7 show the same grafted region after one week and six months after the surgical procedure, respectively.



Figure 4 - Initial x-ray of the case, where large pneumatization of the maxillary sinus can be observed.

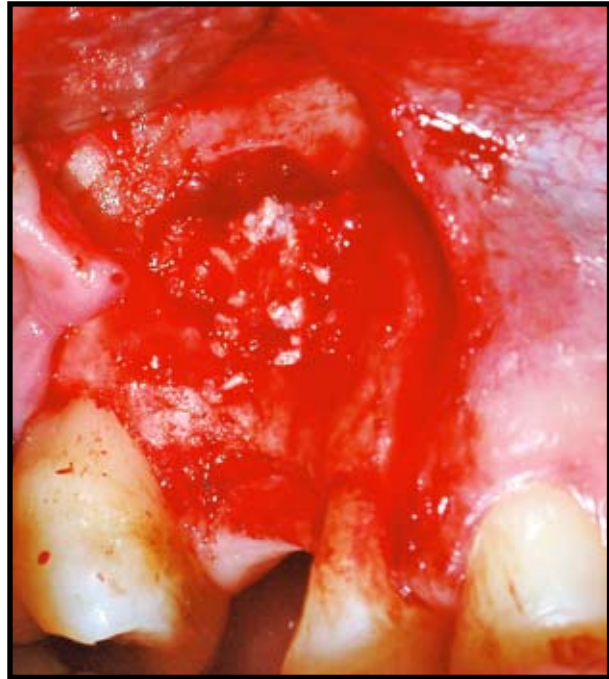


Figure 5 - Filling of the maxillary sinus to increase bone height.



Figure 6 - X-ray one week after placing the filling material..



Figure 7 - X-ray of the grafted region after six months.



Figure 8 - Drilling period with a trephine for collection of bone from the grafted site for histological study and placement of the implant.



Figure 9 - A) Detail of the trephine with collected block of bone and **B)** collected block of bone.

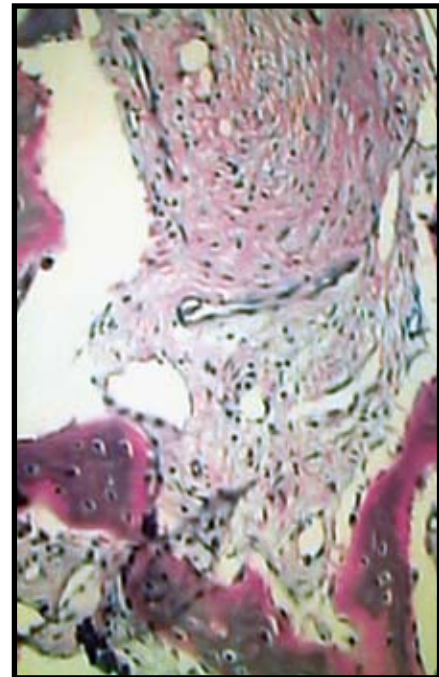


Figure 10 - Histological image of the neoformed bone, where the large bone tissue growth can be observed.



Figure 11 - X-ray indicating pneumatization of the maxillary sinus.

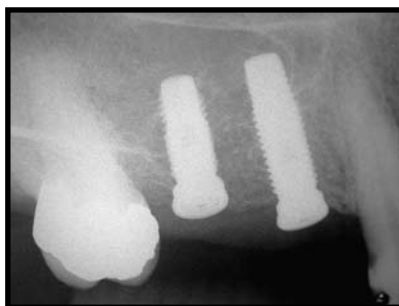


Figure 12 - Implants and graft placed in the region of the maxillary sinus.

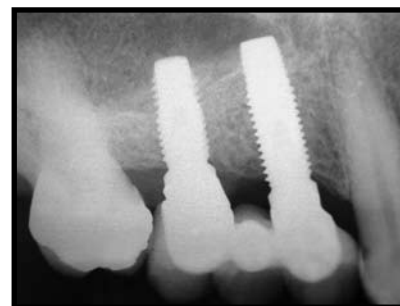


Figure 13 - Prosthesis installed in masticatory function.

Figure 8 shows the time of drilling to remove the block of bone for histological analysis. Figures 9A and 9B show the trephine used for preparing the site that will receive the implant and for collection of the block for histological study.

Figure 10 shows the result of the histological analysis of a slide stained with toluidine blue, where the growth of bone tissue can be observed in the region where the HA graft was placed, six months after the surgery.

Figures 11, 12 and 13, clinical case II, show x-rays of the region showing the increased bone volume in the maxillary sinus, the placement of two implants together with the filling material and the prosthesis placed in masticatory function.

The x-rays show, in both presented cases, in the situations prior and after the graft surgery

with hydroxyapatite, a considerable increase of bone tissue, enabling the placement of implants and subsequent placement of prosthesis in function.

CONCLUSIONS

The characterization of the material showed that the powder produced by the precipitation method shows phase associated only to hydroxyapatite, without the coexistence of other phases referring to the Ca-P systems. The powder is composed of fine particles, forming uniform and dispersed agglomerates. The clinical cases showed that this product is efficient for promoting bone tissue growth.

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ABSTRACT

The use of bone substitutes for recovery of lost function represents a constant search within the field of Dentistry, more specifically in contemporary Implantology. For this reason, biomaterials have received a great deal of attention from the international scientific community. The clinical use of hydroxyapatite, mainly in the maxillary sinus, for bone height gain and placement of dental implants has demonstrated very satisfactory results; either when used by itself or in conjunction with other materials, such as PRP (Platelet-Rich Plasma), autogenous bone, or other grafting materials. In this study, hydroxyapatite powder was prepared using the precipitation method. Scanning electron microscopy, infrared spectroscopy and x-ray diffraction were used to characterize the obtained material. Clinical cases were undertaken with the objective of verifying the promotion of bone tissue growth for implant placement. Results showed that the obtained powder is composed exclusively of hydroxyapatite, and that phase precipitation of the calcium phosphate system did not take place. Histological studies of the grafted areas and x-rays demonstrated that the receptor site displayed adequate bone tissue growth, making possible the placement of the implants and subsequent support of masticatory loads.

KEY WORDS: Hydroxyapatite. Precipitation. Dental implants. Bone remodeling.

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