EFFECT OF MILLING TIME ON PROPERTIES OF CALCIUM PHOSPHATE CEMENTS BASED ON ALPHA-TRICALCIUM PHOSPHATE

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ABSTRACT

Nowadays, α-tricalcium phosphate is receiving growing attention as a raw material for several injectable hydraulic bone cements, biodegradable bioceramics and composites for bone repair. However the calcium phosphate cements used as bone substitute, generally have low mechanical strength compared with the bones of the human body. One alternative to reduce this problem is to determine the particle size of the material which can results in greater mechanical strength. This work aims to study the influence of the milling time on the mechanical properties of the α-tricalcium phosphate cement. The α-tricalcium phosphate was obtained by wet reaction from calcium nitrate and phosphoric acid. The powder obtained was characterized by particle size analysis and X-ray diffraction. Specimens were prepared and evaluated by apparent density, scanning electron microscopy and compression strength. The results demonstrated the influence of the milling time on the mechanical properties of the cement.

Keywords: alpha-TCP, cement, biomaterials, nitrate, particle size.

INTRODUCTION

During the past 50 years, advances in many specialty bioceramics such as alumina, zirconia, hydroxyapatite, tricalcium phosphates and bioactive glasses have made significant contribution to the development of modern health care industry and have improved the quality of human life. These are the ceramics, which can be used inside the body without rejection to augment or replace various diseased or damaged parts of the musculoskeletal system [1,2]. They are primarily used as bone substitutes in the biomedical industry due to their biocompatibility, low density, chemical stability, high wear resistance, and for calcium phosphates, mainly for their compositional similarity with the mineral phase of bone. But the potential of any ceramic material to be used as an implant in vivo depends upon its ability to withstand complex stresses at the site of application and its compatibility with the biological environment. Calcium

phosphates are the materials of choice in both dentistry and medicine. They have been used in the field of biomedical engineering owing to the range of properties that they offer, from tricalcium phosphates being resorbable to hydroxyapatite being bioactive; they are undeniably the current rage for clinical usage [2-4]. They exhibit considerably improved biological affinity and activity compared to other bioceramics.

Despite the existence of various systems of calcium phosphate cements (CFC) studied, the ones based on α-tricalcium phosphate (α-TCP) are of particular interest due to the formation, during the setting reaction, of calcium deficient hydroxyapatite, similar to bone hydroxyapatite. Hydroxyapatite promotes bone growth where implanted, establishing links of chemical nature between the hydroxyapatite and the bone (bioactivity), allowing the proliferation of fibroblasts, osteoblasts and other bone cells [5-7]. It has been reported that α-TCP is a metastable phase and can only be obtained after heat treatment of β-TCP up to 1250 °C for 15 hours, followed by rapid cooling, resulting in a material composed by α-TCP as major phase [8].

The particle size and hence the specific surface area of cement reactants is commonly altered by milling the solid phase in wet or dry conditions. By prolonged milling, crystalline salts can become amorphous [9], hence increasing both the kinetic and thermodynamic solubility of the material rendering the powder more reactive [9-11]. This concept has recently been applied to crystalline water-free calcium phosphates [12] such as tricalcium phosphate cements [13] and tetracalcium phosphate [14]. Alpha-tricalcium phosphate (α-TCP) are widely used in tissue engineering for the regeneration of bone tissue, due to their biocompatibility and biodegradability [15,16]. However, the brittle nature of α-TCP confines clinical application to non-load-bearing repair and substitution.

This work aimed to investigate the influence of prolonged milling of α-TCP in the particle size and mechanical properties of alpha-tricalcium cements. The powder was obtained by the wet precipitation process, using calcium nitrate and phosphoric acid as reagents. To obtain the powder was not used rapid cooling and the holding times was reduced during the calcinations. In order to prepare the calcium phosphate cement the powders were mixed with an accelerator in an aqueous solution, for making specimens. The specimens were soaked in SBF (Simulated Body Fluid) for until seven days prior to assess the mechanical properties.

MATERIALS AND METHODS

The TCP was synthesized in laboratory using calcium nitrate ($Ca(NO_3)_2.4H_2O$) and phosphoric acid (H_3PO_4), according to the following reaction:

$$9\ Ca(NO_3)_2\ .\ 4\ H_2O + 6\ H_3PO_4 \rightarrow\ 3\ Ca_3(PO_4)_2 + 36\ H_2O + 18\ HNO_3$$

The Ca/P ratio was maintained in 1.5 and the reagents concentration was 2.0 M. The solution obtained by mixing the reagents was maintained at a temperature of 90 ± 2 °C for 24 hours. After this period, the solution was dried at 120 ± 2 °C for 24 hours and then calcined at 1500°C for 1 hour.

After the calcination, the reaction product was grounded using mortar and pestle to obtain a fine powder # 325 sieved.

The milling time and the identification of the samples are summarized in the Table 1. The samples were milled in a ball milling using alumina balls with 10.80 mm in diameter. Each gram of powder was milled with four alumina balls and using 2 ml of anhydrous ethyl alcohol.

Table 1. Milling time and identification of the samples

Sample	M00	M05	M1	M2	M4	M8	M16
Milling time (h)	0.0	0.5	1.0	2.0	4.0	8.0	16.0

The particle size analysis was performed using laser diffraction. The detection range of the instrument is from 0.04 to 2500 µm. Measurements were made on a laser diffractometer Cilas 1180.

The evaluation of the phase composition of powder after calcination and without mill in ball milling (M00 sample) was performed using X-ray diffraction. Phillips X'Pert MPD diffractometer with a copper tube (Kα radiation = 1.5418 Å) was used for this analysis. The voltage and current used in the tube were 40 kV and 40 mA, respectively. The scanning speed of the goniometer was 0.05 °/s, and the scan interval (2Ɵ) from 10 to 40°.

Cement pastes were prepared by addition of a 2.5% w/v Na_2HPO_4 aqueous solution to the powder. The liquid/powder ratio employed varied with the powder used, as shown in Table 2. The samples were conformed in a mold according to ASTM F 451-95 [17]. The mold used was stainless steel containing cavities of

6.0 ± 0.1 mm in diameter and 12.0 ± 0.1 mm in height. The demoulded samples were kept in a 100% humid environment for 24 hours, and then immersed in SBF solution for different periods of time.

Table 2. Liquid/powder ratio necessary to prepare the cement paste

Sample	M00	M05	M1	M2	M4	M8	M16
L/P ratio ($mL.g^{-1}$)	0.29	0.29	0.30	0.31	0.32	0.33	0.34

A liter of simulated body fluid (SBF) was prepared by dissolving NaCl 7.995 g, $NaHCO_3$ 0.353 g, KCl 0.224 g, $K_2HPO_4.3H_2O$ 0.228 g, $MgCl_2.6H_2O$ 0.305 g, $CaCl_2$ 0.227 g, and Na_2SO_4 0.071 g into distilled water. The solution was buffered at pH 7.4 by adjusting the volume amount of Tris (tris-hydroxymethylaminomethane) and HCl at 36.5 °C. The *in vitro* studies were performed by immersing the specimens in a customized static chamber at 37°C. The SBF was replaced daily. The specimens were immersed in SBF for seven days.

The apparent density was performed based on ASTM C20 - 00 (2010) [18] and the compressive strengths after SBF immersion were determined using a universal mechanical testing apparatus (Instron, 3369) at ambient temperature. Samples were compressed between platens with a constant deformation rate of 1.0 mm/min. All results were the averages of five measurements.

Scanning electron microscopy (SEM), was performed on the fracture surface of specimens used in compression test, using a JEOL scanning electron microscope, model JSM 6060.

RESULTS AND DISCUSSION

The TCP obtained by wet reaction, allowed the obtaining of α-TCP phase with high purity, as discussed below. In this study the obtained powder was milled at different times and their properties were measured and evaluated. The laser diffraction analysis allowed to determine the average particle size, which are shown in Table 3.

Table 3. Average particle size of powders obtained

Sample	M00	M05	M1	M2	M4	M8	M16
Particle size (μm)	21.94	16.80	13.33	9.07	7.19	6.19	3.61

The average particle size determined by laser diffraction decreases about 24% in only 0.5 hours of milling, compared to the M00 sample. With 16 hours of milling the reduction was approximately 83%. This variation in average particle size influenced the compression strength of the samples, as shown below.

The wet milling of α-tricalcium phosphate in ethanol allowed the breaking of the aggregates with the consequent decrease in average particle size. After two hours of milling the value obtained lies in the range described as required to obtain the calcium phosphate cements (≤ 10 μm), however in this case de cement were injectable [19]. Santos *et al.* [8] obtained an average particle size of 8 μm after five hours of milling. Both studies obtained the cement from solid state reaction of the reactants. There are no published studies of calcium phosphate cement from powders obtained by wet reaction.

X-ray diffraction (XRD) patterns show that the α-TCP obtained show high purity. The peaks pointed in the diffractograms of M00 sample (Fig. 1), correspond to the diffraction pattern data sheets number 00-029-0359 (α-TCP) of the Joint Committee on Powder Standards Diffraction - JCPDS.

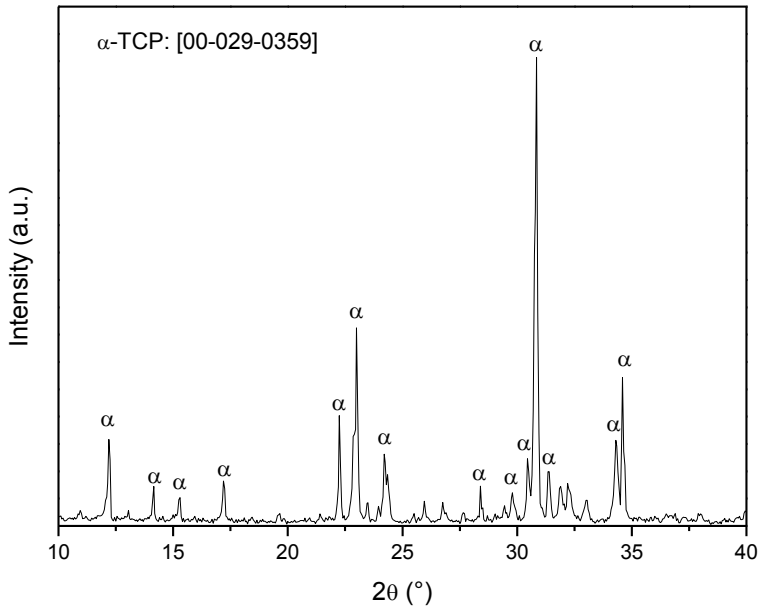

Figure 1. X-ray diffraction patterns of the M00 sample.

It is possible to observe the presence of phase α-TCP only. Currently, there are no known data on literature about pure α-TCP for use as cement, independently of the synthesis method used [20]. The rate of transformation of β-TCP in α-TCP is

reported in literature as low and reversible. For obtaining α-TCP of high purity, the use of high holding times, at temperatures higher than 1200 °C, and quenching is necessary to preserve the α-TCP phase at room temperature. In this work, it was not use high holding times and not uses quenching for obtaining the α-TCP.

Table 4 shows the values of apparent density and apparent porosity measured by Archimedes method and the compressive strength of samples prepared for each studied milling time.

Table 4. Compression strength, apparent porosity and apparent density of the samples.

Sample	Apparent Porosity (%)	Apparent density ($g.cm^{-3}$)	Compression strength (MPa)
M00	30.43 ± 1.87	1.82 ± 0.02	25.73 ± 2.74
M05	31.54 ± 3.50	1.75 ± 0.04	20.82 ± 3.08
M1	32.89 ± 1.83	1.75 ± 0.02	22.42 ± 2.86
M2	36.05 ± 2.45	1.69 ± 0.03	23.67 ± 2.25
M4	38.02 ± 2.32	1.66 ± 0.03	20.19 ± 2.63
M8	38.54 ± 1.72	1.67 ± 0.02	24.17 ± 2.81
M16	39.68 ±1.51	1.66 ± 0.02	19.20 ± 2.49

It can be noted an increase in the apparent porosity of the cements with the increase of milling time of the powders. Contrary result was waiting, although the method of preparation of the powder results in a very reactive powder which may have causing the formation of agglomerates and thus result in increased in apparent porosity of the cement.

The best results of compressive strength were observed for the sample unmilled in ethanol and for the sample with eight hours of milling. With these results we can conclude that it is not necessary to perform the milling in ethanol of α-TCP prepared by this methodology, to obtain best results of compressive strength. Unless the α-TCP is used as injectable cement, which requires an average particle size less than 10 μm [19].

Another factor that may have influenced the higher compressive strength of the M00 sample is the smallest amount of liquid required for the conformation of the specimens (as shown in Table 2).

The increase of mechanical strength of calcium phosphate cements, is attributed to hydration of α-TCP, resulting in hydroxyapatite. Hydroxyapatite crystallites are nucleated on the surface of α-TCP particles by supersaturated solution of Ca and P that surrounds the particles after the initial dissolution of the particles. Subsequent dissolution of α-TCP particles and growth of hydroxyapatite crystals are controlled by the diffusion process, by the layer of hydroxyapatite crystallites. The characteristics of hardening of the cement depend on the formation of a network of interlocking crystals, through this process of diffusion. Thus, the increased mechanical strength of some samples may be associated with greater interlacing of hydroxyapatite crystals [(21)].

The Fig. 2 shows the fracture surface micrographs of samples immersed in SBF solution, for different times.

In micrographs can be observe hydroxyapatite needles formation in all the samples, however in different amounts and sizes. In the M00 and M8 samples it can be seen that the hydroxyapatite needles are greater than other samples.

The increase in mechanical strength observed in the M00 and M8 samples, occurs as a result of the formation of the intersection of hydroxyapatite needles precipitate after solubilization of α-TCP, when immersed in SBF solution [(22)]. In general, the compressive strength increases due to the growth and interlocking of these needles or crystals.

Thus possibly, the presence of hydroxyapatite needles increases the mechanical strength. The mechanical strength observed for the specimens developed can be considered low if it is taken into account the requirements related to trabecular bone, in which the main request is in compression. However, it should be noted that the behavior observed *in vitro* may not correspond to the behavior *in vivo*, once the bone has little irrigation and that should occur osteoconduction for the cement implant site. These factors can result an increase in mechanical strength at the implant site.

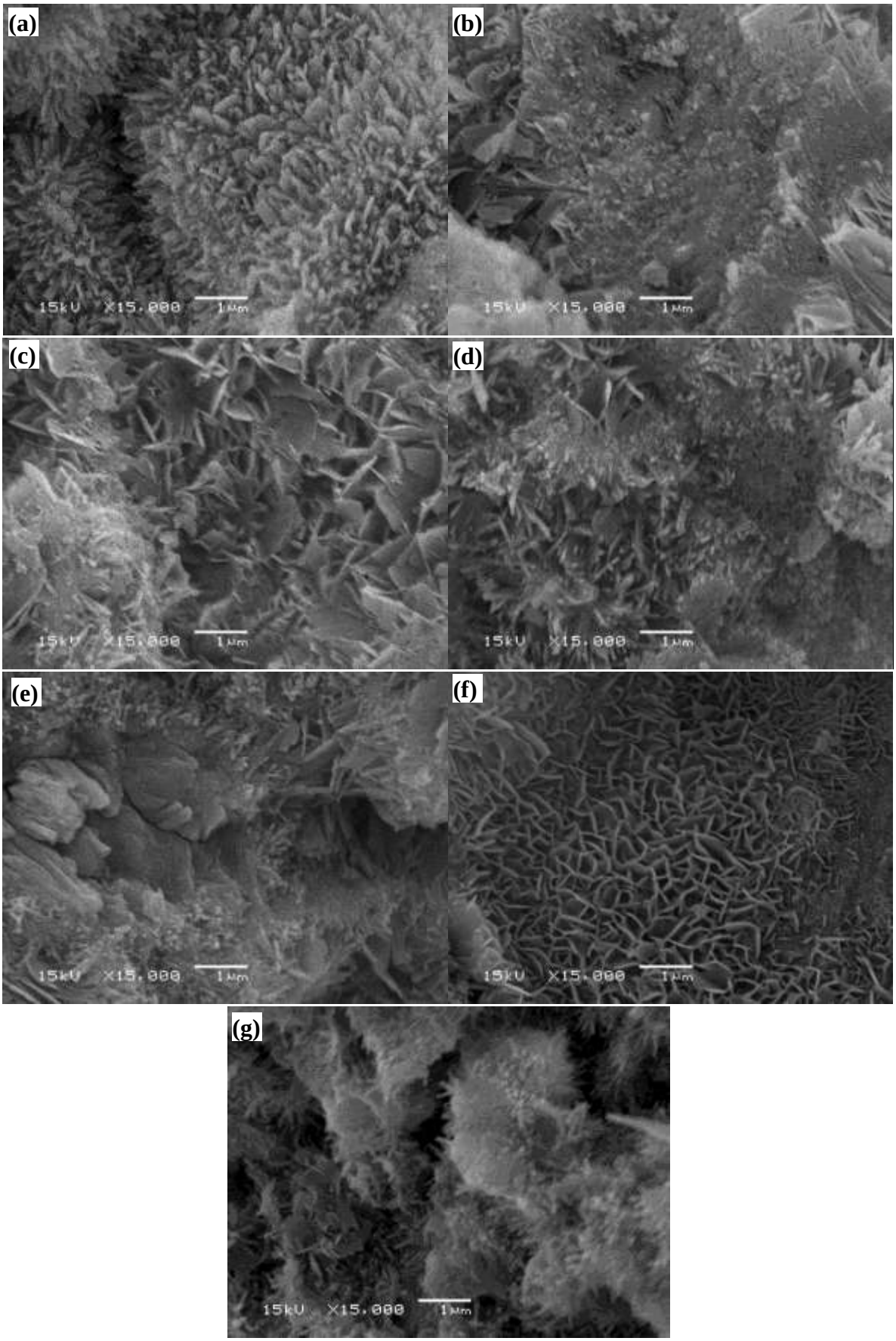

Figure 2. Fracture surface micrographs of (a) M00, (b) M05, (c) M1, (d) M2, (e) M4, (f) M8 and (g) M16.

CONCLUSIONS

By wet precipitation method, was obtained powder of α-tricalcium phosphate with high purity (100% α-tricalcium phosphate by x-ray diffraction). Evaluating the influence of milling time in ethanol on the properties of the powders, we conclude that the average particle size is strongly influenced by the milling time of the powder samples. The average particle size is a determinant factor of the liquid/powder ratio required for obtaining a paste with a consistency suitable for the conformation of the specimens. Powders with higher average particle size, ie without milling in ethanol, required smaller quantity of liquid for the conformation of the specimens, resulting in higher compressive strength and low apparent porosity. By immersion of specimens in SBF solution for seven days, was possible to verify the variation in the morphology of the samples. The sample unmilled in ethanol and for the sample with eight hours of milling the hydroxyapatite needles are greater than other samples. For α-TCP prepared by wet reaction, using calcium nitrate and phosphoric acid as reagents, wet milling in the ball milling, was not efficient to increase the compressive strength of the specimens.

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