BONE CEMENT COMPOSITE BASED ON ALPHA-TRICALCIUM PHOSPHATE AND HYDROGELS

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ABSTRACT

The calcium phosphate cements developed generally have low mechanical strength compared with bones in the body. Aiming to improve the mechanical properties of cement and address the needs of bone substitutes was developed in this work, composites based on alpha-tricalcium phosphate/hydrogels. The tricalcium phosphate, consisting essentially of alpha-tricalcium phosphate was obtained by wet precipitation process using phosphoric acid and calcium nitrate. The composites were prepared using the powder synthesized and three different types of hydrogels: poly (N-vinyl-2-pyrrolidone), poli (acrylic acid) and poly (N-vinyl-2-pyrrolidone-co-acrylic acid). The characterization of the composites was performed by compressive strength, apparent density, X-ray diffraction and scanning electron microscopy. The composites showed values of compressive strength, around 45 MPa. The soaking of the samples in Simulated Body Fluid (SBF) caused the formation of hydroxyapatite needles in the surface of the samples, demonstrating the bioactivity of the composites developed.

Keywords: alpha-TCP, cement, biomaterials, bone substitute, mechanical strength.

INTRODUCTION

Calcium phosphates are 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 (1, 2, 3). They exhibit considerably improved biological affinity and activity compared to other bioceramics.

Despite the existence of several 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 (4, 5, 6). 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 (7).

There are some disadvantages which have limited the specific use of calcium phosphate for replacing hard tissue, such as their fragile nature, the reality off between mechanical strength and porosity and the problem of particle migration. Because bone is a tissue that requires some degree of mechanical stress to maintain its vitality, long-term security of a permanent implant calcium phosphate is questionable. In order to minimize or even overcome these problems, one solution is to mix it with a suitable binder. In this sense, have been used several biopolymers such as collagen fibril glue, gelatin, chitosan and alginate (8, 9 10).

The composite, generally, have attracted the maintenance of many researchers because of the possibility to combine the advantages of different materials. In bone, calcium phosphate/hydrogel interaction has an important role in determining their mechanical properties. A fundamental aspect in the synthesis of the composite of calcium phosphate/hydrogel is the growth of calcium phosphate in aqueous medium similar to natural bone, structurally and compositionally. Some studies show that the mechanical properties of composites are narrower in scope than the values obtained for the bone (11).

The aim of this work was the study and the development of composites based on alpha-tricalcium phosphate cement/hydrogels with improved mechanical properties for use as bone substitutes.

MATERIALS AND METHODS

The TCP was synthesized in laboratory using calcium nitrate ($Ca(NO_3)_2.4H_2O$) and phosphoric acid (H_3PO_4), both with 2 M concentration, 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 solution obtained by the mixing of reagents was maintained at a temperature of 90 ± 2 °C for 24 hours. After this period, the solution was dried in an oven at 120 ± 2 °C for 24 hours and then calcined at 1300 °C for 1 hour.

After calcination, the reaction product was unagglomerate using mortar and pistil to obtain a fine powder sieved in # 325 ASTM sieve.

The powder obtained was characterized by X-ray diffraction, to evaluate the phase composition. Phillips X'Pert MPD diffractometer with a copper tube (Kα radiation = 1.5418 Ǻ) 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 (2Θ) from 10 to 40°.

Specimens were prepared from α-tricalcium phosphate synthesized and hydrogel formulations as shown in Table 1.

Table 1. Composition of hydrogels used in the manufacture of the specimen.

Sample	NVP (mol/L)	AA (mol/L)	MBAM (%)	AIBN (%)	TEMED (%)	Na_2HPO_4 (%)
C	-	-	-	-	-	2.5
VA30	30.0	-	2.0	3.0	0.2	2.5
VA03	-	14.0	2.0	3.0	0.2	2.5
VA33	30.0	14.0	2.0	3.0	0.2	2.5

The hydrogels polymerization occurred by free radical polymerization using N-Vinyl-2-pyrrolidone (NVP) (Merck, Germany) and Acrylic Acid anhydrous (AA) (Aldrich, Germany). As initiator was used Azo-bis-isobutironitrila (AIBN) (Aldrich, Germany). The bifunctional compound N,N'-methylenebisacrylamide (MBAM) (Aldrich, Germany) were used as crosslinking agent and N,N,N',N'-tetramethylethylenediamine (TEMED) was used as catalyst. Bibasic sodium phosphate was used as accelerator of the reaction.

To prepare the specimens, the paste was conformed in a mold according to ASTM F 451-95 standard (12). The mold used was stainless steel containing cavities 6 mm ± 0.1 mm in diameter and 12 mm ±0.1 mm in height. The ratio liquid/powder remained around 0.3 $mL.g^{-1}$. The mold with the specimens remained for one hour in an oven at 70 ± 2 °C for the polymerization reaction. Thereafter, the samples removed from the molds and kept in a humid environment for 24 hours at 37 ± 2 °C, then immersed in SBF (Simulated Body Fluid) for seven days at 37°C, and then characterized. The SBF solution was replaced daily.

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 surface of the samples were observed by SEM to determine if there was precipitation of hydroxyapatite, following procedures of ISO 23317 (13) standard for determination of bioactivity.

The apparent density of the samples was determined based on the ASTM C20-00 standard (14). The mechanical strength of the samples were evaluated using an Instron universal testing machine, Model 3369 at ambient temperature. Samples were compressed between plates with a constant deformation rate of 0.5 $mm.min^{-1}$. All results were the average of five measurements.

For scanning electron microscopy (SEM) were analyzed fracture surfaces of the specimens used in the compression test. For this analysis we used a JEOL scanning electron microscope, model JSM 6060.

RESULTS AND DISCUSSION

The peaks pointed on the diffractogram of Fig. 1 correspond to diffraction pattern data sheets number 00-009-0348 and 00-029-0359 (phase α) of the Joint Committee on Powder Difraction Standards – JCPDS.

Looking at Fig. 1 it can be seen only the presence of phase α-tricalcium phosphate in the powder synthesized by wet reaction, but should not exclude the presence of a small amount of phase β-tricalcium phosphate (detection limit of 5%), however may be considered pure by X-ray diffraction. Generally the transformation of β-tricalcium phosphate to α-tricalcium phosphate is slow and reversible and is required to obtain the phase α-tricalcium phosphate higher purity, the use of high holding times at temperatures above 1200 °C in order to assure maximum conversion, and quenching to preserve the α phase at room temperature. In this work it was not necessary to use high holding times, neither the execution of quenching to stabilize the α phase.

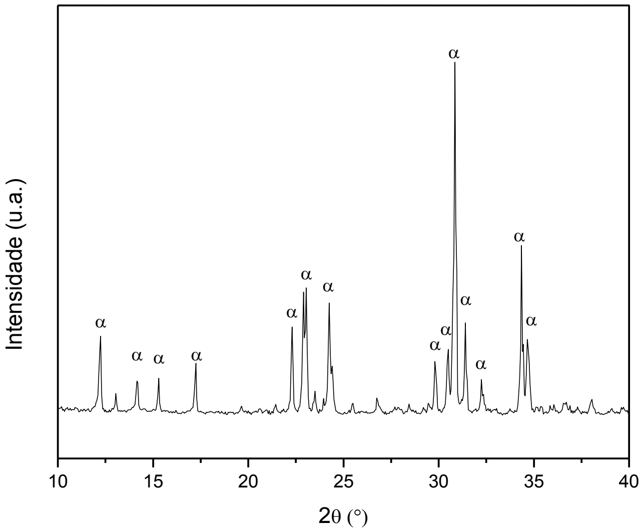

Figure 1. X-ray diffractogram obtained for the sintered powder.

In Fig. 2, are shows the results of apparent density of C sample and composites.

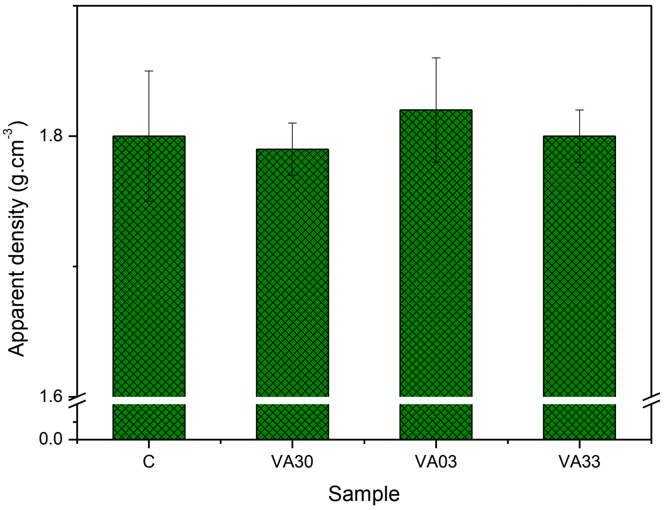

Figure 2. Apparent density of the samples.

The results of mechanical strength are shown in Table 2. The compression strength test shown that composites have similar mechanical strength (within the error of the measured values), demonstrating that the addition of hydrogels had no significant influence on this property.

Table 2. Mechanical strength of the samples.

Sample	Mechanical strength (MPa)
C	50,59 ± 12,20
VA30	46,88 ± 9,75
VA03	38,94 ± 4,19
VA33	46,71 ± 3,99

The increased mechanical strength of the cement occurs as a result of formation of intercrossing of hydroxyapatite crystals precipitated after solubilization of α-tricalcium phosphate (4). In general, the compressive strength of the calcium phosphate cements increases with increasing immersion time in SBF due to interlocking and growth of crystals.

Moreover, the presence of cement accelerators such as Na_2HPO_4, can produce a decrease in mechanical properties (15). Probably the slightly higher mechanical strength of the hydrogel-free composition is due to the separation of hydroxyapatite crystals promoted by hydrogel polymer in the composite structure.

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 to the cement implant site. These factors can result an increase in mechanical strength at the implant site.

The Fig. 3 shows the surface fracture micrographs of samples, placed in SBF for seven days.

Analyzing the micrographs for the fracture surface of the samples can be observed the formation of hydroxyapatite crystals. The presence of hydroxyapatite crystals appears to have contributed to increase the mechanical strength of the samples.

In the VA03 sample can be observed hydroxyapatite needles formation but also the presence of Hadley grains (16). The formation of this structure begins with the dissolution of α-TCP particles in the liquid phase of cement. The liquid phase receives Ca and P becoming supersaturated in these ions and precipitating the more stable phase (CDHA) on the surface of the particles of α-TCP, the site of highest concentration of ions and the lowest surface energy. The α-TCP particles continues to solubilize, however ions have now to overcome the layer of precipitated CDHA, which becomes thicker as the reaction takes place. At the end of the reaction, when nearly all α-TCP was consumed, there will be a shell of hydroxyapatite (CDHA) with an empty inside (Hadley grains), where the particle originally α-TCP was completely solubilized (16). The formation of this structure generally decreases the mechanical strength of the material and this also occurred with this sample.

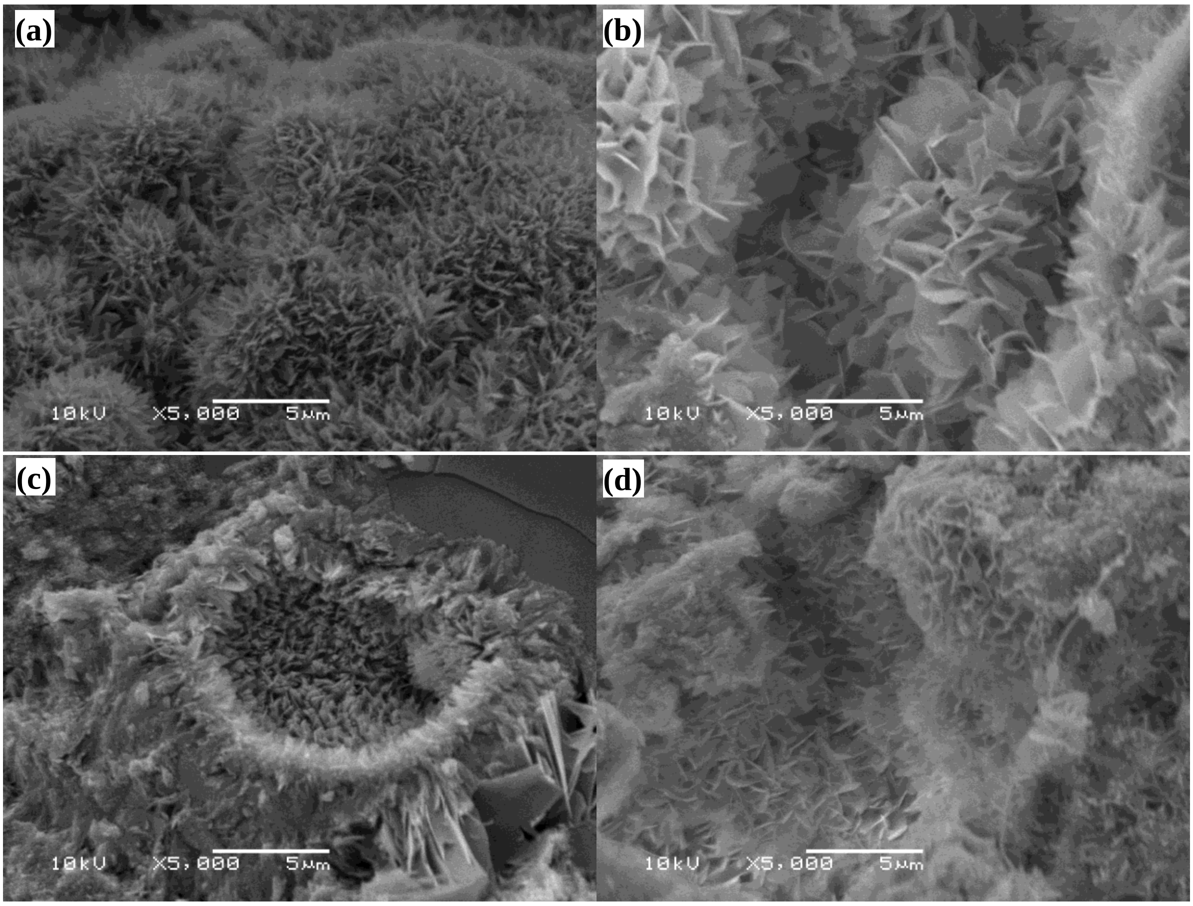

Figure 3. Fracture surface micrographs of (a) C, (b) VA30, (c) VA03 and (d) VA33.

Figure 4 shows the X-ray diffraction patterns obtained for samples from the specimens used in testing of mechanical strength after milling. The samples presented in its composition the phase α-tricalcium phosphate and hydroxyapatite.

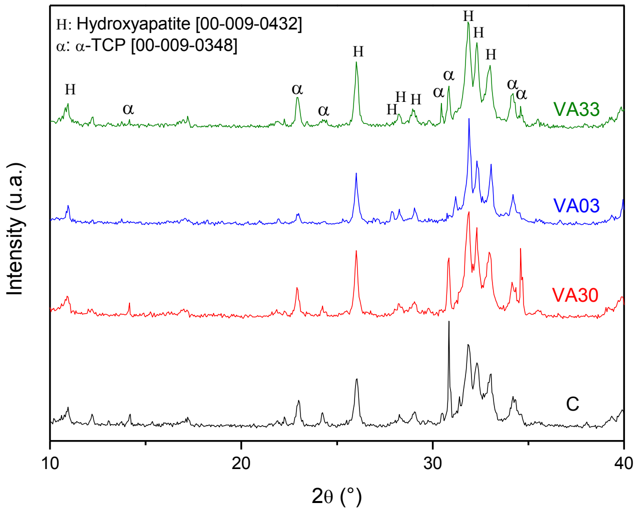

Figure 4. XRD patterns for samples obtained after immersion in SBF and compression test.

X-ray diffraction (XRD) patterns show that the presence of hydrogel and the type of hydrogels used influences the final products. The peaks pointed in the diffractograms in the Fig. 4, correspond to the diffraction pattern data sheets number 00-029-0359 (α-TCP) and 00-009-0432 (Hydroxyapatite) of the Joint Committee on Powder Standards Diffraction - JCPDS.

The result shows that all samples showed the hydroxyapatite formation after immersion in SBF, but not all α-TCP was converted into hydroxyapatite. 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 (17).

CONCLUSIONS

It was possible to obtain α-tricalcium phosphate by the method of synthesis by wet reaction, not described in literature. The synthesis method proved to be an efficient process to obtain calcium phosphates. The samples prepared with only 2.5%

Na2HPO4 solution, showed higher average mechanical strength when compared to samples prepared with hydrogels. The composite α-tricalcium phosphate/hydrogel proved to be a material with potential for use as bone substitute, but later studies with reduction of the amount of liquid, and with consequent increase of mechanical strength, are required. The formation of hydroxyapatite crystals on the samples surface indicates the bioactivity of the synthesized powder, and the developed composite.

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