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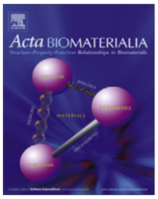

# Relevance of the setting reaction to the injectability of tricalcium phosphate pastes

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ABSTRACT

The aim of the present work was to analyze the influence of the setting reaction on the injectability of tricalcium phosphate (TCP) pastes. Even if the injection was performed early after mixing powder and liquid, powder reactivity was shown to play a significant role in the injectability of TCP pastes. Significant differences were observed between the injection behavior of non-hardening β-TCP pastes and that of self-hardening α-TCP pastes. The differences were more marked at low liquid-to-powder ratios, using fine powders and injecting through thin needles. α-TCP was, in general, less injectable than β-TCP and required higher injection loads. Moreover, clogging was identified as a mechanism hindering or even preventing injectability, different and clearly distinguishable from the filter-pressing phenomenon. α-TCP pastes presented transient clogging episodes, which were not observed in β-TCP pastes with equivalent particle size distribution. Different parameters affecting powder reactivity were also shown to affect paste injectability. Thus, whereas powder calcination resulted in an increased injectability due to lower particle reactivity, the addition of setting accelerants, such as hydroxyapatite nanoparticles, tended to reduce the injectability of the TCP pastes, especially if adjoined simultaneously with a $Na_2HPO_4$ solution. Although, as a general trend, faster-setting pastes were less injectable, some exceptions to this rule were found. For example, whereas in the absence of setting accelerants fine TCP powders were more injectable than the coarse ones, in spite of their shorter setting times, this trend was inverted when setting accelerants were added, and coarse powders were more injectable than the fine ones.

## 1. Introduction

Minimally invasive surgery techniques offer many benefits for the patient over traditional surgeries, such as fewer associated injuries, quicker recovery time and less pain. Moreover, shorter hospital stays are needed, often allowing outpatient treatments that result in reductions in health costs [1]. However, these techniques require a new generation of injectable biomaterials, able to be implanted through small incisions, using laparoscopic devices. When targeting bone-grafting applications, calcium phosphate cements (CPC) are promising candidates because, together with their potential injectability, they have the ability to harden in an appropriate period of time, giving stability to the implantation site. For these reasons, CPC are increasingly being used as synthetic bone grafts in areas such as orthopedic surgery, dentistry and maxillofacial surgery, with excellent biocompatibility and in vivo osteoconductivity [2–4]. Moreover, the possibility of loading them with drugs, proteins or growth factors increases their potential for bone regeneration applications [5].

In addition to the direct application as bone grafting materials, recent developments have raised the possibility of using CPC in combination with different low-temperature processing techniques to obtain pre-set scaffolds [6–9]. Also, in some of these processing techniques, injectability becomes a critical issue. This is the case of robocasting, where a CPC slurry is injected through a thin nozzle to print three-dimensional macroporous structures layer by layer [10].

In spite of many contributions over the last decade [11–20], there are still many unresolved issues in the understanding of the phenomena underlying the injectability of CPC. The complexity of the system comes from the fact that clinically useful CPC are hydraulic biphasic pastes, with high solid content (>50%) and broad particle size distributions (PSD), which make them non-Newtonian fluids with viscoelastic rheological behavior that depends on shear rate [21–24]. Moreover, the evolution of the rheological properties during the setting reaction adds further complexity to the system.

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Alpha tricalcium phosphate ($\alpha$-$Ca_3(PO_4)_2$; $\alpha$-TCP) is one of the most common reactants in CPC. In spite of its low solubility ($-\log K_s = 25.5$; at 25 °C [25]), when $\alpha$-TCP is mixed with water, it hydrolyzes to a calcium deficient hydroxyapatite ($Ca_9(HPO_4)(PO_4)_5OH$; CDHA) according to the reaction in Eq. (1). This conversion produces an entangled network of CDHA crystals that is responsible for the setting and progressive hardening of the cement paste [26,27].

$$3\alpha\text{-}Ca_3(PO_4)_2(s) + H_2O(l) \rightarrow Ca_9(HPO_4)(PO_4)_5OH(s) \quad (1)$$

In order to identify the most important parameters controlling the injectability of CPC, some authors focused their attention on less reactive calcium phosphate pastes [28–30]. In these studies, beta tricalcium phosphate ($\beta$-TCP) was proposed as a model because of its similarity to $\alpha$-TCP and its lower solubility in water ($-\log K_s = 28.9$; at 25 °C [31]), which prevents it from setting in physiological conditions. This model explained some of the mechanisms underlying the process of injection of hydraulic pastes and identified the most relevant variables, including liquid to powder (L/P) ratio, PSD, particle morphology, plastic limit (PL) and viscosity of the liquid phase [28]. However, this model did not take into account the evolution of the rheological properties with the advance of the setting reaction.

The aim of the present work was to analyze the influence of the setting reaction on the injectability of tricalcium phosphate (TCP) pastes. During the study, $\alpha$-TCP was tested as self-setting cementitious material, whereas $\beta$-TCP was employed as a control material to discern the effect of the advancement of the setting reaction on injectability. Moreover, the role of different additives commonly used to accelerate the setting of $\alpha$-TCP-based cements [32–34] and their influence on the injectability of the pastes were analyzed: (i) the incorporation of precipitated hydroxyapatite nanoparticles (pHA), used as seeds to promote heterogeneous nucleation of the precipitation product [35]; and (ii) the use of a $Na_2HPO_4$ solution as a liquid phase, which, based on the common ion effect [36], modifies the chemical equilibrium of Eq. (1), promoting the precipitation of CDHA.

## 2. Materials and methods

### 2.1. Powder fabrication and characterization

A stoichiometric mixture of calcium carbonate ($CaCO_3$; Sigma-Aldrich C4830) and monetite ($CaHPO_4$; Sigma-Aldrich C7263) was used to synthesize $\beta$-TCP and $\alpha$-TCP in a furnace (Hobersal CNR-58) at 1100 and 1400 °C, respectively. In the case of $\beta$-TCP, after the thermal treatment, the material was cooled down inside the furnace. For $\alpha$-TCP, quenching was performed to stabilize the alpha phase. Powders were obtained by dry milling the material in a planetary mill (Pulverisette 6, Fritsch Gmbh) using an agate jar and balls. The milling protocols were adjusted to obtain similar PSD in the two starting powders. Fine (F) powders were obtained using 10 balls ($d = 30$ mm) for 60 min at 450 rpm, followed by a second milling for 60 min at 500 rpm with 100 balls ($d = 10$ mm). In the case of coarse (C) powders, two different milling protocols were applied, 15 min at 450 rpm for $\alpha$-TCP and 70 min at 150 rpm for $\beta$-TCP (10 balls of 30 mm in both cases).

Since the milling process has been shown to affect the reactivity of the powders [37], the behavior of as-milled and calcined powders was compared. A calcination process was applied to previously milled fine $\alpha$-TCP and $\beta$-TCP powders, by applying a thermal treatment at 500 °C for 24 h.

The PSD of both F and C powders was analyzed by laser diffraction (LS 13 320 Beckman Coulter) and the specific surface area (SSA) was determined by nitrogen adsorption according to the Brunnauer–Emmet–Teller (BET) method (ASAP 2020 Micromeritics). The phase composition of the different powders was assessed by high resolution X-ray diffraction (XRD; PANalytical, X'Pert PRO Alpha-1). The X-ray powder diffraction measurements were obtained by scanning in Bragg–Brentano geometry using Cu $K_\alpha$ radiation. The experimental conditions were: $2\theta$ scan step 0.020° between 4° and 100°, counting time 150 s per point, voltage 45 kV and intensity 40 mA. The diffraction patterns were compared with the Joint Committee on Powder Diffraction Standards for $\alpha$-TCP (JCPDS No. 9-348), $\beta$-TCP (JCPDS No. 9-169) and HA (JCPDS No. 9-432). The presence of an amorphous phase was assessed by the external standard method, adding a known amount of a zinc oxide standard (Panreac 141786-1210) to the different studied powders. Rietveld refinements were carried out using the FullProf Suite software package in order to quantify the phases present using the Inorganic Crystal Structure Database (ICSD) including structural models for $\alpha$-TCP (ICSD No. 923), $\beta$-TCP (ICSD No. 6191), HA (ICSD No. 151414) and ZnO (ICSD No. 26170). Powder morphology was observed by field emission scanning electron microscopy (FE-SEM; JEOL J7000) in Au–Pd-coated samples.

### 2.2. Experimental design

The experiments were performed according to two factorial designs. The first one aimed to elucidate whether a different behavior in terms of injectability was observed when comparing $\alpha$-TCP and $\beta$-TCP polymorphs. A $2^3$ factorial design was followed, where the three independent variables were the type of polymorph ($\alpha$ vs. $\beta$), the PSD (F vs. C) and the L/P ratio (0.35 vs. 0.45 ml $g^{-1}$). The second set of experiments aimed to assess the effect of some additives commonly used to accelerate the setting reaction on PL, injectability, initial setting time and final setting time of the paste. In this case, the experiments were performed according to a $2^4$ factorial design, selecting four variables, namely, type of polymorph ($\alpha$ vs. $\beta$), powder granulometry (F vs. C), use or not of $Na_2HPO_4$ as liquid phase (0 vs. 2.5 wt.% in the liquid phase) and addition or not of pHA as a seed (0 vs. 2 wt.% in the solid phase). The L/P ratio was fixed at 0.35 ml $g^{-1}$.

### 2.3. Paste preparation

$\alpha$-TCP fine ($\alpha$-TCP/F) and coarse ($\alpha$-TCP/C), as well as $\beta$-TCP fine ($\beta$-TCP/F) and coarse ($\beta$-TCP/C) were used as solid phases. In some cases, pHA nanoparticles (2 wt.%; Alco, 1.02143) with a SSA of 55.5 $m^2\,g^{-1}$ were homogeneously mixed with the powders. Ultrapure water or an aqueous solution of 2.5 wt.% $Na_2HPO_4$ (Panreac 131679.1210) was employed as the liquid phase. The different pastes were obtained by mixing thoroughly in a glass mortar the corresponding amount of solid phase with 2 ml of liquid phase, using L/P ratios between 0.35 and 0.55 ml $g^{-1}$.

### 2.4. PL and setting times

The PL was determined according to standard ASTM D-4318-00 [38] adapted to small quantities of material [28]. Briefly, 0.2 ml of liquid phase was added to 1 g of solid phase and mixed. Afterwards, liquid phase was added dropwise, mixing the paste after each addition. The blend was weighed once it had acquired the consistency of a workable paste. Then, the blend was dried and weighed again to obtain the total amount of water used. The corresponding L/P ratio was considered as the PL.

Setting times were assessed with a Gillmore needle testing set according to standard ASTM C266-99 [39]. As in the case of PL, setting times were determined at room temperature.