Manuscript Number: AB-12-1460R1

Title: Relevance of the setting reaction to the injectability of tricalcium phosphate pastes

Article Type: Full Length Article

Keywords: Calcium phosphate cement; hydroxyapatite; injectability; setting reaction; tricalcium phosphate; minimally invasive surgery; injectable scaffolds.

Corresponding Author: Professor Maria-Pau Ginebra, Ph.D.

Corresponding Author's Institution: Technical University of Catalonia (UPC)

First Author: Edgar-Benjamin Montufar, Ph.D.

Order of Authors: Edgar-Benjamin Montufar, Ph.D.; Yassine Maazouz; Maria-Pau Ginebra, Ph.D.

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 shown to affect also 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 were found to this rule. For example, whereas in absence of setting accelerants fine TCP powders were more injectable than the coarse ones in spite of their shorter setting times, when setting accelerants were added this trend was inverted, and coarse powders were more injectable than the fine ones.

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

E.B. Montufar^{a,b,c†}, Y. Maazouz^{a,b†}, M.P. Ginebra^{a,b,*}

^aBiomaterials, Biomechanics and Tissue Engineering Group, Dept. Materials Science and Metallurgical Engineering, Technical University of Catalonia, Spain.

^bBiomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain.

^cInstitute for Bioengineering of Catalonia, Spain.

* Corresponding author, Tel: +34 93 401 7706; Fax: +34 93 401 6706; E-mail: maria.pau.ginebra@upc.edu

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 shown to affect also 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 were found to this rule. For example, whereas in absence of setting accelerants fine TCP powders were more injectable than the coarse ones in spite of their shorter setting times, when setting accelerants were added this trend was inverted, and coarse powders were more injectable than the fine ones.

Keywords: Calcium phosphate cement; hydroxyapatite; injectability; setting reaction; tricalcium phosphate; minimally invasive surgery; injectable scaffolds.

†EBM and YM contributed equally to this work

1. Introduction

Minimally invasive surgery techniques offer many benefits for the patient over traditional surgeries, such as less associated injuries, quicker recovery time and less pain. Moreover, shorter hospital stays are needed, often allowing outpatient treatments that result in reducing 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 (CPCs) 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, CPCs are being increasingly 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 put forward the possibility of using CPCs 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 3D 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 CPCs. The complexity of the system comes from the fact that clinically useful CPCs are hydraulic biphasic pastes, with high solid content (above 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.

Alpha tricalcium phosphate (α - $\text{Ca}_3(\text{PO}_4)_2$; α -TCP) is one of the most common reactants in CPCs. In spite of its low solubility ($-\log K_s = 25.5$; at 25 °C [25]), when α -TCP is mixed with water, it hydrolyzes to a calcium deficient hydroxyapatite ($\text{Ca}_9(\text{HPO}_4)(\text{PO}_4)_5\text{OH}$; 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].

In order to identify the most important parameters controlling the injectability of CPCs, some authors focused their attention on less reactive calcium phosphate pastes [28-30]. In these studies beta tricalcium phosphate (β -TCP) was proposed as a model due to its similarity to α -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 allowed to understand some of the mechanisms underlying the process of injection of hydraulic pastes and to identify 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. Along the study α -TCP was tested as self-setting cementitious material, whereas β -TCP was employed as 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 α -TCP based cements [32-34] and their influence on the injectability of the pastes was analyzed: i) the incorporation of precipitated hydroxyapatite nanoparticles (pHA), used as a seed 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 β -TCP and α -TCP in a furnace (Hobersal CNR-58) at 1100 and 1400 °C respectively. In the case of β -TCP, after the thermal treatment the material was cooled down inside the furnace. For α -TCP, a 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 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 α -TCP and 70 min at 150 rpm for β -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 α - and β -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 α radiation. The experimental conditions were: 2 θ 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 α -TCP (JCPDS No. 9-348), β -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 reference No. 141786-1210) to the different studied powders. Rietveld refinements were carried out using the software package FullProf Suite, in order to quantify the phases present using the Inorganic Crystal Structure Database (ICSD) including structural models for α -TCP (ICSD No. 923), β -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 was aimed at elucidating whether a different behavior in terms of injectability was observed when comparing α - and β -TCP polymorphs. A 2³ factorial design was followed, where the three independent variables were the type of polymorph (α vs. β), the PSD (F vs. C) and the L/P ratio (0.35 vs. 0.45 mL g⁻¹). The second set of experiments was aimed at assessing 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 2⁴ factorial design, selecting four variables, namely, type of polymorph (α vs. β), powder granulometry (F vs. C), use or not of Na₂HPO₄ 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

α-TCP fine (α-TCP/F) and coarse (α-TCP/C), as well as β-TCP fine (β-TCP/F) and coarse (β-TCP/C) were used as solid phases. In some cases pHA nanoparticles (2 wt.%; Alco, 1.02143) with a SSA of 55.5 m² g⁻¹ were homogenously mixed with the powders. Ultrapure water or an aqueous solution of 2.5 wt.% Na₂HPO₄ (Panreac,131679.1210) were employed as liquid phases. The different pastes were obtained by mixing thoroughly in a glass mortar the correspondent 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. Plastic limit and setting times

The plastic limit (PL) was determined according to ASTM D-4318-00 standard [38] adapted to small quantities of material [28]. Briefly, 0.2 mL of liquid phase were 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 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 Gilmore needle testing set according to ASTM C266-99 standard [39]. As in the case of PL, setting times were determined at room temperature.

2.5. Injectability test

An extrusion test was performed, as described in [40]. Briefly, after homogenization the paste was introduced in a commercial syringe with an aperture of 2 mm of diameter (13 mm diameter cartridge with nominal capacity of 5 mL). The syringe was placed in a customized injection setup in a universal testing machine (BIONIX, MTS, MN). 2.5 min after mixing the solid and liquid phases, the paste was injected at a constant velocity of 15 mm min⁻¹. The load was recorded as function of the plunger run. The test was stopped when the applied load reached 100 N. The injectability percentage was calculated applying the following equation:

$$Inj = (W_F - W_A) / (W_F - W_E) * 100 \quad (\text{eq. 2})$$

Where: lnj is the Injectability percentage, W_E is the weight of the empty syringe, W_F is the weight of the syringe full of paste and W_A is the weight of the syringe after the injection.

In addition to the quantification of the injectability percentage, the yield load and the injection load were obtained from the extrusion curves (load-plunger run), where the plunger run was considered as the percentage of displacement of the syringe plunger respect to its initial position.

In some specific cases the injection was started after different post-mixing times of 2.5, 5, 60 or 240 min. In other tests a 3 cm-length tapering cannula with a final inner diameter of 0.84 mm was used. All experiments were performed at least in triplicate for statistical analysis.

2.6. Phase composition and microstructure

The phase composition of α - and β -TCP/C pastes prepared at L/P ratio of 0.35 mL g^{-1} without additives after 7 days immersion in water at 37°C was determined by XRD, and the microstructure was observed by FE-SEM, after coating with Au-Pd. In addition, the evolution of the SSA during the early stages of the setting reaction was assessed. To this end, the setting reaction was stopped by quenching the paste in liquid nitrogen at different time points after mixing with water the as-milled or the calcined α - and β -TCP/F powders, followed by lyophilization. Then, the SSA was measured by nitrogen adsorption following the BET theory.

2.7. Dynamic viscosity

The effect of the incorporation of pHA and/or Na_2HPO_4 on the dynamic viscosity of α -TCP/F was studied using a controlled stress rheometer (RC20-CPS, RheoTec GmbH). In order not to overcome the torque limit of the equipment and prevent the breaking down of the material at high shear rate, the L/P ratio of the paste was set to 0.55 mL g^{-1} . Parallel plate geometry was employed, with 25 mm diameter plates onto which an adhesive grinding paper with controlled roughness (Buehler, GP 240) was glued in order to avoid slipping phenomena. The paste was placed onto the bottom plate and the upper plate was slowly set to a gap of 2 mm, thus avoiding any exudation of the liquid phase from the paste. Two minutes after the mixture of solid and liquid, the sample was subjected to a pre-shear at 15 s^{-1} during 30 s, followed by a reconstruction step of 1 min in order to reset the structural memory of the material. During the test, the shear rate was increased linearly from 0 to 100 s^{-1} in 30 seconds.

2.8. Hardening reaction

The effect of the incorporation of pHA and/or Na_2HPO_4 on the hardening kinetics of α -TCP/C was determined by assessing the compressive strength of the cement specimens after different reaction times. Cylindrical specimens (6 mm diameter and 12 mm height) were prepared at a L/P ratio of 0.35 mL g^{-1} in Teflon moulds and immersed in Ringer's solution (0.9 % NaCl) at 37°C for different time intervals (1, 2, 4, 8, 16, 32, 64 and 360 h). Then, the samples were quenched in acetone to stop the hydrolysis reaction and tested in a universal testing machine (BIONIX, MTS, MN) equipped with a load-cell of 2.5 KN, at a crosshead speed of 1 mm min^{-1} . Six specimens were tested for each time and formulation.

2.9. Statistical analysis

The factorial designs were analyzed in terms of the main effects of the independent variables and their interactions using the software Minitab 15 (Minitab Inc.). Results are reported as mean $\pm$ standard deviation between series. Analysis of variance (ANOVA) of the responses was used to statistically analyze the data.

3. Results

3.1. Powder characteristics

The volumetric PSD of the coarse and fine powders of the two TCP polymorphs are presented in **Figure 1**. Similar PSD were obtained for the α -TCP and β -TCP powders, as confirmed by the SSA values, displayed in **Table 1**. The morphology of the particles is shown in Figure 1. The phase composition of the different powders, obtained by Rietveld refinement of the XRD diagrams, with an intrinsic error of 1 wt.% is also displayed in Table 1. As expected, F powders contained a higher proportion of amorphous phase, which was eliminated after calcination, while the content of β -TCP slightly increased.

3.2. Injection behavior of alpha and beta tricalcium phosphate pastes

In the first set of experiments, the injection behavior of as-milled α -TCP and β -TCP pastes was studied varying powder granularity (F vs. C) and L/P ratio (0.35 vs. 0.45 mL g^{-1}). Higher L/P ratios were not tested because complete injectable pastes were obtained at 0.45 mL g^{-1} irrespective to the polymorph or particle size used. **Figure 2** shows the extrusion curves (load vs. plunger run) during paste injection. Three relevant stages were found in the reported curves. The first stage consisted in an initial transient regime in which the applied load increased rapidly until reaching the yield load that corresponded to the load needed for the

paste starting to flow. In the second stage, pastes flowed in steady state conditions at a constant load, referred to as injection load. In the third and final stage, the load increased again. This was caused either by the fact that the syringe was empty or because the paste was not further injectable. In the former case, a sharp load increase was registered at the end of the plunger run, whereas in the latter case, the load increased less abruptly until reaching the maximum load allowed in the testing protocol (100 N). In the particular case of α -TCP/C, force spikes were found systematically in the extrusion curve, in all the samples tested, caused by clogging of the syringe aperture (see Figure 2b). Nonetheless, the applied load was able to unblock the syringe, allowing the paste to flow again, until completion of the extrusion test. In particular, the α -TCP/C paste prepared at a nominal L/P ratio of 0.45 mL g^{-1} , was totally injectable at a constant load aside from the clogging load peaks (see Figure 2b). In order to assess if clogging was associated to a change in the L/P ratio, the paste injected before and after a clogging load spike was collected for the α -TCP/C paste (Figure 2b). The L/P ratio of the extruded paste was quantified by determining the weight loss of the paste before and after quenching in liquid nitrogen and lyophilization ($n=3$). The values obtained were $0.47 \pm 0.02 \text{ mL g}^{-1}$ (before) and $0.46 \pm 0.04 \text{ mL g}^{-1}$ (after). The LPR of the paste immediately after mixing was $0.45 \pm 0.03 \text{ mL g}^{-1}$. The statistical analysis of the data confirmed that there were no significant differences between these values, thus the LPR was kept constant along the injection ($p > 0.05$).

The average values and standard deviations of the yield load, injection load and injectability obtained for the different pastes, together with the setting times, are summarized in **Table 2**. In general, setting times were longer for β - than for α -TCP, and for C powders than for F ones. In agreement with previous works, injectability and setting times increased with increasing L/P ratio, while yield load and injection load decreased. At low L/P ratio (0.35 mL g^{-1}), α -TCP pastes were less injectable than β -TCP ones and required higher injection loads. These differences were more pronounced for F powders, while for C powders they were almost negligible. Moreover, F powders required lower injection loads and were more injectable than C ones, being the differences larger for β -TCP than for α -TCP. In contrast, at high L/P ratio (0.45 mL g^{-1}) the injectability, yield and injection loads did not change significantly between TCP polymorphs or powder granulometry.

Although β -TCP was chosen as a model for a non-setting or non-curing ceramic paste, in fact, as shown in Table 2, well-defined initial and final setting times were found for the as-milled β -TCP pastes using the Gillmore needles method. However, the XRD patterns displayed in **Figures 3a** and **b** demonstrated that, whereas α -TCP totally hydrolyzed to a calcium deficient

hydroxyapatite, β -TCP did not hydrolyze after 7 days in contact with water, as no new phase was detected by XRD. Only a slight increase in the intensity and a reduction in the width of the XRD peaks was observed. Furthermore, SEM images showed very different microstructures. Whereas a network of entangled crystals was observed after 7 days in the α -TCP sample (Figure 3c), with no trace of the α -TCP particles, in the case of β -TCP (Figure 3d) the starting particles were clearly visible, and only small precipitates were observed, probably in a non-detectable amount by XRD.

In order to assess whether this partial reaction of the β -TCP powders could be attributed to an amorphisation produced during milling, and more importantly, to clarify up to what extent this could affect the injectability of the pastes, the behavior of as-milled and calcined powders was compared. As shown in **Figure 4a**, when as-milled or calcined powders were injected at different times after mixing powder and liquid, the behavior was very different. First, the injectability of the calcined β -TCP was higher than that of uncalcined β -TCP, and was kept constant for over 4 h, as expected for a non-self-setting paste. In contrast, the injectability of the uncalcined β -TCP powder decreased around 50 % after 1 h and the paste could not be injected at all after 4 h. Second, the injectability of the α -TCP pastes was initially similar for the calcined and the uncalcined powders, and lower than that of calcined β -TCP. However, it decreased over time, to a higher extent for the as-milled than for the calcined α -TCP powder. Interestingly, there was a correlation between this behavior and the SSA evolution at early reaction stages, as shown in **Figure 4b**. The SSA of the uncalcined β - and α -TCP increased over time, indicating the precipitation of a new phase with high SSA, even at short times. In contrast, the SSA of the calcined powders did not increase after mixing with water, even after 60 min.

The effect of the internal needle diameter on the injectability of uncalcined α - and β -TCP pastes with different granularities is shown in **Figure 5**. Two different injection configurations were used: 1) injection without cannula, through the aperture of the syringe (inner diameter 2 mm); and 2) injection through a needle with a tapered tip, with a final inner diameter of 0.84 mm. The injectability of α -TCP was significantly reduced when reducing the inner diameter of the needle, in particular for C powders. In contrast, injectability was hardly altered in the β -TCP pastes. Interestingly, no statistically significant differences in yield and injection loads were observed when reducing the aperture size of the cannula. When a small inner diameter cannula was employed, the extrusion curves of the α -TCP pastes (F and C) presented a sharp increment of load at the end of the steady state plateau (Figures 5c and d). This abrupt load increment matched with the sudden stop of the injection. As in the case of α -TCP/C without

cannula (Figure 5d), the sharp load increase was probably produced by the clogging of the syringe aperture which, in the small-diameter needle, was not overcome by the applied force. No indications of clogging were found in the β -TCP pastes, irrespective of the inner diameter of the cannula or the particle size.

3.3. Effect of the incorporation of setting accelerant additives on the injectability of the pastes

The second set of experiments was aimed at determining the effect of the incorporation of setting accelerants on the injectability of TCP pastes. The separate and combined effects of the Na_2HPO_4 solution and/or the pHA nanoparticles on the PL, setting times, injectabilities, yield loads and injection loads of α - and β -TCP pastes are summarized in **Table 3**. It is important to note that no statistically significant differences were found in the PL of the different formulations, which in average was $0.23 \pm 0.01 \text{ mL g}^{-1}$. A summary of the statistical analysis of the factorial design is shown in **Table 4**.

As expected, the most significant effect on the setting time was the type of polymorph. The setting time measured with Gillmore needles was significantly lower in α - than in the β -TCP pastes ($p < 0.001$), this effect being more marked for the C than for the F powders. In this experiment as-milled powders were used, this being the reason for this apparent setting of β -TCP measured by the Gillmore needles. The second most significant variables were the particle size ($p < 0.008$) and the use of the Na_2HPO_4 solution ($p < 0.01$). The addition of pHA seeds was also significant, but to a lesser extent ($p < 0.03$). Moreover, the incorporation of both additives at the same time produced the highest reduction of the setting times, for both β - and α -TCP.

When looking at the injectability, the addition of pHA nanoparticles was the most significant parameter ($p < 0.0009$), especially when the accelerant solution was used, and for the fine powder, as shown by the significance of the interactions between these two parameters (pHA/ Na_2HPO_4 and pHA/PSD respectively). The type of polymorph was also significant, being more injectable the β - than the α -TCP pastes ($p < 0.002$). Although from the analysis of the main effects it could seem that **in general** the addition of the sodium phosphate salt in the liquid phase tended to reduce the injectability ($p < 0.005$), a closer examination of its interaction with pHA revealed that, in fact, in absence of pHA the sodium phosphate solution slightly increased injectability **except for β -TCP/F**, whereas when pHA was present, a strong reduction was found. Thus, the simultaneous incorporation of pHA and Na_2HPO_4 considerably reduced the injectability, although to a different extent on the two polymorphs and PSD. Whereas this reduction was very significant for the α -TCP, in the case of β -TCP pastes it was significant only for the F powder, being irrelevant for the C powder.

The influence of the different parameters on the yield load was similar to that described for injectability. Although the independent incorporation of pHA or Na_2HPO_4 solution increased the yield loads, it was the simultaneous incorporation of these two additives that had the most significant effect, in particular for α -TCP powders, as revealed by significance of the two-order ($\text{Na}_2\text{HPO}_4/\text{pHA}$) and three-order (TCP/ $\text{Na}_2\text{HPO}_4/\text{pHA}$) interactions.

These trends are illustrated in **Figure 6**, where representative extrusion curves for α - and β -TCP pastes containing the different additives are shown.

The dynamic viscosity of the α -TCP/F pastes with the independent or simultaneous incorporation of the two additives is shown in **Figure 7**. The increase in viscosity due to the incorporation of Na_2HPO_4 was lower than the increase produced by the incorporation of pHA. The highest viscosity was found when simultaneously incorporating Na_2HPO_4 and pHA. All the pastes presented a shear thinning behavior and, as a consequence, the differences in viscosity amongst the different samples decreased with increasing shear rate.

Finally, the effect of the accelerants on the hardening kinetics of CPCs is illustrated in **Figure 8**, where the evolution of the compressive strength of the α -TCP/C paste with time is shown. No significant increase in compressive strength with time was observed in β -TCP pastes. The samples prepared with α -TCP and water as liquid phase presented an induction period of about 8 h during which a very small increase in compressive strength was found, slightly higher in presence of pHA. Afterwards, the hardening rate became faster, reaching after 360 h a compressive strength of 37.6 ± 2.7 MPa in absence of seed or 70.5 ± 2.7 MPa when 2 wt.% pHA was added. In contrast, when the Na_2HPO_4 solution was used as the liquid phase, the hardening of the cement started increasing since the first hours of reaction, reaching a value of 53.9 ± 1.8 MPa after 360 h in absence of seeds, whereas a lower value (37.9 ± 1.5 MPa) was found when both additives were used simultaneously.

4. Discussion

4.1. General considerations

One of the objectives of the present work was to determine the influence of the setting reaction on the injectability of calcium phosphate pastes. α - and β -TCP polymorphs were selected as a model, since they have different solubilities. In fact, α -TCP is known to have a self-setting ability through its hydrolysis at room or body temperature to a CDHA (see eq.1), and for this reason it is used as a reactant in CPCs. In contrast, β -TCP, which is less soluble, does not present this self-curing capacity. Moreover, other parameters affecting the kinetics of

the setting reaction in CPCs were considered, such as the calcination of the powder, or the addition of accelerant agents in the cement formulations. Even if, as a matter of fact, the injection of the paste is supposed to take place immediately after mixing the liquid and the powder phases of the cement, we were interested in evaluating the influence of the incipient setting reaction on the injectability of the pastes.

Since one of the factors that influence most the injectability is the particle size, the first step was to obtain similar PSD for both TCP polymorphs to be able to compare them. Although β -TCP with the same PSD could be obtained by heat treatment of α -TCP, it was preferred to use the same method (i.e. sintering followed by milling) to obtain the two polymorphs, since milling is known to alter the reactivity of the powders. This allowed in addition to evaluate the effect of calcination on both α - and β -TCP powders. To obtain similar PSD, SSA and particle morphologies (Figure 1) it was necessary to adjust the milling protocols in the case of the coarse powders. This can be explained by the fact that, due to higher sintering temperatures and times, α -TCP comminution required more energy than β -TCP. However, in the case of fine powders, where a more energetic milling was applied, the same milling protocol resulted in similar PSD for the two polymorphs.

Surprisingly, the PL was the same irrespective of the polymorph, PSD or incorporation of the additives that reduced the setting time, as shown in Table 3. For this reason the results were analyzed in terms of L/P ratio.

In this study, the injectability was characterized by monitoring the load needed for the paste to be extruded through a given aperture, at constant injection velocity until a maximum load of a 100 N. The shape of the load vs. plunger run curves was indicative of different phenomena taking place during paste extrusion, such as phase separation or clogging of the syringe aperture. In fact, when phase separation occurred (see for instance Figure 2), the load necessary for the paste to be injected gradually increased, due to the progressive decrease of the L/P ratio of the paste inside of the syringe [29]. In contrast, when clogging of the syringe aperture happened (Figures 2b, 5c-d and 6a-b), there was an abrupt increase in the load, either transient or permanent, due to the impermeability of the aggregates formed, which prevented further injection [41]. By contrast, if no phase separation or clogging happened, the paste was injected at a constant load until emptying the syringe. Even though both clogging and filter pressing result in a load increase during injection, they should be considered as different phenomena hindering injectability. In fact, filter pressing is as gradual and irreversible process, caused by the progressive liquid phase separation from the solid phase. In contrast, in

clogging impermeable aggregates are formed that block the syringe and stop completely the flow of the paste, resulting in a sudden and transient increase of load. No phase separation is produced in this case, and when the syringe is unblocked, the load returns to the constant steady state value (Figure 2b, α -TCP/C paste, L/P 0.45 mL g⁻¹). This was corroborated by the fact that the L/P ratio of the paste did not change before and after a clogging episode, as described in the results section. However, it is true that in some cases clogging can happen in parallel to filter pressing, as in the case of α -TCP/C paste prepared at low L/P ratio (see Figure 2b).

4.2. Differences between self-setting and non-self-setting pastes

When comparing the injection behavior of α - and β -TCP polymorphs using 2 mm aperture syringes (i.e., more than 20 times larger than the particle size range of the powders, which is 0.1-20 μ m for the F powders and 0.1-80 μ m for the C powders), it was observed that, in general, α -TCP was less injectable than β -TCP and required higher injection loads (Figure 2). However, when analyzing in more detail the data obtained, the following trends were found: i) at high L/P ratio the injection parameters (namely injectability, yield load and injection load) were similar, irrespective of the particle size; ii) at low L/P ratio, the differences amongst α - and β -TCP pastes were more marked, especially for F powders. This was associated to the fact that, since the hydrolysis reaction is surface-mediated, the difference in reactivity between α - and β -TCP should be more pronounced the higher the SSA of the powders; and iii) α -TCP/C pastes presented transient clogging episodes, which were not observed in β -TCP/C pastes with equivalent PSD.

These differences were more pronounced when the pastes were injected using cannulas with smaller apertures, such as 0.84 mm (only ten times larger than the largest C particles) (Figure 5). Whereas the injectability of β -TCP was unaltered (F powder) or only slightly reduced (C powder) when reducing needle diameter, the injectability of the α -TCP paste strongly decreased, in particular for C powders. Furthermore, the sharp profile at the end of the extrusion curves suggested that, with thin cannulas, the phenomenon behind the sudden interruption of injection of α -TCP was clogging of the cannula rather than phase separation. Although both clogging and phase separation were fostered by the reduction of the transversal section at the entrance of the cannula, which induces particle aggregation, the difference between them lies on the size and the permeability of the aggregates formed. As expected, thinner cannulas have greater probability to be clogged than thick ones. Thus, while with thick cannulas clogging only happened using α -TCP/C powders and was transitory, with thin

cannulas clogging occurred for both F and C α -TCP pastes. No clogging was found for β -TCP paste, irrespective of PSD or needle diameter, suggesting a major particle aggregation in the case of the more reactive polymorph.

4.3. Apparent setting of β -TCP and its effect on injectability

Although β -TCP was used as control and considered *a priori* as a non-self-setting material, in fact an apparent setting was found according to the Gilmore needles method (Tables 2-4). This can be attributed to an increased reactivity of the powder due to a partial amorphisation during milling [37,42], as corroborated by the amorphous phase quantified by Rietveld, which increased with milling time (Table 1). In fact, this was in agreement with the SEM images and the XRD evolution with time (Figure 3). The increase in crystallinity found in the β -TCP after 7 days, the observation of small precipitates on the surface of the particles (Figure 3d) and the increase in SSA (Figure 4b) could be explained by the dissolution of the amorphized fraction formed on the surface of the particles during milling. The fact that no other phase than β -TCP was found by XRD can be attributed to the low amount of hydrolyzed material, which in fact was not enough to produce a cementitious self-hardening reaction. After calcination at 500 °C, no amorphous phase was detected in the β -TCP powder by Rietveld (Table 1), which was consistent with the decrease in reactivity suggested by the unaltered SSA with time (Figure 4b). Interestingly, this had a significant effect in the injectability of the β -TCP paste. Not only the injectability at 2.5 minutes after mixing was higher in the calcined powder, but this injectability was maintained constant if the paste was injected long after mixing powder and liquid, even after 4 hours (Figure 4a). In contrast, in the uncalcined powder the injectability decreased when injection was delayed for 60 minutes, and the paste was not injectable at all after 4 hours. As expected, a similar trend was observed with α -TCP, the injectability decreasing less markedly with post-mixing time for less reactive calcined powders than for the as-milled ones. In both cases, the effect of powder reactivity on the injectability of the pastes was clear, and showed that even at very short times, the incipient precipitation from solution fostered the interactions between neighboring particles [43,44], which ultimately constrained paste injectability.

4.4. Effect of setting accelerants

CPCs should set within minutes after implantation. This allows a fast grafting surgery, without extravasation risk. Although the cement hardening was studied only for the α -TCP/C, it is known that in the range studied, the PSD does not significantly affect the final compressive strength of the cement, and it is only relevant for the hardening rate, being faster in the case of fine

powders [45,46]. In the present work, it was observed that both Na_2HPO_4 dissolved in the liquid phase and the incorporation of pHA nanoparticles to the solid phase reduced in a similar way the setting times of TCP pastes, especially in the case of α -TCP (Table 3). However, it is interesting to note that the setting of the cement pastes, as measured by the Gilmore needles, and the strength acquisition (Figure 8) were not linked phenomena. In fact, only the Na_2HPO_4 solution produced a faster hardening (Figure 8), whereas the addition of pHA did not prevent a long induction time prior to strength increase. Keeping in mind that strength development in this type of materials is the result of the entanglement of precipitated crystals [26], in the case of Na_2HPO_4 , the early strength acquisition proves that the common ion effectively accelerated the precipitation of CDHA, and this could also be behind the setting time reduction. In contrast, in the case of pHA, the fact that no hardening acceleration was found suggests that the setting acceleration mechanism was associated to the physical interactions in the paste, due to the increase in SSA caused by the addition of the nanoparticles, rather than to an acceleration of the CDHA precipitation. Whereas both mechanisms would affect the setting times, only the former would have a real accelerating effect in the hardening kinetics.

These two different mechanisms are also probably behind the uneven effect of the two additives on the injectability of the pastes. The reduction in injectability produced by pHA was higher than that of Na_2HPO_4 , and in absence of pHA the sodium phosphate salt even increased paste injectability. The changes in injectability can be related to the effect of the two additives on the dynamic viscosity of the pastes (**Figure 7**). In fact, the addition of pHA increased the viscosity to a higher extent than Na_2HPO_4 . As for the setting time reduction, the increment of viscosity caused by the addition of pHA can be associated to the increase of SSA in the paste, which also impaired paste injection. In contrast, a different mechanism is probably acting in the case of Na_2HPO_4 . In fact, an increase in viscosity could be expected from the accelerating effect of the setting reaction by the common ion effect. However, this might be counterbalanced in part by a reduced particle-particle electrostatic attraction due to the change in pH. Indeed, in contrast to distilled water (pH = 6), the pH of the 2.5 wt.% Na_2HPO_4 solution is 9.1. Taking into account that the isoelectric point of α - and β -TCP is 6.7 ± 0.1 [47], the higher difference between the isoelectric point and the pH of the liquid phase results in a negative charge on the particles, which gives rise to enhanced particle repulsions within the paste, leading in turn to an improved, or at least a less impaired injectability. However, when the two additives were added simultaneously, a synergistic effect was found, resulting in a strong reduction of both setting time and injectability.

In order to analyze possible correlations between injection properties of the TCP pastes and their setting times, injectability and injection loads were represented in **Figure 9** as a function of the initial setting times, for different paste formulations with L/P ratio of 0.35 mL g⁻¹ (see values in Table 3). Although with a considerable dispersion, the general trend was that when the initial setting time increased the injectability of the pastes tended to increase, both parameters being higher for β - than for α -TCP. However, it is interesting to note that there were two parameters that influenced in opposite directions injectability and setting times: 1) when no additives were used, F powders were more injectable than C ones, even if the initial setting times were slightly shorter; 2) the addition of Na₂HPO₄ significantly reduced the setting times but at the same time increased injectability. In contrast, the addition of pHA or the simultaneous addition of the two setting accelerants, resulted in a reduction of both injectability and setting times. On the other side, no correlation was found between injection loads and setting times.

5. Conclusion

Powder reactivity was shown to play a significant role in the injectability of TCP pastes. Even if the injection was performed at very short times, significant differences were observed between the injection behavior of the non-hardening β -TCP pastes and self-hardening α -TCP pastes, α -TCP being less injectable than β -TCP and requiring higher injection loads. Moreover, the parameters affecting powder reactivity were shown to affect also paste injectability. Thus, whereas powder calcination resulted in an increased injectability, the addition of setting accelerants tended to reduce the injectability of the TCP pastes.

6. Acknowledgments

Authors acknowledge the financial support of the Spanish Ministry of Science and Innovation in the MAT 2009-13547 project. Support for the research of MPG was received through the prize "ICREA Academia" for excellence in research, funded by the Generalitat de Catalunya.

7. References.

- [1] Park A, Kavic SM, Lee TH, Heniford BT. Minimally invasive surgery: the evolution of fellowship. *Surgery* 2007; 142: 505-11; discussion 511-3.
- [2] Driessens FCM, Planell J, Boltong M, Khairoun I, Ginebra MP. Osteotransductive bone cements. *Proc Inst Mech Eng H* 1998; 212: 427–435.
- [3] Dorozhkin SV. Calcium orthophosphate cements for biomedical application. *J Mater Sci* 2008; 43: 3028-3057.
- [4] Del Valle S, Miño N, Muñoz F, González A, Planell JA, Ginebra MP. In vivo evaluation of an injectable Macroporous Calcium Phosphate Cement. *J Mater Sci Mater Med* 2007; 18:353–361.
- [5] Ginebra MP, Canal C, Espanol E, Pastorino D, Montufar EB. Calcium phosphate cements as drug delivery materials. *Adv Drug Deliv Rev* 2012, Epub ahead of print, doi:10.1016/j.addr.2012.01.008
- [6] Ginebra MP, Espanol M, Montufar EB, Perez RA, Mestres G. New processing approaches in calcium phosphate cements and their applications in regenerative medicine. *Acta Biomater* 2010; 6: 2863-73.
- [7] Ginebra MP, Delgado JA, Harr I, Almirall A, Del Valle S, Planell JA. Factors affecting the structure and properties of an injectable self-setting calcium phosphate foam. *J Biomed Mater Res* 2007; 80A: 351-361.
- [8] Montufar EB, Traykova T, Planell JA, Ginebra MP. Comparison of a low molecular weight and a macromolecular surfactant as foaming agents for injectable self-setting hydroxyapatite foams: Polysorbate 80 versus gelatin. *Mat Sci Eng C* 2011; 32:1498-1504.
- [9] Perez RA, Altankov G, Jorge-Herrero E, Ginebra MP. Micro- and nanostructured hydroxyapatite/collagen microcarriers for bone tissue engineering applications. *J Tissue Eng Regen Med* 2012, Epub ahead of print, doi:10.1002/term.530,
- [10] Butscher A, Böhner M, Hofmann S, Gauckler L, Müller R. Structural and material approaches to bone tissue engineering in powder-based three-dimensional printing. *Acta Biomater* 2011; 7: 907-20.
- [11] Wang X, Ye J, Wang H. Effects of additives on the rheological properties and injectability of a calcium phosphate bone substitute material. *J Biomed Mater Res B* 2006; 78: 259–264.
- [12] Barralet J, Grover L, Gbureck U. Ionic modification of calcium phosphate cement viscosity. Part II: hypodermic injection and strength improvement of brushite cement. *Biomaterials* 2004; 25: 2197–2203.

- [13] Gbureck U, Barralet JE, Spatz K, Grover LM, Thull R. Ionic modification of calcium phosphate cement viscosity. Part I: hypodermic injection and strength improvement of apatite cement. *Biomaterials* 2004; 25: 2187–2195.
- [14] Ishikawa K. Effects of Spherical Tetracalcium Phosphate on Injectability and Basic Properties of Apatitic Cement. *Key Eng Mater* 2003; 240-242: 369-372.
- [15] Ratier A, Freche M, Lacout J, Rodriguez F. Behaviour of an injectable calcium phosphate cement with added tetracycline. *Int J Pharm* 2004; 274: 261–268.
- [16] Alves HLR, Dos Santos LA, Bergmann CP. Injectability evaluation of tricalcium phosphate bone cement. *J Mater Sci Mater Med* 2008; 19: 2241–2246.
- [17] Qi X, Ye J, Wang Y. Improved injectability and in vitro degradation of a calcium phosphate cement containing poly(lactide-co-glycolide) microspheres. *Acta Biomater* 2008; 4: 1837-45.
- [18] Xu HHK, Weir MD, Burguera EF, Fraser AM. Injectable and macroporous calcium phosphate cement scaffold. *Biomaterials* 2006; 27: 4279–4287.
- [19] Burguera EF, Xu HHK, Weir MD. Injectable and rapid-setting calcium phosphate bone cement with dicalcium phosphate dihydrate. *J Biomed Mater Res B* 2006; 77: 126–134.
- [20] Jack V, Buchanan F, Dunne N. Particle attrition of alpha-tricalcium phosphate: effect on mechanical, handling, and injectability properties of calcium phosphate cements. *Proc Inst Mech Eng H* 2008; 222: 19–28.
- [21] Liu C, Shao H, Chen F, Zheng H. Effects of the granularity of raw materials on the hydration and hardening process of calcium phosphate cement. *Biomaterials* 2003; 24: 4103-4113.
- [22] Liu C, Shao H, Chen F, Zheng H. Rheological properties of concentrated aqueous injectable calcium phosphate cement slurry. *Biomaterials* 2006; 27: 5003-13.
- [23] Fatimi A, Tassin JF, Axelos MV, Weiss P. The stability mechanisms of an injectable calcium phosphate ceramic suspension. *J Mater Sci Mater Med* 2010; 21: 1799-809.
- [24] Ribeiro M, Ferreira J, Labrincha J. Plastic behaviour of different ceramic pastes processed by extrusion. *Ceram Int* 2005; 31: 515–519.
- [25] Fowler BO, Kuroda S. Changes in heated and in laser-irradiated human tooth enamel and their probable effects on solubility. *Calcif Tissue Int.* 1986; 38: 197-208.
- [26] Ginebra MP et al., Setting reaction and hardening of an apatitic calcium phosphate cement. *J Dent Res* 1997; 76: 905-12.
- [27] Ginebra MP, Fernández E, Driessens FCM, Planell JA. Modeling of the Hydrolysis of α -Tricalcium Phosphate. *J Am Ceram Soc* 2004; 82: 2808-2812.

- [28] Böhner M, Baroud G. Injectability of calcium phosphate pastes. *Biomaterials* 2005; 26: 1553–1563.
- [29] Habib M, Baroud G, Gitzhofer F, Böhner M. Mechanisms underlying the limited injectability of hydraulic calcium phosphate paste. *Acta Biomater* 2008; 4: 1465–1471.
- [30] Habib M, Baroud G, Gitzhofer F, Böhner M. Mechanisms underlying the limited injectability of hydraulic calcium phosphate paste. Part II: particle separation study. *Acta Biomater* 2010; 6: 250-6.
- [31] Blitterswijk CA. Solubility of β -Ca₃(PO₄)₂ in the System Ca(OH)₂-H₃PO₄-H₂O at 5, 15, 25, and 37 °C. *J Res Natl Bur Stand (U. S.)* 1974; 78: 667-675.
- [32] Ginebra MP, Fernández E, Boltong MG, Bermúdez O, Planell JA, Driessens FCM. Compliance of an apatitic calcium phosphate cement with the short-term clinical requirements in bone surgery, orthopaedics and dentistry. *Clin Mater* 1994; 17: 99-104.
- [33] Khairoun I, Boltong MG, Driessens FCM, Planell JA. Effect of calcium carbonate on the compliance of an apatitic calcium phosphate bone cement. *Biomaterials* 1997; 18: 1535-9.
- [34] O'Hara RM, Dunne NJ, Orr JF, Buchanan F. Optimisation of the mechanical and handling properties of an injectable calcium phosphate cement. *J Mater Sci Mater Med* 2010; 21: 2299-305.
- [35] Driessens FCM, Boltong MG, Bermudez O, Planell JA. Formulation and setting times of some calcium orthophosphate cements: a pilot study. *J Mater Sci Mater Med* 1993; 4: 503-508.
- [36] Fernández E, Boltong MG, Ginebra MP, Bermúdez O, Driessens FCM, Planell JA. Common ion effect on some calcium phosphate cements. *Clin Mater* 1994; 16: 99-103.
- [37] Böhner M, Luginbühl R, Reber C, Doebelin N, Baroud G, Conforto E. A physical approach to modify the hydraulic reactivity of alpha-tricalcium phosphate powder. *Acta Biomater* 2009; 5: 3524-35.
- [38] ASTM International D4318-00 - Standard test methods for liquid limit, plastic limit and plasticity index of soils 2000.
- [39] ASTM International C266-99 - Standard test method for time of setting of hydraulic-cement paste by Gillmore needles 2007.
- [40] Ginebra M, Rilliard A, Fernández E, Elvira C, San Román J, Planell JA. Mechanical and rheological improvement of a calcium phosphate cement by the addition of a polymeric drug. *J Biomed Mater Res* 2001; 57: 113-8.
- [41] Mason MS, Huang T, Landers RG, Leu M. Aqueous-based extrusion of high solids loading ceramic pastes: Process modeling and control. *J Mater Process Technol* 2009; 209: 2946-2957.
- [42] Gbureck U, Grolms O, Barralet J, Grover L, Thull R. Mechanical activation and cement formation of beta-tricalcium phosphate. *Biomaterials* 2003; 24: 4123–4131.

- [43] Bergstrom L. Colloidal Processing of Ceramics. In : Holmberg K, editor. Handbook of Applied Surface and Colloid Chemistry. John Wiley & Sons, Ltd, 2001: 202-218.
- [44] Lewis JA. Colloidal Processing of Ceramics. Processing 2000; 59: 2341-2359.
- [45] Ginebra MP, Driessens FCM, Planell JA. Effect of the particle size on the micro and nanostructural features of a calcium phosphate cement: a kinetic analysis. Biomaterials 2004, 25, 3453-3462.
- [46] Espanol M, Perez RA, Montufar EB, Marichal C, Sacco A, Ginebra MP. Intrinsic porosity of calcium phosphate cements and its significance for drug delivery and tissue engineering applications. Acta Biomater 2010;5:2752-2762.
- [47] Mezzasalma SA. Hydration pressure induced by solid surface dissolution in ion adsorption from aqueous media: Role of structural force in proton/oxydyle isotherms of α -tricalcium phosphate/water systems. J Chem Phys 1997; 107: 9214.

Figure captions

Figure 1. Particle size distribution of the two TCP polymorphs studied along this work (α - and β -TCP), a) Fine (F) particles; b) coarse (C) particles. In the bottom the scanning electron micrographs show the respective unreacted powders (scale bar 5 μm).

Figure 2. Representative injection curves (load vs. plunger run) corresponding to a) fine and b) coarse powders. The captions near the curves represent the TCP polymorph (α -TCP solid line; β -TCP dashed line) and the L/P ratio in mL g^{-1} .

Figure 3. X-ray diffraction patterns for α -TCP/C (a) and β -TCP/C (b) before ($t = 0$) and after immersion in water at 37 $^{\circ}\text{C}$ ($t = 7$ days). SEM images correspond to the material morphology after the same period: α -TCP/C (c) and β -TCP/C (d) (scale bar 5 μm). L/P ratio of 0.35 mL g^{-1} .

Figure 4. a) Injectability and b) specific surface area of α (circles) and β (squares) TCP/F powders, either as-milled (non-calcined; open symbols) or calcined (close symbols), as function of post-mixing time (the time delay between mixing and characterization). L/P ratio of 0.45 mL g^{-1} .

Figure 5. a and b) Injectability of β -TCP and α -TCP, F and C, without cannula (internal diameter of 2 mm) and with cannula (internal diameter of 0.84 mm); c and d) representative injection curves (load vs. plunger run) corresponding to the values presented in a and b. L/P ratio of 0.35 mL g^{-1} .

Figure 6. Representative injection curves (load vs. plunger run) corresponding to a) α -TCP/F, b) α -TCP/C, c) β -TCP/F and d) β -TCP/C pastes prepared without additives (none), with only one additive (pHA or Na_2HPO_4) or both additives (pHA+ Na_2HPO_4). L/P ratio of 0.35 mL g^{-1} . None stands for no additive.

Figure 7. Dynamic viscosity of the paste, as function of the shear rate, for α -TCP/F containing or not pHA (2 wt.%), and mixed either with water or with a 2.5 wt.% Na_2HPO_4 solution. L/P ratio of 0.55 mL g^{-1} . None stands for no additive.

Figure 8. Compressive strength as function of reaction time, for α -TCP/C containing or not pHA (2 wt.%), and mixed either with water or with a 2.5 wt.% Na_2HPO_4 solution. L/P ratio of 0.35 mL g^{-1} .

Figure 9. a) Injectability and b) injection load, as function of the initial setting time. The captions near the points represent the additives incorporated in the paste. L/P ratio of 0.35 mL g⁻¹. None stands for no additive.

Injection curves for

Non-self-setting
 β -TCP paste

Self-setting
 α -TCP paste

Figure 1
[Click here to download high resolution image](#)

Figure 2
[Click here to download high resolution image](#)

Figure 3
[Click here to download high resolution image](#)

Figure 4
[Click here to download high resolution image](#)

Figure 5
[Click here to download high resolution image](#)

Figure 6
[Click here to download high resolution image](#)

Figure 7
[Click here to download high resolution image](#)

Figure 8
[Click here to download high resolution image](#)

Figure 9
[Click here to download high resolution image](#)

Table 1. Specific surface area (SSA) and phase composition of α - and β -TCP powders with different granulometry (coarse: α -TCP/C and β -TCP/C; and fine: α -TCP/F and β -TCP/F) and after calcination (α -TCP/F-cal and β -TCP/F-cal).

	SSA [m ² g ⁻¹]	Phase composition [wt.%] ^a			
		α -TCP	β -TCP	HA	Amorphous
α -TCP/C	0.98 ± 0.01	97.3	1.4	1.3	0.0
α -TCP/F	3.34 ± 0.09	94.2	2.2	0.2	3.5
α -TCP/F-cal	2.9 ± 0.01	94.9	4.4	1.1	0.2
β -TCP/C	0.84 ± 0.01	3.0	94.9	1.1	1.0
β -TCP/F	3.41 ± 0.01	1.4	92.0	2.6	4.1
β -TCP/F-cal	3.03 ± 0.02	0.6	97.5	2.0	0.0

^a Error ± 1 %

Table 2. Effects of particle size and L/P ratio on initial setting time, final setting time, injectability, yield load and injection load of α - and β -TCP pastes.

TCP	Powder	L/P ratio [mL g ⁻¹]	Initial setting time [min]	Final setting time [min]	Injectability [%]	Yield load [N]	Injection load [N]
α	Fine	0.35	15 $\pm$ 4	23 $\pm$ 4	60.5 $\pm$ 0.8	15.8 $\pm$ 0.8	14.7 $\pm$ 1.0
		0.45	35 $\pm$ 2	55 $\pm$ 2	96.2 $\pm$ 0.4	8.4 $\pm$ 1.1	6.8 $\pm$ 1.3
	Coarse	0.35	15 $\pm$ 3	27 $\pm$ 4	48.5 $\pm$ 3.2	18.5 $\pm$ 1.7	16.4 $\pm$ 1.9
		0.45	37 $\pm$ 4	88 $\pm$ 4	91.6 $\pm$ 3.1	6.8 $\pm$ 1.0	5.4 $\pm$ 1.1
β	Fine	0.35	44 $\pm$ 4	71 $\pm$ 1	92.0 $\pm$ 1.1	10.1 $\pm$ 0.6	8.2 $\pm$ 0.2
		0.45	52 $\pm$ 3	92 $\pm$ 5	96.6 $\pm$ 0.5	8.5 $\pm$ 1.8	6.8 $\pm$ 1.6
	Coarse	0.35	52 $\pm$ 2	85 $\pm$ 2	56.0 $\pm$ 1.8	17.7 $\pm$ 2.0	16.0 $\pm$ 1.9
		0.45	73 $\pm$ 4	> 120	90.9 $\pm$ 0.5	9.9 $\pm$ 1.8	8.6 $\pm$ 2.1

Table 3. Effects of TCP polymorph (α vs. β), particle size distribution (PSD), and additives (2 wt.% pHA or 2.5 wt.% Na_2HPO_4 solution on the plastic limit, initial setting time, final setting time, injectability, yield load and injection load. L/P = 0.35 mL g^{-1} . Setting times determined through Gilmore method.

TCP	PSD	Additives	Plastic limit [mL g^{-1}]	Initial setting time [min]	Final setting time [min]	Injectability [%]	Yield load [N]	Injection load [N]
α	Fine		0.23 ± 0.01	15 ± 4	23 ± 4	60.5 ± 0.7	15.8 ± 0.8	14.7 ± 1.0
		Na_2HPO_4	0.23 ± 0.01	9 ± 1	18 ± 1	63.1 ± 0.8	13.0 ± 0.2	12.1 ± 0.6
		pHA	0.24 ± 0.01	6 ± 2	11 ± 2	26.3 ± 2.8	16.0 ± 1.5	
		pHA - Na_2HPO_4	0.23 ± 0.02	4 ± 1	8 ± 2	0.7 ± 0.6	> 100	
	Coarse		0.24 ± 0.01	15 ± 3	27 ± 4	48.5 ± 3.3	18.5 ± 1.7	16.4 ± 1.9
		Na_2HPO_4	0.22 ± 0.01	12 ± 4	24 ± 4	56.2 ± 0.6	17.3 ± 0.7	15.4 ± 0.7
		pHA	0.25 ± 0.02	11 ± 2	24 ± 4	36.4 ± 2.8	13.0 ± 0.7	12.3 ± 0.4
		pHA - Na_2HPO_4	0.23 ± 0.01	8 ± 2	15 ± 3	4.0 ± 2.0	> 100	
β	Fine		0.23 ± 0.01	44 ± 4	71 ± 1	92.0 ± 1.1	10.1 ± 0.6	8.20 ± 0.2
		Na_2HPO_4	0.23 ± 0.01	26 ± 2	47 ± 3	84.1 ± 1.3	14.5 ± 2.7	11.9 ± 2.1
		pHA	0.24 ± 0.01	39 ± 2	51 ± 2	83.1 ± 1.4	11.1 ± 1.0	9.5 ± 1.3
		pHA - Na_2HPO_4	0.24 ± 0.01	22 ± 3	40 ± 3	11.6 ± 2.3	59.7 ± 4.1	57.1 ± 1.6
	Coarse		0.23 ± 0.01	52 ± 2	85 ± 2	55.9 ± 1.8	17.7 ± 2.0	16.0 ± 1.9
		Na_2HPO_4	0.22 ± 0.01	45 ± 3	78 ± 4	64.9 ± 0.3	17.4 ± 2.1	14.9 ± 2.1
		pHA	0.24 ± 0.02	45 ± 3	80 ± 3	54.1 ± 1.1	15.1 ± 2.3	12.7 ± 2.1
		pHA - Na_2HPO_4	0.22 ± 0.01	43 ± 2	65 ± 5	37.8 ± 2.2	18.7 ± 2.7	17.9 ± 2.9

Table 4: Statistical analysis of the second factorial design (2^4). L/P = 0.35 ml g⁻¹.

	Initial setting time [min]	Injectability [%]	Yield load [N]
	Average: 24.75	Average: 48.7	Average: 28.61
Factors	Most significant effects	Most significant effects	Most significant effects
A: TCP polymorph ($\beta \rightarrow \alpha$)	A (-29.5; $p < 0.0007$)	C (-33.91; $p < 0.0009$)	B (+27.91; $p < 0.0004$)
B: Na ₂ HPO ₄ (0 $\rightarrow$ 2.5 wt.%)	D (+8.25; $p < 0.008$)	A (-23.47; $p < 0.002$)	C (+26.16; $p < 0.0004$)
C: pHA (0 $\rightarrow$ 2 wt.%)	AD (-5.25; $p < 0.02$)	B (-16.81; $p < 0.004$)	BC (+27.91; $p < 0.0004$)
D: PSD (F $\rightarrow$ C)	B (-7.25; $p < 0.01$)	BC (-19.64; $p < 0.003$)	A (+16.16; $p < 0.001$)
	C (-5.00; $p < 0.03$)	CD (+10.58; $p < 0.008$)	ABC (+15.87; $p < 0.001$)