J Mater Sci: Mater Med (2008) 19:2241–2246
DOI 10.1007/s10856-007-3329-6

Injectability evaluation of tricalcium phosphate bone cement

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Received: 4 February 2006 / Accepted: 19 November 2007 / Published online: 1 December 2007

Abstract Calcium phosphate cements are biomaterials made from a mixture of calcium phosphate powder in aqueous solutions that forms a paste that reacts at the body temperature and hardens as a result of precipitation reactions. These cements are commonly used in dentistry and orthopedic bone filling surgeries, which require extremely invasive procedures. The challenge consists in formulating an injectable paste by additives incorporation. In this work, three different additives (carboxymethylcellulose, agar polymer and sodium alginate) were incorporated to tricalcium phosphate, in concentrations of 0.4, 0.8, 1.6, 3.2 and 6.4 wt.%. Injectability was evaluated through a new method developed for this purpose. Results showed that it was possible to obtain injectable compositions of α-tricalcium phosphate cement. It was verified that the injectability depends on the rheological behavior of the pastes and injection time. In this study, pastes with viscosity suitable for good homogenization and injection were obtained.

1 Introduction

The great progress of the field of biomaterials is due to the increase on life expectation and car accidents. Calcium phosphate cements have been successfully used for bone repair in the last decade [1]. This kind of cement can be prepared by mixing a calcium phosphate salt with water or with aqueous solution of inorganic or organic salts, forming a paste that can react at room or body temperature and hardens due to the interconnection of precipitates of calcium phosphate crystals (e. g. hydroxyapatite) [2]. Although poly(methylmethacrylate) is the most popular cement used for orthopedic surgeries, this polymer has some inconveniences like the high exothermic polymerization reaction that can cause bone tissue necrosis [3]. One important remark: despite the similar names, no comparison can be made between calcium phosphate cements and PMMA. CaP cements are hydraulic cements, while acrylic cements set by polymerization; mechanical properties are totally different (compression strength about 70 MPa for PMMA and 30 MPa for calcium phosphate cement); applications are diverse: CaP cement is not used neither indicated for the anchorage and fixation of cemented stems in hip prostheses.

Researches concerning bioactive bone cements are growing, mainly because of the lower probability of causing side effects in the living tissue [4]. These cements are used mainly for bone replacement that requires extremely invasive surgical procedures. The current challenge is the development of an injectable composition, in such a way that the biomaterial could be placed into the body with minimal invasive surgery techniques [5]. Thus, the possibility of cement injection is an extraordinary improvement. The advantages concerning injection of cement are: in situ preparation, reduction of surgery time, very small cavity for implantation and perfect adjustment to the bone defect increasing life quality of the patient [6]. Previous studies [7, 8] showed that it is possible to improve cement injectability by additives incorporation without significant modifications in chemical reactions [9]. A good additive must improve injectability without avoiding hydroxyapatite precipitation [10].

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The main objective of this work is to study the variables involved in the injection process of alpha-tricalcium phosphate (α-TCP) cements.

2 Materials and methods

2.1 α-TCP preparation

The methodology reported by Driessens et al. [3] was used to synthesize the α-TCP cement powder. An equimolar mixture of calcium carbonate (Nuclear, Brazil) and calcium pyrophosphate [$Ca_2P_2O_7$-γ] (IQUIMM, Brazil) was calcined for 15 h at 1,300 °C, followed by quenching in air [11, 12]. The material was then crushed in a porcelain mortar and submitted to wet milling during 6 h in a plastic bottle containing ethanol and zirconia balls with diameter of 10 mm. After milling, the material was dried at 90 °C for 72 h; the resulting mean particle size was 3.35 μm, and it was measured by means of laser granulometry (CILAS, model 1180).

2.2 Paste preparation

Three different powdered additives (VETEC, Brazil) were incorporated to the α-TCP: carboxy-methyl-cellulose (CMC), agar (red seaweed polysaccharide) and sodium alginate (SA), in concentrations of 0.4, 0.8, 1.6, 3.2 and 6.4 wt.%. All of these components are biocompatible, as already reported in the literature [8, 10].

2.3 Consistency evaluation

An aqueous solution of 2.5 wt.% disodium hydrogen phosphate [Na_2HPO_4] (VETEC, Brazil) was used as liquid phase [9, 13]. Liquid fractions were limited in function of the rheological response of each paste composition. Aliquots of 0.5 mL of liquid were gradually added in 20 g of TCP powder until a bright and fluid homogeneous paste was obtained, as described in ASTM C427 [14] standard.

2.4 pH measurements

After 2 min of mixing the liquid into the powder, pH was measured at room temperature (26 ± 1 °C) by introducing the probe of a digital pH meter into the paste (DIGIMED, model DM-20).

2.5 Pastes rheology

The paste viscosity was measured by a Brookfield viscosimeter (BROOKFIELD, model RVDVII+) at room temperature, with a RV7 spindle rotating at 100 rpm. Viscosity was monitored during 1 h, according to ASTM D2196 standard [15]. Initial viscosity was considered to be the mean value of the two first minutes of the test.

2.6 Injectability test

The apparatus used to evaluate the injectability is shown in Fig. 1. Injectability was quantified in terms of the residual mass of cement retained into the syringes after loading with a constant force of 50 N [16] during 5 and 30 s. Polymeric disposable syringes of 5 mL and a stainless steel cannula 80 mm high and diameter of 3 mm were used. Injectability tests were performed after 2 min of mixing.

The syringe was filled with the paste using a metallic spatula and a vibratory table. The syringe piston was placed in contact with the paste, and care was taken to minimize air retention inside the syringe. The injectability was calculated according to the equation shown in Fig. 2, where M_0 is the empty syringe mass, M_1 is the filled syringe mass, and M_2 is the syringe mass after injection. All syringe mass measurements were made without the cannula.

2.7 Mechanical strength

Specimens were made by placing the paste in a stainless steel mold, according to ASTM F 451 standard [17]. Mechanical strength was measured with a load rate of 5 mm/min on five cylindrical specimens 12 mm height and 6 mm of diameter after 3 days of immersion in deionized

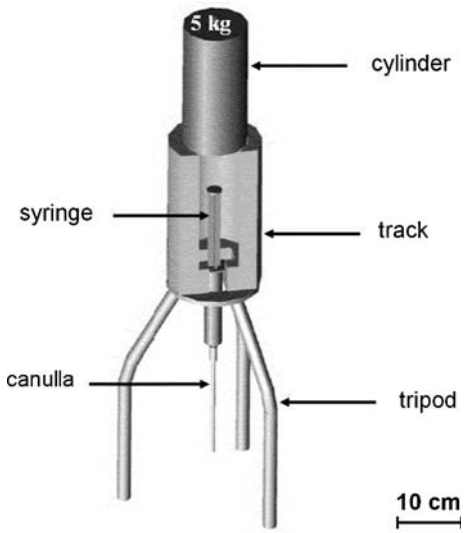

Fig. 1 Apparatus for injectability test. It is composed by a tripod, a track, and a metallic cylinder weight

$$Injectability(\%) = \left[\frac{(M_1 - M_0) - (M_2 - M_0)}{(M_1 - M_0)}\right] \times 100$$

Fig. 2 Equation used to calculate the cements injectability

water at room temperature. An ATS model 1105C equipment was used for that.

3 Results and discussion

3.1 Consistency evaluation

The liquid-to-powder (L/P) ratio necessary to obtain a bright homogeneous and fluid paste lied between 0.6 mL/g (similar to pure cement) and 0.87 mL/g, as shown in Table 1. The differences in the L/P ratio values show the influence of both content and type of additive in the cement, which can absorb liquid and increase viscosity.

It was noted that the L/P ratio should be increased by increasing additive content in order to the powder mixtures get completely wet. It was observed that high CMC content did not cause a significant increase in paste fluidity, and a higher L/P ratio (0.87) was necessary for higher additive contents. Agar and sodium alginate (SA), which are classified as hydrocolloids, have a tendency to retain liquids, forming an insoluble gel [18]. It was verified that all compositions containing sodium alginate showed some consistency change, leading to fast increase of L/P ratio. Even so a high L/P ratio could make the cement application impossible due to the high resulting porosity and consequent low mechanical strength. Controlled porosity can be extremely beneficial as far as osteoconduction is concerned [10, 19].

3.2 pH measurements

pH is one of the main factors that influence rheological properties of bone cements, and it can cause several problems to the implant and to the human body as a whole [20]. If ceramic grafts are associated to metallic prostheses, a low pH can remove the protective oxides of some metals, leading to prostheses corrosion [5]. Materials with pH within the range of 6.5–8.0 are considered suitable for implants [3]. Pure cement had an initial pH of 9.0, while pastes containing additives showed a pH ranging from 7.9 to 9.4. At the end of setting, the pH in water for all formulations was around 7.0. As long as the setting reaction occurs ($3Ca_3(PO_4)_2 + H_2O \rightarrow Ca_9(HPO_4)(PO_4)_5OH$), the hydration process neutralizes pH [19], due to hydroxyapatite formation. This shows that the additives can be used without changing the pH to undesirable values and provoking biological response [21].

Table 1 Liquid to powder ratio necessary for a bright homogeneous and fluid paste

Compositions (wt.%)	L/P ratio (mL/g)		
	CMC	AGAR	SA
0.4	0.60	0.60	0.67
0.8	0.60	0.67	0.73
1.6	0.60	0.67	0.87
3.2	0.67	0.67	0.87
6.4	0.87	0.87	0.87

3.3 Rheology

It was verified that the viscosity of the compositions do not exhibit a constant tendency with increasing additives content, as shown in Fig. 3. This behavior may be related to the non-uniform distribution of additives within the cement mass, since it is difficult to manually obtain homogeneous distributions in such a short time for a material with paste consistency. The viscosity of investigated compositions lied between 17,000 and 30,000 cP, with the exception of compositions containing 3.2 and 6.4 wt.% of agar, which had viscosity around 7,000 cP. Viscosity of compositions containing agar increased up to a fraction of 1.6 wt.%, due to gel formation. However, higher agar contents were detrimental to the setting reaction, since an abrupt viscosity decrease (defloculation) was observed, without any hardening effect.

The rheological behavior of the compositions containing additives in the first hour of setting (see Table 2) shows that higher additives content causes gradual decrease in viscosity, while for pure cement the viscosity increased abruptly up to approximately 30 min, according to the experimental observations. The decrease in viscosity may

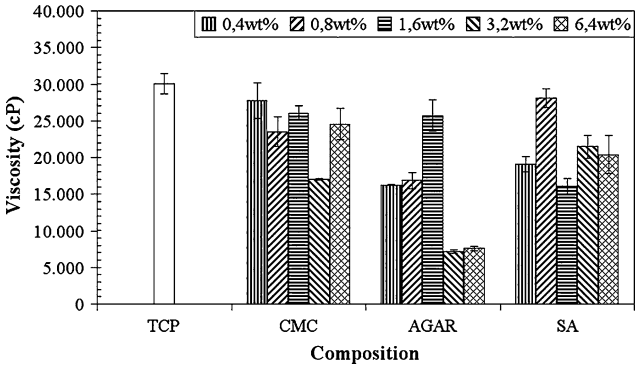

Fig. 3 Viscosity after 2 min of mixing for all pastes compositions

Table 2 Rheological behavior in the first hour of setting for proposed compositions

Compositions (wt%)	Viscosity change		
	CMC	AGAR	SA
0.4	++	+	+
0.8	+	+	+
1.6	+	++	+
3.2	–	+	–
6.4	–	=	–

++, abrupt viscosity increase; +, viscosity increase; –, viscosity decrease; =, constant

be related to the possible deflocculating effect of the additives.

The decrease in viscosity may be related to a possible deflocculating effect of these additives. This effect decreases viscosity in a less effective way because additives migration towards the surface within the cement paste is difficult; hence, huge amounts of additives are needed to cause the steric deflocculating effect.

In order to decrease the viscosity of ceramic suspensions, the typical value of deflocculant addition is about 0.5 wt.%, considering the amount of solids in suspension. However, the additives used in this work are not typical deflocculants, and their deflocculating affect must be less significant than those industrially used (such as ammonia polyacrylate, sodium polyacrylate, sodium silicate, among others), and higher amounts are needed. Considering additive-free cements, or cements with small amounts of additives, the sudden increase in viscosity is a consequence of the cement seting reaction.

According to the literature [6], the bone cement must be placed in the implantation site in about 7 min. Within this time, mixing, homogenization and injection times must be considered. Shorter setting times after such processes are desirable in order to avoid cement migration to regions outside injection site and washout. The aim of this work was not to develop cement for clinical use, but to study the injectability. The setting time can be adjusted for clinical usage with the increase in the amount of disodium hydrogen phosphate [13]. Although viscosity is an essential parameter to evaluate injectability, no information about it was found elsewhere, showing the need of more research concerning viscosity properties and injectability of cements.

3.4 Injectability

3.4.1 Influence of liquid-to-powder ratio on injectability

A directly proportional correlation between L/P ratio and injectability was observed, while the correlation between L/P ratio and compressive strength was found to be inversely proportional [19]. Figure 4 shows that the injectability increases and the compressive strength of the cement decreases with increasing liquid-to-powder ratio. It was noted that the injection process is fundamentally controlled by the rheology of the paste during syringe loading, i.e., it depends on the paste consistency right before injection [18]. The increase in liquid fraction make paste injection easier, since viscosity is lower, as well as the friction between cement paste and syringe walls and between the cement particles themselves.

For a successful clinical application of bone cement, a good injectability is necessary but not enough; a suitable mechanical strength after setting is also needed. Therefore, the composition must have a low L/P ratio in order to lead to an acceptable mechanical strength after hardening and to prevent migration of cement to undesirable sites. The mechanical strength decrease of the cement pastes caused by higher liquids amount is related to porosity. When the amount of liquid is higher than the necessary for stoichiometric setting reaction of the cement, it will fulfill the spaces among cement particles and, after drying, it evaporates and leads to the formation of pores. Porosity has a deleterious effect on the properties of ceramic materials, acting as stress raisers and reducing mechanical strength. Hence, the lowest possible amount of liquid should be added to cement, keeping mechanical resistance as high as possible.

3.4.2 Influence of injection time

Significant increase of injectability was obtained even for compositions with low content of additives due to the lubricant/coagulant effect of these additives. The injectability value of the pure cement is associated to mass loss of the liquid phase, as a consequence of liquid segregation

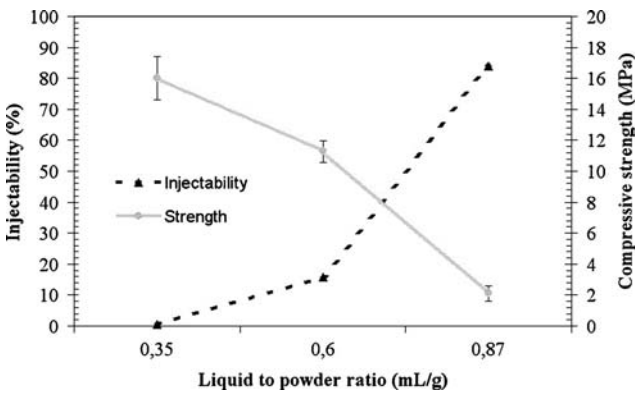

Fig. 4 Injectability for 30 s of injection time and compressive strength of pure cement in three liquid to powder ratios

(splitting of liquid and solid fractions). Figure 5 shows that the additives increased the cement injectability even for an injection time of only 5 s. However, the injectability calculated by equation A does not reflect the homogeneity of the paste that was injected; it just tells that there was mass loss in the syringe after injection. Some liquid segregation during injection process was observed for all compositions. During injection, the force applied by the syringe must exceed the material resistance, as well as the friction of the syringe walls themselves. The flow stress is higher close to the syringe walls and decreases along the syringe axle. Material flow can occur by wall slipping or laminar flow inside the injected material, away from the syringe walls. Another concern during the injection process and that makes it harder is the angle of reduction of sectional area from the syringe to the cannula. The higher this angle, the higher the compression stresses needed to drive the particles to the cannula and inject them. Hence, if too high stresses occur because of the friction at the mold walls or friction/compression among particles, segregation of liquid phase can take place.

CMC confers both cohesion and workability to the cement. Additions of CMC promote electrostatic changes and introduce lateral polymer chains to the cement particles. Repulsion forces and the increase in the space among particles caused by the lateral chains of polymer maintain the system dispersed [10]. Hence, when the cement hydration process starts, the electrostatic repulsion effect is lost, but the polymer chains keep the particles dispersed and raise the working time of the cement, since the cement solubilization is avoided [8]. All compositions containing agar also improved cement injectability, possibly because of the formation of a liquid film around the particles, allowing them to slide against each other and improving injectability [14]. Sodium alginate increased the paste consistency, adsorbing a significant fraction of liquid and avoiding particles wetting and dispersion, as reported in the literature [22]. This fact is in agreement with previous studies [20], which report that sodium alginate forms an insoluble gel in the presence of calcium ions. This insoluble hydrogel formed by sodium alginate can inhibit or even avoid completely cement dispersion in the liquid [20]. According to Ishikawa et al. [22], even low contents of sodium alginate promote good particle dispersion, and this was also observed in this work. Additives of high molecular weight reduce liquids migration. A film of adsorbed gel over the hard cement particles reduces friction among particles, and the colloid–liquid or additive–liquid matrix decreases the particles consolidation degree, decreasing effective stress and flow shear stress. Additives also promote mechanical resistance after injection [23]. Segregation is undesirable and depends on the properties of each additive. Such phenomenon allows the pressured liquid to migrate with subsequent purge of liquid phase, causing undesirable compaction of the solid fraction. This compaction leads to cannula clogging.

Observations of injected pastes with 5 s of injection time showed that all compositions led to the formation of a more homogeneous injected paste, which is desirable to the process. So, in order to check more significant differences in the effect of these additives in the cement, the injectability test was carried out with an injection time of 30 s.

Injectability of all investigated compositions improved by more than 30% for a injection time of 30 s, as shows in Fig. 6.

Segregation was visually observed for all compositions containing additives during injection tests, as well as for pure cement, in different severity degrees.

Injectability of most pastes was higher than 30%. For pure cement injection, injectability was 15.7%, and a significant part of it is related to the mass loss that is associated to segregation and liquid fraction loss. Injectability of compositions containing 0.4 wt.% and 0.8 wt.% of CMC was even lower than additives-free calcium phosphate cement. However, the homogeneity of the injected material from these compositions was better than those with no additives. For the other ones, injectability

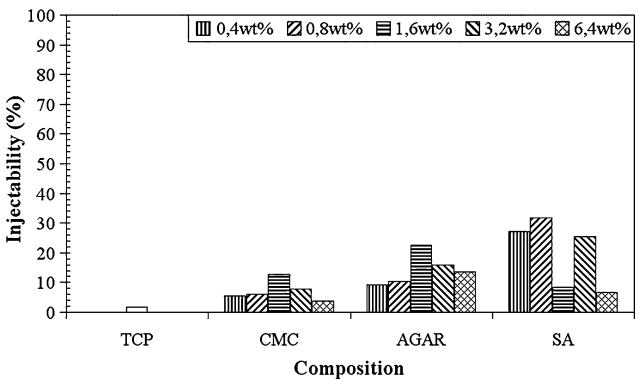

Fig. 5 Injectability of all cements for 5 s of injection time

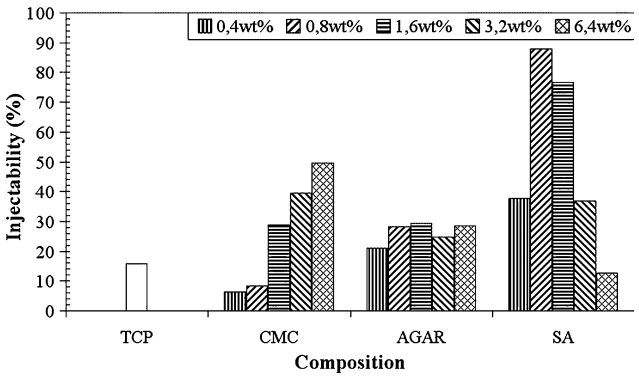

Fig. 6 Injectability comparison among all cements for injection time of 30 s

increased with increasing additives amount. Nevertheless, higher injectability values do not mean that the material is suitable for the intended usage, since only compositions containing up to 1.6 wt.% carboxymethylcellulose, 1.6 wt.% agar and 0.8 wt.% sodium alginate shown good homogeneity in the injected paste (minimal segregation). Segregation was observed for all other composition with higher additives content, which demonstrates that there is an optimal amount of additive for material injection. With these two aspects in mind, the composition containing 0.8 wt.% of sodium alginate was the most suitable one, with high injectability (ca. 90%) and good homogeneity of the injected material.

4 Conclusions

All proposed compositions showed an improvement on injectability of α-TCP cement. The method proposed to evaluate injectability was effective to compare all compositions. Injectability is not the only parameter to be considered for calcium phosphate cement injection; the homogeneity of the injected paste, that must have low liquid/powder segregation, is a key factor of the process. For clinical usage the setting time must be adjusted by the amount of disodium hydrogen phosphate in the liquid phase. A directly proportional correlation among cement injectability, liquid-to-powder ratio, and injection time was found. Compositions containing up to 1.6 wt.% carboxymethylcellulose, 1.6 wt.% agar and 0.8 wt.% sodium alginate had the best results regarding injectability and homogeneity. The composition containing 0.8 wt.% sodium alginate was the best composition for injection procedures.

Acknowledgements The authors thank the additives suppliers for material donation and the Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES) for financial support.

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