J Mater Sci: Mater Med (2010) 21:2049–2056
DOI 10.1007/s10856-010-4078-5

Injectable and fast resorbable calcium phosphate cement for body-setting bone grafts

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Received: 11 December 2009 / Accepted: 29 March 2010 / Published online: 13 April 2010

Abstract In this work a calcium phosphate (CPC)/polymer blend was developed with the advantage of being moldable and capable of in situ setting to form calcium deficient hydroxyapatite under physiological conditions in an aqueous environment at body temperature. The CPC paste consists in a mix of R cement, glycerol as a liquid phase carrier and a biodegradable hydrogel such as Polyvinyl alcohol, which acts as a binder. Microstructure and mechanical analysis shows that the CPC blend can be used as an injectable implant for low loaded applications and fast adsorption requirements. The storage for commercial distribution was also evaluated and the properties of the materials obtained do not significantly change during storage at −18°C.

1 Introduction

Calcium phosphate based ceramics (such as hydroxyapatite HA) are promising materials for orthopedic and dental surgery. They closely resemble the mineral phase of the bone extracellular matrix, so they are expected to be osteoinductive and osteoconductive, promoting regeneration of the damaged bone tissue [1–3].

Unfortunately, HA containing materials have a limited resorption rate in vivo due to the low solubility of these materials at physiological pH [4, 5]. For some clinical applications, it would be desirable to obtain a more rapid resorption and better osteointegration of the implant.

A calcium phosphate cement (CPC) was developed with the advantage of being moldable (the CPC paste intimately adapts to the bone cavity) and capable of in situ setting to form hydroxyapatite [6–10]. The ability to obtain biological apatite in an aqueous environment at body temperature has been one of the most important factors in the creation of a new class of bone substitute implants. CPCs generally consist of a powder and an aqueous liquid which are mixed to form a paste [11, 12]. The paste is placed into a defect as a substitute for the damaged part of the bone [13]. Most conventional CPCs are mixed with an aqueous solution immediately before application. In the clinical situation, the ability of the surgeon to properly mix the cement and then place the cement paste into the defect within the prescribed time is a crucial factor in achieving optimum and reproducible results [2, 14]. One approach to control handling is to use a mixing machine where the human factor only plays a minor role, hence reducing failure risks. But more innovative approach is to combine calcium phosphate cement with glycerol to form a paste that can be injected into the defect [15–17] and it sets upon contact with body fluids [18–20]. The potential advantages of the injectable calcium phosphate cements include: easy placement in surgery, ability to be used in difficult surgical sites and capability of filling narrow defects facilitating minimally invasive techniques [16, 21, 22].

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Next generation of biomaterials must combine bioactive and bioresorbable properties to activate in vivo mechanism of tissue regeneration, stimulating the body to heal itself and to facilitate replacement of the scaffold by the regenerating tissue [23, 24]. For implantation of apatitic cements many surgeons want a material which after implantation in bone is completely resorbed within the shortest possible time, at least when this resorption is compensated by growth of the new bone [25, 26].

R cement has a higher solubility than conventional CPC and its reaction products are similar to the mineral phase of bone (CDHA). A previous study [27] indicated that R cement promoted the formation of bone tissue in vivo and vascularization. R cement showed good osseointegration, a significant level of cement resorption and the creation of osteons and capillaries. This result was in contrast with previous cell culture studies with human bone marrow cells, where it was found that R cement was cytotoxic for cell growth, producing poor adhesion, negligible growth and cell death [27].These findings are probably attributable to the acidification of the media and, therefore, the increase of degradation, leading to a higher level of released Na^+ and K^+ ions.

The aim of the present work was to develop an injectable R cement in which the paste is previously prepared to avoid storage hardening. The injection properties of R cement can be improved by substituting the liquid phase with glycerol [6]. Glycerol was selected as a liquid phase because it is nontoxic, biocompatible and is also water-miscible [6]. When CPC–glycerol paste is immersed in water, there is a glycerol–water exchange, resulting in cement hardening [28]. Calcium phosphate cement paste must harden in an aqueous environment, which implies that the cement should not disintegrate upon contact with body fluids [21]. To improve the cohesion properties of the R cement paste, polyvinyl alcohol (PVA) have been used. PVA is a hydrophilic biocompatible polymer widely used in biomedical applications. It improves the cohesion and the injectable properties of the R cement paste. In this sense, the polar nature of PVA facilitates the formation of hydrogen bonds. It is expected that addition of PVA to the R cement/glycerol composition should improve "the stability" of the paste during the storage. Furthermore, PVA is a highly hydrophilic polymer and easily swells when absorbs water. This aspect would be a key advantage in order to absorb residual water from glycerol from the cement to avoid undesired settings before implantation.

In the present project, we designed and prepared an injectable calcium phosphate paste composition. The influences of the glycerol and polyvinyl alcohol on the physico-chemical properties of the R cements as well as the stability of the paste during storage were studied.

2 Materials and methods

2.1 Preparation of the R cement samples

The R cement was prepared as a mixture of two powders: CPSP [calcium potassium sodium phosphate; $Ca_2KNa(PO_4)_2$] and MCPM [monocalcium phosphate monohydrate; $Ca(H_2PO_4)_2{\cdot}H_2O$, Panreac] in the molar ratio of 2.5:1 giving a Ca/P ratio of 0.86.

The CPSP was synthesized by a solid-to-solid reaction between K_2CO_3 (Merck), $NaCO_3$ (Merck) and $CaHPO_4$ (Merck) at a temperature of 1200°C for 12 h followed by cooling in the furnace and milling.

As a liquid phase we have used a mixture of glycerol (Aldrich) and polyvinyl alcohol (PVA from Aldrich-Sigma, degree of hydrolysis 80%, molecular weight: 9,000–10,000 g/mol). PVA–glycerol solutions were prepared by partial dissolution of the PVA powder in glycerol under constant stirring for 6 h. The optimum quantity of the PVA required in the formulation was found by adding it in different weight ratio to the liquid part of the cement and monitoring the changes in paste cohesion evaluations. The chosen concentration was 0.5% of PVA mass fraction for the liquid phase. The liquid phase (glycerol mixed with polyvinyl alcohol) and the R cement powder were mixed in a mortar to obtain a water-free paste with workable consistency, using a liquid/powder ratio of 0.46 ml/g and then placed into the syringe. This mass fraction was chosen because it improved the cohesion and the injectable properties of the paste.

In order to determine the stability of the paste after two months, syringes with two different paste compositions were stored in the freezer: A^* (R cement/glycerol) and B^* (R cement/glycerol/PVA).

2.2 Characterization of the R cement samples

The initial setting time (t_I) and the final setting time (t_F) for each of the R cement composites, before and after two months storage in the freezer, were measured using the standard Gillmore needles method. For this study the cement paste was placed into a mold of 10 mm diameter and 3 mm depth and immersed in a Ringer's solution. Ringer's solution was prepared by dissolving 9 g of sodium chloride (NaCl, Panreac) in 1 l of water.

The injectability test was performed at a constant injection speed of 15 mm/min using a MTS 858 BIONIX. Three specimens were tested for each formulation. The injectability was reported as a weight loss percentage between the cement extruded from the syringe and the cement remained inside the syringe.

XRD was used to determine the crystalline phase after setting and to find out if a conversion to CDHA took place or if

remnants of a starting material were present. The XRD patterns were recorded with a powder X-ray diffractometer Bragg–Brentano PANalytical X'Pert PRO MPD (Cu-Kα_1 radiation, $\lambda = 1.5406$ Å, 40 mA, 45 kV, step size of 0.017°).

Fourier-transformed infrared (FTIR) spectroscopy (Bomem MB-120) was used to characterize the R cement samples as a function of immersion time. The FTIR absorption spectra were obtained within the range between 4000 and 400 cm^{-1}, with a step size of 1.93 cm^{-1} using the KBr pellet method.

The porosity and the pore size distribution of the cement samples were measured by a mercury intrusion technique (Autopore IV 9500).

To evaluate the compressive strength of the cement, the paste was molded into cylinders (6 mm in diameter, 12 mm in length). Then the samples were incubated in Ringer's solution for 1, 3, 7 and 14 days and then the compressive strength was measured. The compressive strength of the specimens was measured using a universal material testing machine (Adamel Lhomargy, DY 32/34, software AUTOTRAC). Each measurement was repeated five times. The compressive strength was calculated by using the fracture load divided by specimen's cross-section area. Immediately after the samples had been tested in compression, the specimens were immersed in acetone and dried to stop reaction. The microstructural development was investigated by scanning electron microscopy (SEM Jeol JSM 6400) on the broken surfaces of specimens previously tested in compression.

3 Results and discussion

The initial setting time, t_I, represents the time after which the cement paste reached a point of stiffness where it becomes unworkable, and t_F, final setting time, is the time required for the mixture to stiffen sufficiently to resist the penetration of a Gillmore test needle [29]. Setting times (t_I, t_F) before and after two months of storage in the freezer, are summarized in Table 1. For R cement/water paste initial setting time was about 10 min. The setting time increased with the presence of glycerol reaching 50–56 min. The samples which were stored in the freezer for two months showed a significantly lower setting time 29–36 min. The addition of PVA into the cement composition had little increase in the initial setting time, in both types of samples before and after storage in the freezer.

All cement pastes were injectable, both before and after 2 month of storage in the freezer. The applied force (Fig. 1) increased at the beginning of the injection and reached a constant level after several mm displacement of the syringe, disrupted only in part by a sudden decrease of the pressure when air bubbles were pressed out through the needle. After about 15–20 mm displacement the applied forces strongly increased up to the limit of 100 N since no further paste remained in the syringe. The extrusion was stopped when the applied force reached 100 N because it is considered the average maximum strength injection during surgery [18].

The injectability of the R cement pastes, reported as a weight loss percentage between the cement extruded from the syringe and the cement remained inside the syringe is present in Fig. 2. Although all types of R cement pastes were nearly fully injectable, there are some changes in the injectability of the pastes before and after storage in the freezer. A small difference is observed in the applied force in the second case, which is about 10 N higher surely due to the small reaction with ambient, moist or water from the glycerol. It is not significant and the blend can be easily injected anyway. However, samples with PVA seem to be not so sensitive to this phenomenon perhaps due to the water absorption from PVA hydrogel. In Fig. 2, two monthly storage samples have a little lower injectability which is even lower in the case of R cement/glycerol.

Figure 3 shows the patterns of X-ray diffraction of the sample A (R Cement/Glycerol) and B (R Cement/Glycerol/PVA) after 1, 3, 7 and 14 days of immersion in Ringer's solution and the same samples A^* and B^* which were placed into the Ringer's solution after two month of storage in the freezer. Both types of R cement showed the similar XRD patterns. After 1 day of immersion characteristic peaks of CPSP were present. PVA showed curious properties: it is able to increase the cement reaction rate. The reason is not clear, but perhaps it can be due to the increase of porosity originated when PVA is dissolved increasing the surface in contact with the fluid.

The pattern of the cement after 7 days of immersion, exhibited the characteristic peaks around $2\theta = 26$ and 32° attributable to hydroxyapatite. There were no CPSP peaks. During 7 days of immersion, all types of R cement samples

Table 1 Setting times for R cement composites before and after storage in the freezer

	Time-zero samples		Two months samples	
	(t_I) (min)	(t_F)	(t_I)	(t_F)
R cement/water	<10	16–20 min	–	–
R cement/glycerol	<50	4 h	<29 min	3 h 30 min
R cement/glycerol/PVA	<56	4 h	<36 min	3 h 30 min

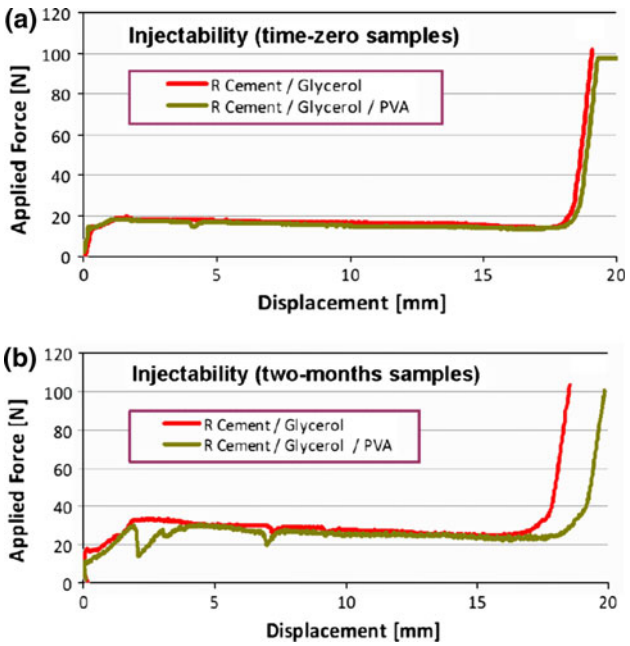

Fig. 1 Typical force/displacement diagrams of injectability experiments with R cement composites before (**a**), and after two month of storage in the freezer (**b**)

were totally converted to CDHA. There were no differences in XRD spectrum between samples.

The porosity values versus immersion time in Ringer's solution are presented in Fig. 4. R Cement/Glycerol/PVA time-zero sample showed a gradual increase between 1 and 14 days whereas R Cement/Glycerol showed a dramatic rise at between time points, either between 1 and 3 days or between 3 and 7 days, this sample also showed a decrease in porosity from 7 to 14 days. Porosity increases due to the dissolution of the starting materials and the precipitation of CDHA from the solution. PVA is highly hydrophilic therefore its presence in the time-zero cement sample seemed to determine the degree of reaction.

As we can observed in Fig. 4—there were small changes in porosity between samples before and after storage in the freezer. Cements are stored in a freezer what can provoke the freezing of the residual water from glycerol, cement or ambient. When water freezes and then melts again at room temperature, it leaves micro and nanopores than can participate in the total recount analysis. In the case of PVA blend, water is mainly absorbed by PVA which still does not freeze.

The porosity of R cement materials after 14 days of immersion in Ringer's solution, as measured by Hg-porosimetry, was about 55%. The mercury intrusion for all types of samples after 7 days of immersion in Ringer's solution shows a bimodal distribution of pore sizes, centered at 0.02 and 0.5 μm (Fig. 5).

The porosity was distributed at different scales: (1) Porosity with pore diameter centered at 0.02 μm is produced by the spaces between crystals and (2) porosity related with pore size of 0.5 μm is corresponding to the space between crystal agglomerates precipitated during the setting reaction (balls). These features are common for all the blends, excepting for time-zero R cement/glycerol, which seems to degrade so fast that at 14 days, this morphology is disappeared.

The effects of PVA addition on mechanical properties of the R cement after different immersion times in Ringer's solution are shown in Fig. 6. In both cements (samples A, B) the maximum hardening in vitro was attained after 1 day of immersion. In order to compare, the compressive strength values of the cement control are also shown, prepared from R cement powder and water, using a liquid/powder ratio of 0.5.

All R cement composites samples show a decrease in the compressive strength in comparison with original R cement (mixed with water). The incubation time and the effects of storage time produced significant effects on compressive strength. The compressive strength of every sample significantly decreased after 7 days of immersion in Ringer's solution. At the same incubation time (7 days), the compressive strength of samples which were stored for two month in the freezer was lower than from time-zero group and remained at 2 MPa until the end of the experiment.

The FTIR analysis of R cement/glycerol (A) and R cement/glycerol/PVA (B) samples before and after storage in the freezer showed all typical absorption characteristics

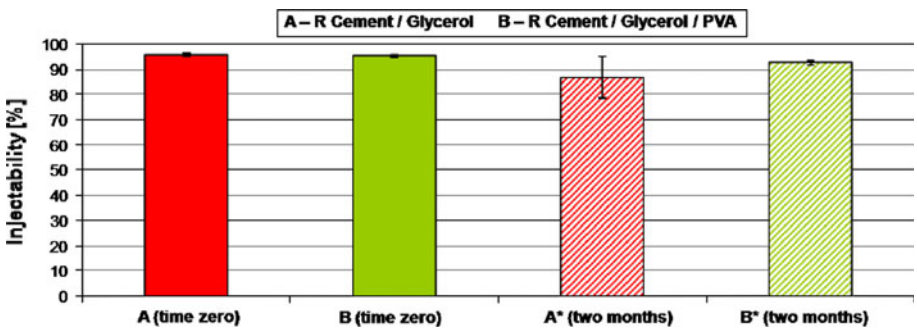

Fig. 2 Injectability of R cement pastes before and after two month of storage in the freezer

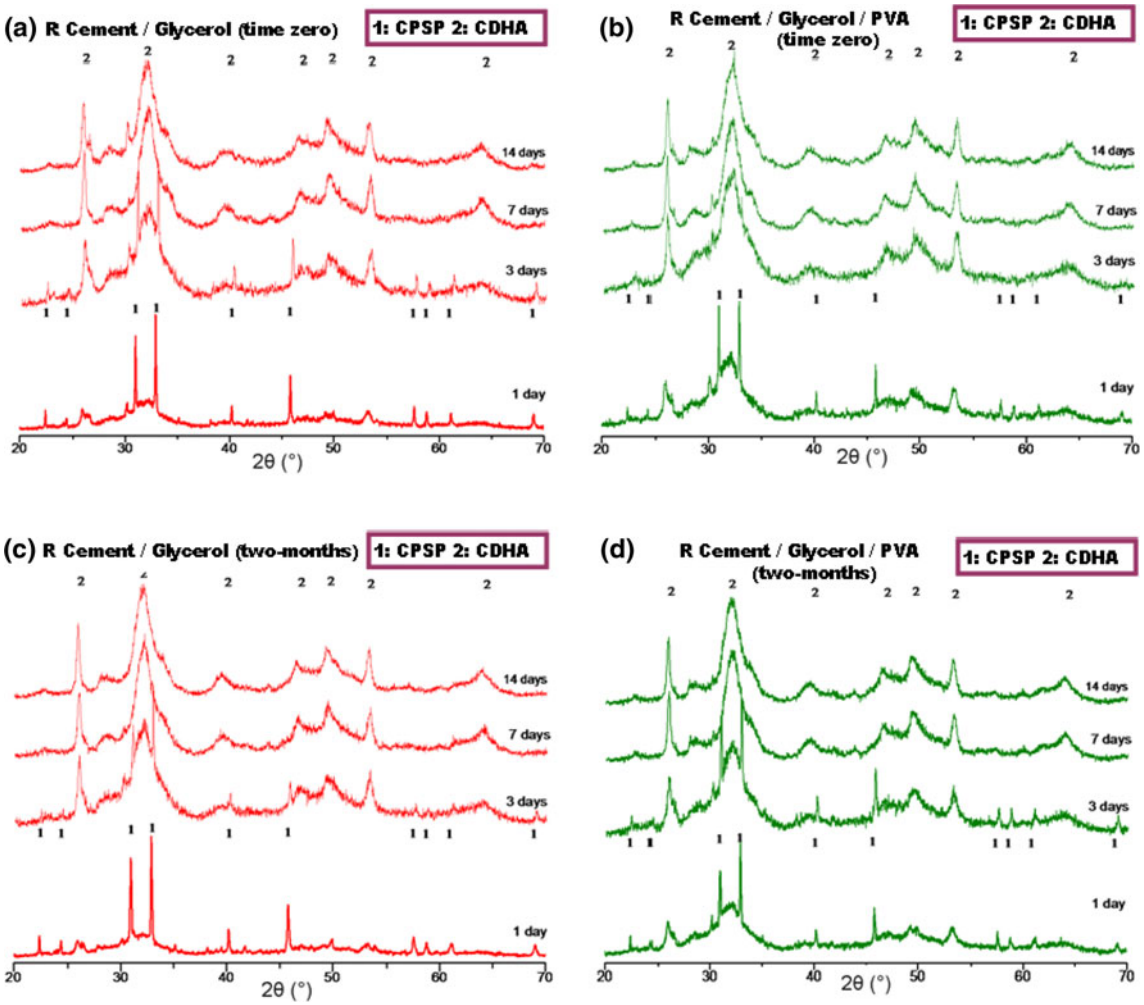

Fig. 3 The X-ray diffraction spectrum of the R cement/glycerol and R cement/glycerol/PVA samples after different times of immersion in Ringer's solution. **a**, **b** time-zero samples; **c**, **d** two month samples

peaks of apatite. Representative FTIR spectra of the R cement/glycerol samples are shown in Fig. 7. The spectra of the samples obtained after two month of storage (Fig. 7b) are very similar to the spectra exhibited in Fig. 7a. The characteristic absorption bands of phosphate appearing at 570, 600 and 961 cm^{-1} were observed for all immersed specimens. Similarly, the peaks at 3571 and 627 cm^{-1} were due to vibration bands of hydroxyl group. In addition, some carbonate content also was seen (CO_3 groups were presented at 877 cm^{-1}), which is an indication of the presence of carbonate apatite. This might have originated through the absorption of carbon dioxide from the atmosphere. The entire spectrum shows the absence of carbonate peaks in the range 1500–1400 cm^{-1} indicating that there was no formation of calcium carbonate or calcium oxide. There were no evidence of polymer content in FTIR analysis which means that it can be dissolved in the first steps of immersion or in contrast, the low concentration does not allow reveal the polymer peaks from the background.

Figure 8 shows SEM images of R cement/glycerol (time-zero samples) and R cement/glycerol/PVA (after two months of storage in the freezer) both specimens after soaking in Ringer's solution for 3 days. From Fig. 8 it can be seen that granular crystals appeared on the surface of specimens soaked in Ringer's solution for 3 days. In both cases SEM micrograph shows the formation of nanosized hydroxyapatite crystals. The surface of sample A showed spherical particles containing tiny crystals which correspond to the apatite. After 1 day of immersion the surfaces were completely covered by flowery-like particles, which changed the original morphology of the specimens completely.

The surfaces of samples which contain PVA were all rough and uneven, with micropores. A high amount of precipitated crystals were observed in both types of R

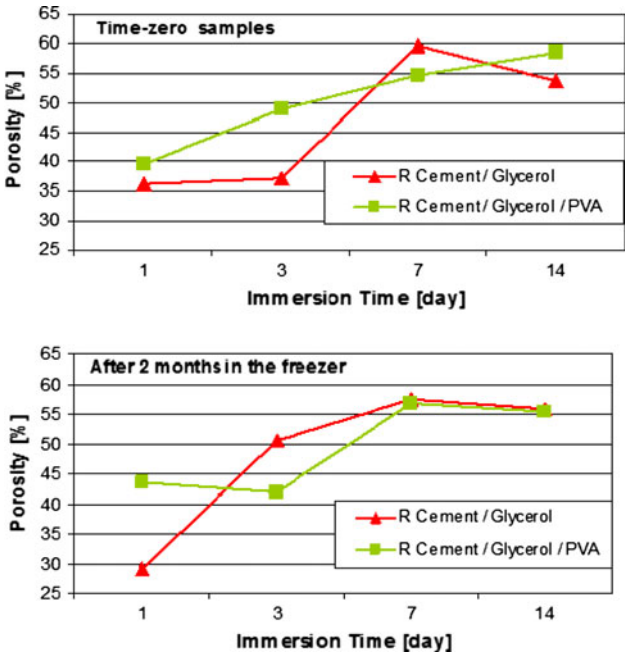

Fig. 4 Porosity of the R cement/glycerol samples after different incubation's times in Ringer's. **a** time-zero samples, **b** samples after two months of storage in the freezer

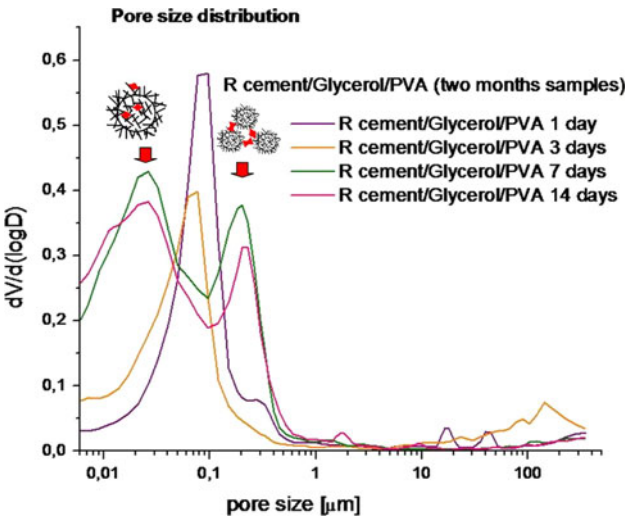

Fig. 5 Typical pore size distribution for R cement/glycerol/PVA compositions after 1, 3, 7 and 14 days in Ringer's solution, as measured by Hg-porosimetry

cement. There were no significant changes in the morphology of R cement before and after two month storage in the freezer.

4 Conclusions

Glycerol addition decreases the injection pressure. No disintegration was observed but the setting time increased.

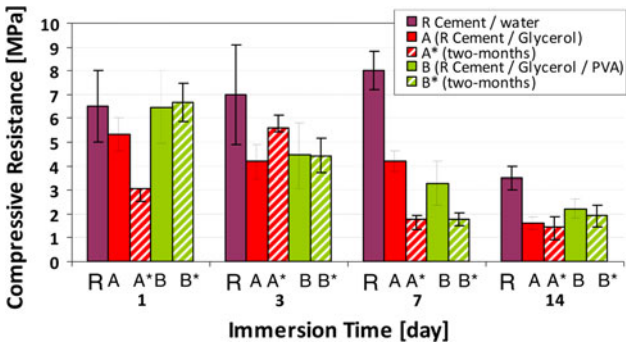

Fig. 6 Compressive strength of the R cement/Glycerol/Polymer composites: *A* (R cement/Glycerol), *A** (R cement/Glycerol after two months of storage), *B* (R cement/Glycerol/PVA), *B** (R cement/Glycerol/PVA after two month of storage in the freezer). As reference the compressive strength of R cement/water is also presented [31]

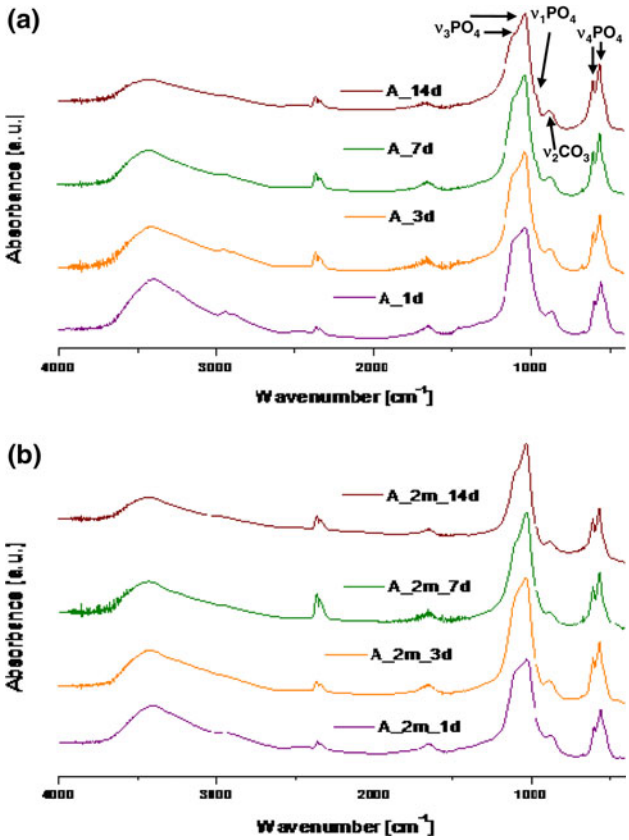

Fig. 7 FTIR spectra of the injectable R cements after different times in Ringer's solution. **a** Time-zero samples, **b** two month samples

When the paste was immersed in Ringer's solution, there was a glycerol-to-water exchange, resulting in cement hardening. The cement setting is the result of a dissolution and precipitation process, and the entanglement of the precipitated crystals is responsible for cement hardening [30]. After 7 days of immersion in Ringer's solution, R cements samples were totally converted to CDHA as

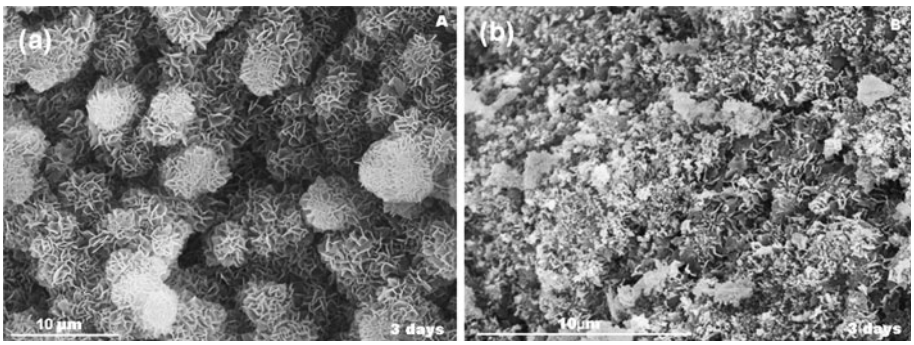

Fig. 8 SEM micrographs of the fracture of both types of R Cement samples: **a** *A* (R cement/Glycerol), and **b** *B** (R cement/Glycerol/PVA) after storage in the freezer. The both samples were immersed during 3 days in Ringer's solution

determined by X-Ray diffraction. XRD results showed no difference between R cement before and after two month of storage in the freezer. SEM results showed that apatite was formed within a short period on R cements samples after its immersion in Ringer's solution, demonstrating high in vitro bioactivity of those materials. The precipitation of apatite increased with increasing immersion time for the materials. Porosity gradually increased due to the degradation of samples when immersed in Ringer's solution, resulting in a compressive strength decrease. After the initial hardening produced by the setting reaction, R cement underwent a rapid loss of mechanical properties in vitro.

As observed in previous studies R cement has a high tendency to dissolve in body fluids [27]. In a closed system, R cement will transform spontaneously into calcium deficient hydroxyapatite with the release of Na^+ and K^+ ions. The dissolution of the R cement in Ringer's solution can not be extrapolated to what happens in vivo, where the extracellular fluids in the bone tissue are saturated with respect to hydroxyapatite [27]. Mechanical study revealed that the compressive strength of materials decrease with immersion time in Ringer's, due to high solubility of the R cement.

PVA not only acts as a cohesive agent in R cement but also provides several improvements, such as increasing mechanical properties and prolong the stability of the paste during the storage.

We have been able to fabricate and characterize a new injectable calcium phosphate cement, applicable as, for example, bone joint in periodontal surgery and other non load bearing applications in tissue engineering, which can be successfully stored in the freezer without changing their properties. R cement paste composition was developed to eliminate the need for on-side powder–liquid mixing during surgery and minimize implant performance variations due to insufficient or inhomogeneous mixing.

Acknowledgments This work was supported by the Spanish Ministry of Education and Science, project "Estancias de jovenes doctores extranjeros en universidades publicas y centros de investigacion españoles" 2007–2008. O. Castano also acknowledges the MICINN for the Ramony Cajal contract.

References

1. Fernandez E, Gil FJ, Ginebra MP, Driessens FCM, Planell JA. Calcium phosphate bone cements for clinical applications Part II: precipitate formation during setting reactions. J Mater Sci. 1999;10:177–83.
2. Xu HHK, Carey LE, Simon CG Jr, Takagi S, Chow LC. Premixed calcium phosphate cements: synthesis, physical properties, and cell cytotoxicity. Dent Mater. 2007;23:433–41.
3. 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;25:3453–62.
4. Tamimi F, Kumarasami B, Doillon C, Gbureck U, Le Nihouannen D, Lopez Cabarcos E, Barralet JE. Brushite-collagen composites for bone regeneration. Acta Biomater. 2008;4:1315–21.
5. Fernandez E, Gil FJ, Ginebra MP, Driessens FCM, Planell JA. Calcium phosphate bone cements for clinical applications Part I: solution chemistry. J Mater Sci. 1999;10:169–76.
6. Carey LE, Xu HHK, Simon CG Jr, Takagi S, Chow LC. Premixed rapid-setting calcium phosphate composites for bone repair. Biomaterials. 2005;26:5002–14.
7. Takagi S, Chow LC. Self-setting calcium phosphate cement and methods for preparing and using them. US Patent 5525148. 1996.
8. Xu HHK, Quinn JB, Takagi S, Chow LC. Synergistic reinforcement of in situ hardening calcium phosphate composite scaffold for bone tissue engineering. Biomaterials. 2004;25:1029–37.
9. Ginebra MP, Fernandez E, De Maeyerl EAP, Verbeeckl RMH, Boltong MG, Ginebra J, Driessens FCM, Planell JA. Setting reaction and hardening of an apatitic calcium phosphate cement. J Dent Res. 1997;76(4):905–12.
10. Takagi S, Chow LC, Hirayama S, Eichmiller FC. Properties of elastomeric calcium phosphate cement-chitosan composites. Dent Mater. 2003;19:797–804.
11. Khairoun I, Driessens FCM, Boltong MG, Planell JA, Wenz R. Addition of cohesion promotors to calcium phosphate cements. Biomaterials. 1999;20:393–8.
12. 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.
13. Xu HHK, Burguera EF, Carey LE. Strong, macroporous, and in situ-setting calcium phosphate cement-layered structures. Biomaterials. 2007;28:3786–96.
14. Habib M, Baroud G, Gitzhofer F, Bohner M. Mechanisms underlying the limited injectability of hydraulic calcium phosphate paste. Acta Biomater. 2008;4:1465–71.
15. Xu HHK, Takagi S, Sun L, Hussain L, Chow LC, Guthrie WF, Yen JH. Development of nonrigid, durable calcium phosphate

cement for use in periodontal bone repair. J Am Dent Assoc. 2006;137:1131–8.
16. Xu HHK, Weir MD, Simon CG. Injectable and strong nano-apatite scaffolds for cell/grow factor delivery and bone regeneration. Dent Mater. 2008;24:1212–22.
17. Bohner M, Baroud G. Injectability of calcium phosphate pastes. Biomaterials. 2005;26:1553–63.
18. Bohner M, Gbureck U, Barralet JE. Technological issues for the development of more efficient calcium phosphate bone cements: a critical assessment. Biomaterials. 2005;26:6423–9.
19. Komath M, Varma HK. Development of fully injectable calcium phosphate cement for orthopedic and dental application. Bull Mater Sci. 2003;26:415–22.
20. Sun L, Xu HHK, Takagi S, Chow LC. Fast setting calcium phosphate cement-chitosan composite: mechanical properties and dissolution rates. J Biomater Appl. 2007;21:299–312.
21. Xu HHK, Weir MD, Burguera EF, Fraser AM. Injectable and macroporous calcium phosphate cement scaffold. Biomaterials. 2006;27:4279–87.
22. Liu H, Li H, Cheng W, Yang Y, Zhu M, Zhou C. Novel injectable calcium phosphate/chitosan composites for bone substitute materials. Acta Biomater. 2006;2:557–65.
23. Costa V, Costa H, Vasconcelos WL. Preparation of hybrid biomaterials for bone tissue engineering. Mater Res. 2007;10(1):21–6.
24. Ishikawa K, Takagi S, Chow LC, Ishikawa Y, Eanes ED. Behavior of a calcium phosphate cement in simulated blood plasma in vitro. Dent Mater. 1994;10:26–32.
25. Driessens FCM, Boltong MG, De Maeyer EAP, Wenz R, Nies B, Planell JA. The Ca/P range of nanoapatitic calcium phosphate cements. Biomaterials. 2002;23:4011–7.
26. Takagi S, Chow LC. Formation of macropores in calcium phosphate cement implants. J Mater Sci. 2001;12:135–9.
27. Sanzana ES, Navarro M, Macule F, Suso S, Planell JA. Of the in vivo behavior of calcium phosphate cements and glasses as bone substitutes. Acta Biomater. 2008;4:1924–33.
28. Takagi S. Rapid-hardering calcium phosphate cement compositions. US Patent 7294187. 2007.
29. ASTM Standard C266, "Standard test method for time of setting of hydraulic-cement paste by gillmore needles," ASTM International; West Conshohocken, 2008. doi:10.1520/C0266-08. www.astm.org.
30. 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 A 2006:351-361.
31. Mestres G, Castaño O, Navarro M, Almirall A, Sanzana ES, Ginebra MP, Planell JA. A novel hybrid calcium phosphate/ACP injectable cement. World Biomaterials Congress 2008. p. P432.