Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 274 (2004) 261–268
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Behaviour of an injectable calcium phosphate cement with added tetracycline

A. Ratier [a], M. Freche [b,*], J.L. Lacout [b], F. Rodriguez [a]

[a] *Laboratoire de Pharmacie Galénique, GEFSoD EA 2631, Faculté des Sciences Pharmaceutiques, 35 chemin des Maraîchers, 31062 Toulouse Cedex, France*

[b] *CIRIMAT Physico-Chimie des Phosphates, UMR CNRS 5085, ENSIACET, 118 route de Narbonne, 31077 Toulouse Cedex, France*

Received 1 August 2003; received in revised form 8 January 2004; accepted 19 January 2004

Abstract

A calcium phosphate cement containing an antibiotic can be used for filling bone defects and to ensure local antibiotherapy. A calcium phosphate cement (already marketed under the name of Cementek®) can become injectable thanks to the addition of silicone. For dental applications, the behaviour of this injectable cement with added tetracycline was investigated. The tetracycline hydrochloride does not allow maturation of the cement: the tetracycline has to be treated with a calcium sulphate solution. The treated tetracycline (TTC) allowed maturation of the cement towards hydroxyapatite. But the setting time was longer and the mechanical properties decreased. Study in a continuous flow cell showed that the tetracycline is released in a continuous manner: thus, after 6 days, 60% of the antibiotic was released into the surrounding medium.

Keywords: Calcium phosphate; Cement; Injectable; Tetracycline

1. Introduction

Calcium phosphate cements are used in surgery or dentistry for filling bone defects (Fukase et al., 1990; Ogilvie et al., 1987). The "in vivo" use of such biomaterials requires preventive antibiotic therapy to avoid the development of postoperative infections. This is achieved either by daily systemic administration (oral or injection) or by "in situ" application. We hope to propose local treatment released "in situ" from the cement. The introduction of active ingredients in such material has been studied for several years (Bohner et al., 1997; Korkusuz et al., 1993; Otsuka et al., 1994).

A non-injectable apatitic cement, Cementek®, has become established in orthopaedic and dental use (Hatim et al., 1997; Lacout et al., 1998). Lacout et al. showed that if silicone was added to the solid phase of this cement, it becomes injectable while preserving its biocompatibility properties (Gonçalvez et al., 2001; Lacout et al., 2000).

The antibiotic chosen for this study was tetracycline because of its large bacteriostatic spectrum active in periodontal diseases (Bercy, 1996; Siegel, 1981). In the presence of tetracycline hydrochloride, the most widely used form, the properties of the cement were strongly modified (Ratier et al., 2001a). The rheological modification was probably due to the presence of traces of magnesium in the antibiotic (Ratier, 2001b). In order to conserve the physicochemical properties of the cement, the tetracycline was initially treated with

* Corresponding author. Tel.: +33-5-62-88-57-77; fax: +33-5-62-88-57-73.
E-mail address: michele.freche@ensiacet.fr (M. Freche).

0378-5173/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2004.01.021

a calcium sulphate solution before being introduced into the cement. It has been checked that the bacteriostatic activity of the active ingredient is preserved. Therefore, the behaviour of an injectable cement containing a tetracycline treated with a calcium sulphate solution was investigated in this study.

2. Materials and methods

2.1. Materials

The cement used in this study is marketed under the name of Cementek® (Teknimed, France). The preparation of the cement consists of mixing a powder with a liquid phase. The solid phase is made up of a mixture of α-tricalcium phosphate (α-TCP; $Ca_3(PO_4)_2$: 38% (w/w)), tetracalcium phosphate (TTCP; $Ca_4(PO_4)_2O$: 49% (w/w)) and sodium glycerophosphate ($C_3H_7Na_2O_6P$: 13% (w/w)). The liquid phase is prepared from lime ($Ca(OH)_2$: 32% (w/w)) and phosphoric acid (H_3PO_4: 68% (w/w)).

In order to render the cement injectable, silicone was added by evaporation of a solution of silicone in cyclohexane (50/50, v/v) mixed in with the solid phase. Two formulae of injectable cements were tested containing, respectively, 1 and 2% (w/w) of silicone in the solid phase.

The antibiotic was introduced into the solid phase of the cement. In order to maintain a liquid to powder weight ratio of 0.43, distilled water was added. Tetracycline hydrochloride ($C_{22}H_{24}N_2O_8$,HCl, Coopération Pharmaceutique Française, batch no. G7601/6) was used as the antibiotic. The treated tetracycline (TTC) was prepared, as previously described, in the laboratory by adding 7 g of tetracycline hydrochloride to 1 l of a solution saturated with calcium sulphate ($CaSO_4 \cdot 2H_2O$, Prolabo, batch no. 87056). After 15 h of stirring in the absence of light, a yellow precipitate formed. This was filtered off and freeze dried. The TTC was kept at 5 °C in the dark. The tetracycline was identified by an inductively coupled plasma method as a tri-hydrated basis tetracycline.

2.2. Samples preparation

Preliminary experiments determined the maximum amount of TTC preserving the mechanical properties and the injectability of the cement: it was fixed at 7% (w/w). The percentages of antibiotic were calculated with regard to the mass of the solid phase. The antibiotic was blended for 5 min with the solid phase before mixing the solid phase with the liquid phase for 2 min. The cements were identified according to the percentages of silicone and treated tetracycline as follows: *Sx* TTCy with *x* the percentage of silicone and *y* the percentage of antibiotic.

2.3. Physicochemical characterisation

2.3.1. Setting time

The setting time of the cement was defined as the time required for a paste to reach a fixed hardness. A TA-XT2 Texture Analyser was used to measure the resistance to penetration of a needle 0.7 mm in diameter and 1.5 cm in length. The speed of the needle penetrating into soft paste was 2 mm/s. Needle displacement was fixed at 5 mm. Specific software was used to draw the curve of "Resistance exerted by the cement on the needle versus the displacement of the needle into the paste". These measurements allowed determination of the "Maximum of resistance versus time". According to convention, setting corresponds to a degree of resistance to cement penetration of 600 g/mm^2.

2.3.2. X-ray diffraction analysis

The X-ray diffraction patterns of the cements were recorded by X-ray powder diffraction analysis (CPS 120 INEL, Co radiation, $\lambda = 1.78892$ Å, 30 mA, 40 kV, duration of measurement: 30 min, $2\theta = 10°$–$70°$).

2.3.3. Scanning electron microscopy

The samples were coated with silver and SEM micrographs were taken with a LEO 435 VP.

2.3.4. Mechanical properties

The samples were prepared from a paste placed in a mould for 1 h. The specimens were removed from the moulds and immersed in distilled water at 38 °C. The distilled water was replaced daily to avoid saturation by the active ingredient. Sample characterisation was performed after 14 days. The samples were dried for 1 day, at room temperature before testing. All testing was performed under compression using a Hounsfield series S machine. Load was applied

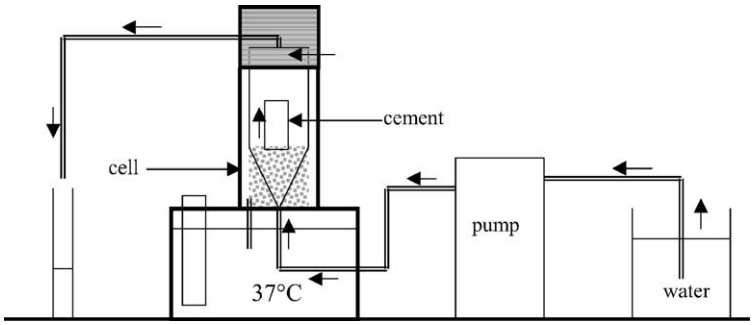

Fig. 1. Schematic representation of the release device.

axially to the specimens with a cross head velocity of 0.1 mm/min. The compressive strength (C) was determined on five cylinders 6 mm in diameter and 6 mm in height. C was calculated with the following formula: $C = F/S$ where F is the maximum load applied and S the cross-sectional area of the cylinder.

2.3.5. Injectability

The injectability was defined as the ability of cement to move through a syringe–catheter device. The force applied to move a 8 cm column of cement through a 3 mm diameter polyethylene catheter was measured using a TA-XT2 Texture Analyser. The time of injectability is the time after which the cement can no longer be injected by an operator: this corresponds to pressure lower than 1.5 bars.

2.4. Kinetics of release

2.4.1. Experimental device

The device used was the cell described in the European Pharmacopoeia, fourth edition, Section 2.9.3. (European Pharmacopoeia, 2002). The equipment (Fig. 1) included three cells wired up in parallel. The dissolution medium, distilled water at 37 °C, circulated by a pump with a flow rate of 0.5 ml/min. After passing through the cell, the solution containing the dissolved active ingredient was collected in tubes. The tubes were changed every 64 min. Every 12 h, samples were frozen at −18 °C until assay. This procedure was used in order to prevent the appearance of degradation products. The whole set-up was kept in the dark. The study was performed for 6 days.

2.4.2. Operating conditions

Four samples (6 mm × 6 mm, total weight 1.24 g) were used for each experiment. One hour after moulding, they were removed from the mould and placed into a continuous flow cell. The four samples contained 61 mg of tetracycline.

2.4.3. Assay

The amount of antibiotic released into distilled water was measured by HPLC (column: Symmetry^{TM} C_8 3.9 mm × 150 mm, mobile phase: 0.1 M sodium phosphate, pH 6.25, methanol 47/55, flow rate: 0.7 ml/min, detection: 280 nm) at different times. After 6 days the samples were ground and the available quantity of antibiotic was determined.

3. Results

3.1. Physical characterisation

Table 1 summarises the duration of injectability, the setting time and the compressive strength obtained for

Table 1
Variation of the mechanical properties with the composition of cement

Formulation	Duration of injectability (min)	Setting time (min)	Compressive strength (MPa)
S0 TTC0	Not injectable	19 ± 1	20 ± 2
S0 TTC7	Not injectable	43 ± 6	13 ± 2
S1 TTC0	13	31 ± 3	19 ± 2
S1 TTC7	15	62 ± 5	9 ± 1
S2 TTC0	12	38 ± 2	14 ± 1
S2 TTC7	15	74 ± 3	8 ± 1

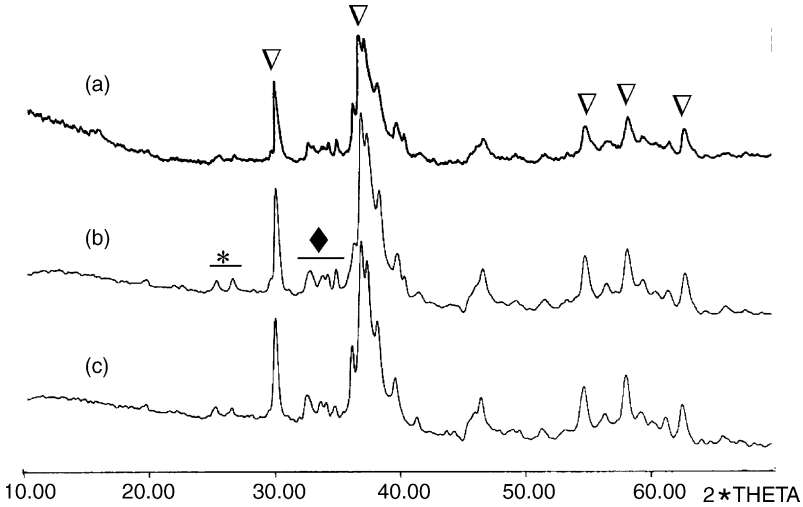

Fig. 2. X-ray diffraction diagrams. (a) Non-injectable cement; (b) cement with 1% (w/w) of silicone; (c) cement with 2% (w/w) of silicone. (▽) HAp, (◆) TTCP, (*) α-TCP.

each kind of cement. The presence of silicone and/or tetracycline modified the physical properties of the cement.

In the absence of an active ingredient, the addition of 1 or 2% of silicone led to the same duration of injectability (S1 TTC0 and S2 TTC0). The cements were injectable for about 12 min. Introducing the antibiotic, the time during which the cement was injectable was slightly increased to 15 min. For manual use, injection seemed to be easier while using a 2% silicone cement.

The setting time of cements containing only silicone (S1 TTC0 and S2 TTC0) was slightly above the one measured for the Cementek® (S0 TTC0) (31 versus 19 min). The compressive strength was about 16 versus 20 MPa for the Cementek®.

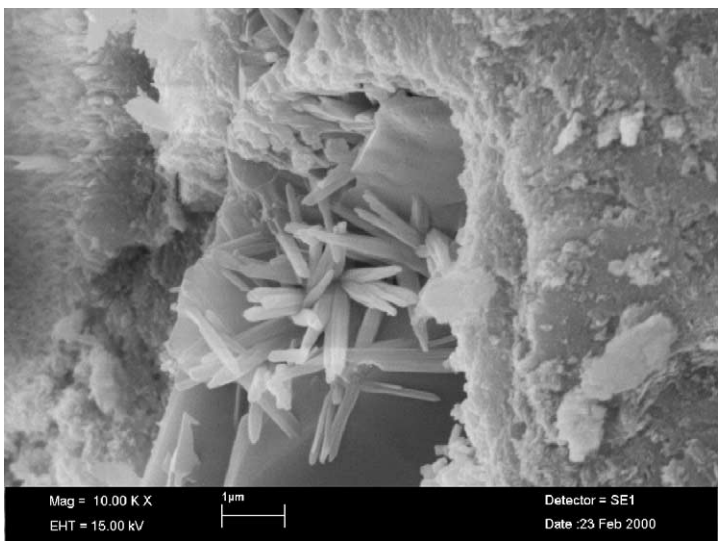

Fig. 3. SEM picture of a cement containing 1% (w/w) of silicone in the solid phase.

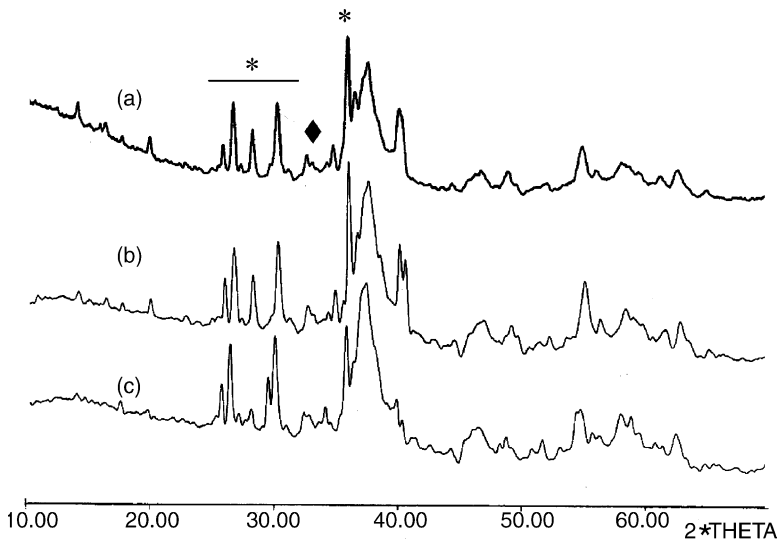

Fig. 4. X-ray diffraction diagrams. (a) Cement S0 TTC7; (b) cement S1 TTC7; (c) cement S2 TTC7. (◆) TTCP, (*) α-TCP.

Concerning the injectable cements containing tetracycline, the setting time increased, it was almost doubled (62 versus 31 min for cements containing 1% silicone) and the mechanical strength decreased to about 9 MPa.

For all the cements without antibiotic, after 14 days of maturation in water, the X-ray diffraction analysis showed a main apatitic phase and two extra phases of starting reagents (TTCP and α-TCP) (Fig. 2). At that moment the reagents had not completely reacted. It was confirmed by the SEM observation: on the SEM picture (Fig. 3), the main phase was composed of needles of apatite, some of them were bigger than 1 μm; nevertheless, some tablet-shaped crystals characteristic of brushite, an intermediate phase formed during the maturation of the cement were also visible.

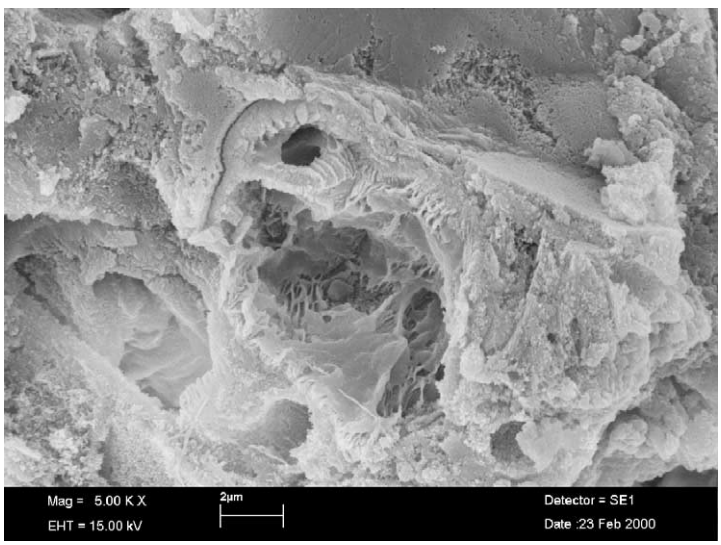

Fig. 5. SEM picture of a cement containing 2% (w/w) of silicone and 7% (w/w) of TTC in the solid phase.

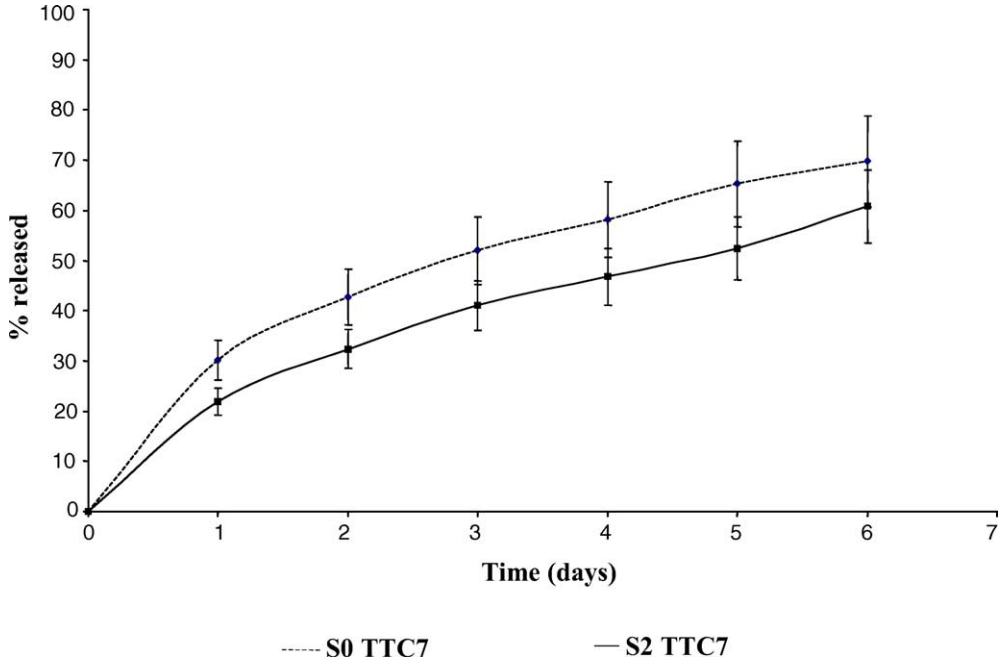

Fig. 6. Curve of release kinetics of tetracycline (calculated regarding the total amount of antibiotic available at the end of the experiment).

In comparison, maturation was strongly modified by the presence of tetracycline. For the cements containing this antibiotic, after 14 days of maturation, the X-ray diffraction patterns (Fig. 4) showed an apatitc phase in small amounts and the initial reagents in large proportions and the SEM did not enable the observation of needles of apatite (Fig. 5).

3.2. Kinetics of release

The study was performed with two kinds of cement containing 7% TTC in the solid phase which corresponds to 4.9% in the final cement: the non-injectable cement (S0 TTC7) and the injectable one (S2 TTC7 containing 2% silicone). The percentage of antibiotic released was calculated versus the total antibiotic quantity available after 6 days.

Fig. 6 which reports the quantity of this released antibiotic percentage versus time allows the distinction of two steps of release. For the two cements, injectable or not, roughly identical curves were observed: a fast first step during 24 h, then a slower, almost linear, second step.

Comparing the kinetics, on the first day, the amount of active principle released by the cement containing the silicone was lower than the amount released by the non-injectable cement (respectively, 22 and 30%). Then, the amounts released became almost identical for the two kinds of cements (about 1.5 mg of antibiotic per day and per gram of cement).

4. Discussion

4.1. Physicochemical characterisation

The addition of treated tetracycline alone (and a liquid/solid ratio equal to 0.43) did not give an injectable product. The contribution of the silicone was absolutely necessary. Three factors act on the rheology of these cements: the presence of silicone, the water added to the liquid phase in order to maintain the liquid/solid ratio equal to 0.43 and the amount of treated tetracycline added.

The effect of the silicone was described previously (Gonçalvez et al., 2001). The silicone is a lubricant and modifies the inter-particle links. It favours the sliding of the particles past one another and the sliding of the cement column along the syringe–catheter device.

During crystallisation, the water acted as a thinning agent. The viscosity of the paste decreased. It also had an effect on the mechanical properties. The excess of water contributed to an increased porosity and it decreased the mechanical properties (Betchem et al., 1997; Hammanishi et al., 1996).

The tetracycline presents a strong adsorption affinity for calcium phosphate. The presence of TTC led to an increase of the setting time that is to say a slowing down of the formation rate of the different phases (brushite, HAp) (Ratier et al., 2001a). This slowing down was correlated with enhanced injectability. Even after 14 days, only a small proportion of hydroxyapatite was formed. This slight amount can be due to a slowing down or a momentary locking of the maturation.

4.2. Kinetics of release

The quantity of available antibiotic is only 41% of the initial quantity. This is in agreement with its strong affinity with calcium ions.

For the two cements, injectable or not, the shape of the release curves was identical. Two phases can be distinguished. These types of curves were described and modelled by Tung for in-vitro release of antibiotic-loaded porous hydroxyapatite cement (Tung, 1995). The first step corresponds to a fast release in the first 24 h. The first day, 22% of the antibiotic available was released by the cement containing 2% silicone and 30% released for the cement without silicone. This high percentage could be due to the dissolution of the active ingredient present on the surface of the sample. The second part of the curve is nearly linear with a weak slope. It corresponds to a step with nearly constant kinetics of release. This can be explained by a dissolution–diffusion phenomenon. First, the active ingredient dissolves into the liquid in the pores and then diffuses from the bulk into the medium. Diffusion of the active ingredient appears able to go on until its almost total release.

The hydrophobic character of silicone can explain the different release rates during the first day (Le Hir, 2001). Thus, the silicone made the surface of the cement grains less wettable. Crossing of the active ingredient from the solid phase was slower. This explains that during the 1 h, the amount of active ingredient released was lower for the cement containing 2% silicone than for the Cementek®.

5. Conclusion

It is possible to make a phosphocalcium cement injectable by adding silicone to the solid phase. To add a tetracycline, it was necessary to treat the tetracycline hydrochloride by a dissolution–precipitation method. The formulation of an injectable cement containing tetracycline imposed maintaining the liquid/solid ratio at 0.43, increasing the amount of water added to the liquid phase. Good results were obtained with cements containing 2% (w/w) of silicone and up to 7% (w/w) of TTC.

The combined effects of the water, the silicone and the treated tetracycline led to a diminution of the mechanical properties, and an increase of the setting time in comparison with cements without antibiotic and good injectability.

The study of the release kinetics showed that the cements prepared allowed progressive release of tetracycline over time. The cements released high concentrations of active ingredient.

References

Bercy, T., 1996. Parodontologie: du diagnostic à la pratique. Parodontology: from Diagnosis to Practice (in French). De Boeck Université, pp. 193–201.

Betchem, F., Michaud, P., Rodriguez, F., Hatim, Z., 1997. Ionic cements: influence of the liquid/solid ratio on porosity and mechanical properties. Bioceramics 10, 179–182.

Bohner, M., Lemaître, J., Van Landuyt, P., Zambelli, P.Y., Merkle, H.P., Gander, B., 1997. Gentamicin-loaded hydraulic calcium phosphate bone cement as antibiotic delivery system. J. Pharm. Sci 86, 565–572.

European Pharmacopoeia, 2002. European Pharmacopoeia, fourth ed. Editions of the European Council, Strasbourg, Section 2.9.3.

Fukase, Y., Eanes, E.D., Takagi, S., Chow, L.C., Brown, W.E., 1990. Setting reactions and compressive strengths of calcium phosphate cements. J. Dent. Res. 69, 1852–1855.

Gonçalvez, S., Brouchet, A., Frèche, M., Rodriguez, F., Delisle, B., Lacout, J.L., 2001. Formulation of an injectable phosphocalcium cement. Key Eng. Mater. 192–195, 789–792.

Hammanishi, C., Kitamoto, K., Ohura, K., Tanaka, S., Doi, Y., 1996. Self-setting, bioactive and biodegradable TTCP–DCPD apatite cement. J. Biomater. Res. 32, 383–389.

Hatim, Z., Frèche, M., Lacout, J.L., 1997. Ciments inoragniques apatitiques: mécanisme de prise, mise en place (French

language). Inorganic Apatitic Cement: Setting Mechanism, Setting Up (in French), vol. IV. Actualités en Biomatériaux, pp. 290–214.

Korkusuz, F., Uchida, A., Shinto, Y., Araki, N., Inoue, K., Ono, K., 1993. Experimental implant-related osteomyelitis treated by antibiotic–calcium hydroxyapatite ceramic composites. J. Bone Jt. Surg. 75-B, 111–114.

Lacout, J.L., Hatim, Z., Frèche, M., 1998. French patent no. 98.03459.

Lacout, J.L., Frèche, M., Gonçalvez, S., Rodriguez, F., 2000. French patent no. 00.02615.

Le Hir, A., 2001. Pharmacie Galénique: Bonnes pratiques de fabrication des médicaments. Pharmacology: Good Practices of Manufacturing of Medicines (in French), eighth ed. Masson, Paris.

Ogilvie, A., Frank, R., Benqué, E.P., Gineste, M., Heughebaert, M., Hemmerle, J., 1987. The biocompatibility of hydroxyapatite implanted in the human periodontium. J. Periodontal Res. 22, 270–283.

Otsuka, M., Nakahigashi, Y., Matsuda, Y., Fox, J.L., Higuchi, W.I., 1994. A novel skeletal drug delivery system using self-setting calcium phosphate cement. 7. Effect of biological factors on indomethacin release from the cement loaded on bovine bone. J. Pharm. Sci. 83, 1569–1573.

Ratier, A., Gibson, I.R., Best, S.M., Frèche, M., Lacout, J.L., Rodriguez, F., 2001a. Setting characteristics and mechanical behaviour of a calcium phosphate bone cement containing tetracycline. Biomaterials 22, 897–901.

Ratier, A., 2001b. Comportement d'un ciment mineral à usage biologique contenant de la tetracycline: étude physico-chimique et libération de l'antibiotique. Behaviour of a Mineral Cement of Biological Uses Containing Tetracycline: Physicochemical Study and Antibiotic Release (in French). Thesis, Université Paul Sabatier.

Siegel, I.A., 1981. Pharmacology of antibiotics used in dentistry. Int. Dent. J. 31, 133–144.

Tung, I.C., 1995. In-vitro release of antibiotic-loaded porous hydroxyapatite cement. Art. Cells Blood Subs. Biotech. 23, 81–88.