Development of Ca-deficient Apatite Cement for Bone Substitution

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Abstract— The objective of the present study is the development of new formulation of ca-deficient apatite cement formed by mixing both calcium phosphate solid and liquid phases with atomic ratio Ca/P equal to 1.63. The solid phase was composed of tetracalcium phosphate powder ($Ca_4(PO_4)_2O$, TTCP), the liquid phase was an aqueous solution comprising phosphoric acid (H_3PO_4), and calcium chloride ($CaCl_2$, $2H_2O$). The mixture of the obtained paste with some additives has been immersed in deionized water at 37°C in order to be characterized by various techniques in different time intervals. The result showed that after mixing, the components turn into a malleable paste that sets in approximately 5-22 min and hardens without disintegration in deionized water. The setting of cement is due to the formation of dicalcium phosphate dihydrate ($CaHPO_4$), $2H_2O$: DCPD) crystallized as entangled platelets. After setting, the paste's physico-chemical and morphological change leads to the hardened cement composed of rich-$HPO4^{2-}$ ion apatite, poorly crystallized as needle-like entangled. The in vivo resorbability of prepared cement can be theoretically predicted to be more interesting comparing to the conventional cement: hydroxyapatite-cement and dicalcium phosphate dihydrate-cement.

Index Terms— Biomaterials, Cement, Tetracalcium phosphate, Calcium chloride, Ca-deficient apatite.

1 Introduction

SELF-SETTING calcium phosphate cements (CPC) have been proposed as bone implants and drug delivery systems for the filling of larger bone defects, e.g. after the resection of bone tumors [1],[2],[3],[4],[5]. The calcium phosphate cements have been used for their biocompatibility, bioactivity and their ability to harden in situ through a low-temperature setting reaction. The composition of the hardened product is of a paramount importance because it determines the solubility and, therefore, in vivo bioresorbability [6]. These properties are directly related to the stoichiometric ratio of calcium / phosphorus as two principal chemical elements. However the solubility of the final hardened product in a physiological medium is inversely proportional to the atomic ratio Ca/P. Many compositions of calcium phosphate cement have been proposed and marketed by different companies. These cements are classified into two categories: brushite-cement structure (CaHPO4, $2H_2O$, molar ratio Ca/P = 1) marketed under names PD-vitalOs® (Eq1) and hydroxyapatite-cement structure ($Ca_{10}(PO_4)_6(OH)_2$, molar ratio Ca/P = 1,67) marketed under names Bone Source®(Eq 2), Cementek® (Eq 3).

$$\left.\begin{array}{l} Ca_4(PO_4)_2O + Ca(H_2PO_4)_2, H_2O + CaO \rightarrow CaHPO_4, 2H_2O \\ \beta Ca_3(PO_4)_2 + H_3PO_4 \rightarrow CaHPO_4, 2H_2O \end{array}\right\} (1)$$

$$2\ Ca_4(PO_4)_2O + 2\ CaHPO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 \quad (2)$$

$$Ca_4(PO_4)_2O + Ca_3(PO_4)_2 + Ca(OH)_2 + H_3PO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 \quad (3)$$

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HAP-cements have drawn much more attention than DCPD-cements due to their similarity to the chemical composition of natural bone. However, the biological application shows that HAP-cement has low resorbability and DCPD-cement has a high resorbability. The high resorbability or the low resorbability of cements limits their use as bone substitutes. The objective of the present study is the development of a new formulation of cement composed of Ca-deficient apatite with adjusted resorbability. The molar ratio of calcium/phosphate is between 1.33 and 1.67. This cement is formed by mixing calcium phosphate solid phase and calcium phosphate liquid phase. The solid phase was composed of $Ca_4(PO_4)_2O$ (TTCP) powder and the liquid phase was an aqueous solution comprising phosphoric acid (H_3PO_4) and dihydrate calcium chloride ($CaCl_2$, $2H_2O$). In order to improve the homogeneity, plasticity and setting time adjustment of obtained paste, we added to the basic formulation the organic and inorganic additives known for their biocompatibility such as lactic acid, xanthan and β-glycerophosphate. The prepared paste sets and hardens in deionized water. In this study, we present the structure, composition and morphological properties, determined by various techniques from paste to the finished cement prepared with molar ratio Ca/P equal to 1.63.

2 Materials and Methods

2.1 Preparation of the solid phase cement

The solid phase of cement was composed of tetracalcium powder (TTCP). This powder, was synthesized by a solid-to-solid reaction between anhydrate dicalcium phosphate

and calcium carbonate (Eq 4) with a Ca/P ratio of 2 at a temperature of 1300 °C with rapid cooling (to prevent uptake of water and formation of HAP (Eq 5) [7],[8]. The obtained powder was milled and sieved. The grain size used is comprised between 40 and 125 µm with an average of 63 µm.

$$2\ CaHPO_4 + 2\ CaCO_3 \rightarrow Ca_4(PO_4)_2O\ + 2\ CO_2 + H_2O \quad (4)$$

$$3\ Ca_4(PO_4)_2O + H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2\ + 2\ CaO \quad (5)$$

In order to promote the cohesion between grains and the dough rheology of paste, we have also included in the solid phase the xanthan as additive with a weight percentage of 4% relative to the formed apatite.

2.2 Preparation of the liquid phase cement

The liquid phase consists of an aqueous solution comprising deionized water, phosphoric acid (H_3PO_4) and dihydrate calcium chloride ($CaCl_2$,$2H_2O$) with the atomic ratio Ca/P equal to 0.5. The pH of the aqueous solution is less than 0.5. In addition, the liquid phase comprises lactic acid ($C_3H_6O_3$) with a weight percentage of 2% relative to the liquid phase. In order to improve the regularity of the setting time, we added a β-glycerophosphate with 12 w% relative to the formed apatite [9],[10],[13]. All of the used reagents were of analytical grade.

2.3 Cement preparation

The cement paste was prepared by mixing the solid and the liquid phase at a liquid (L) to powder (S) according to 0.6 ml g^{-1} and the molar Ca/P ratio of cement was 1.63. The mixture of the two phases is done manually in a mortar with a pestle for 2 min. After mixing, the wet paste was placed in deionized water at laboratory atmosphere (25± 2°C and 50–60 % humidity) and in a body temperature (37± 2°C and > 90 % humidity) for setting and hardening characterization.

2.4 Methods for characterization

2.4.1 Setting times

After mixing solid and liquid phases, the components turn into a moldable paste that sets to a firm mass without disintegration after immersion in deionized water at 25 and 37°C. The initial and final setting times of the pastes were determined with Gillmore needles according to the C266-ASTM standard.

2.4.2 Morphology and physico-chemical evolution of cement

2.4.2.1 Methodology

In this study a method has been used to enable precise composition, structure and microstructure of the various species formed during the evolution of the cement. This method involves blocking the reaction by removal of unbound water necessary for conversion of the reactants. At the required moment of examination, a CPC cement sample is taken, washed with ethyl alcohol and then frozen. The samples are subsequently characterized by various analytical techniques.

2.4.2.2 Phase composition and morphology of cement

The phase compositions, the structure and microstructure of the set and hardened calcium phosphate samples were assessed using the X-Ray Diffraction, Infrared Spectroscopy (FTIR) and Transmission Electron Microscopy (TEM).

Diffractometer Bruker D8 Advanced was used. Copper Kα radiation (λ= 1.5406) produced at 50 kV and 20 mA scanned the diffraction angles 2θ in the 2θ range 10-65°, using a step size of 0.02° and a step time 30s. Crystallographic identification of the phases of prepared powder was accomplished by comparing the experimental XRD patterns to standards complied by the joint committee on powder diffraction standards (JCPDS). Fourier Transform IR Spectroscopy (FTIR) (SHIMADZU FTIR-8400S) with a resolution of 4 cm^{-1} and 20 scans are used. 1% of the powder samples were mixed with 99% KBr, and then pressed in a 13 mm die, the range of FTIR-spectrum was taken of 400 to 4000 cm^{-1}. The morphology of the sample was examined under a Transmission Electron Microscopy (TEM) (Philips CM 100) equipped with an energy dispersive spectroscopy (EDS).

3 Results

3.1 Measurement of setting time at 25°C and at 37°C

The initial and final setting time of calcium phosphate cement are measured in a humidity chamber at body temperature (37 ± 2°C and > 90 % humidity) and in normal laboratory atmosphere (25 ± 2 °C and 50–60 % humidity). The setting times are presented in Figure 1.

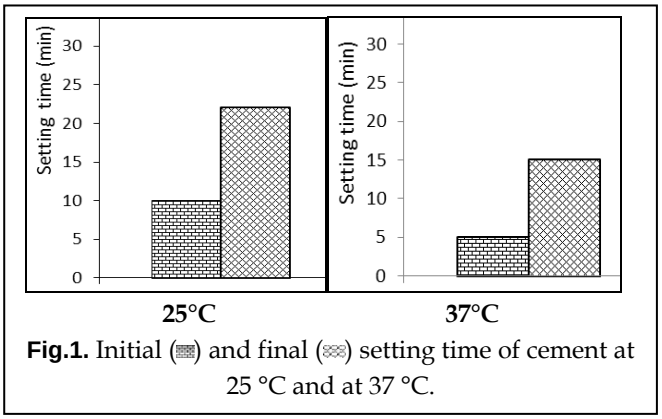

Fig.1. Initial (▦) and final (▩) setting time of cement at 25 °C and at 37 °C.

The results show that calcium phosphate cement has the setting times of 5 to 22 min. The setting time at 25 °C, is higher than that at 37°C. At 25 °C, the final setting time is 22 min; it decreased to 15 min at 37 °C. Setting time of cement increases as the temperature decreases.

The setting time can also be changed by the amount of water in the liquid phase or by adding certain additives such as pyrophosphate [11],[12].

3.2 X-ray Diffraction analysis

Fig.2 and Fig.3 shows the X-ray diffraction patterns of samples taken during the evolution of the cement in water at 37 °C. The X-ray diffraction pattern presented in fig.2 (a) (5 min after mixing) shows the formation of DCPD phase characteristics by the presence of peaks at 11.62°, 20.92° and 29.24°of 2θ. The peaks at 29.83° and 29.23° of 2θ are characteristics of precursor powder (TTCP). The X-Ray diffraction pattern presented in fig.2 (b) (60 min after mixing) shows a decrease concerning the peaks of TTCP and DCPD and a net increase of peak characteristics of apatite phase. The X-Ray diffraction pattern presented in fig.2 (c) (24 h after mixing) shows the disappearance of DCPD and TTCP phases and a formation of a poorly crystallized apatite structure. After 3 months of evolution in the water the cement still shows the presence of the poorly crystalline apatitic phase (fig.3).

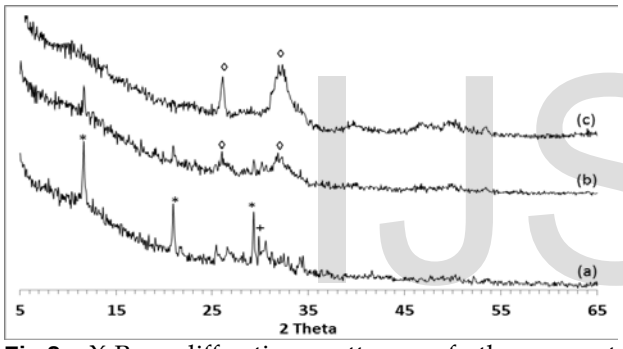

Fig.2. X-Ray diffraction patterns of the cement samples after immersion in deionized water at 37°C: (a) 5 min; (b) 60 min; (c) 24 h.
The main reflections of DCPD are indicated by (*); the TTCP are indicated by (+); the 002 reflection of Ca apatite is indicated with (◊).

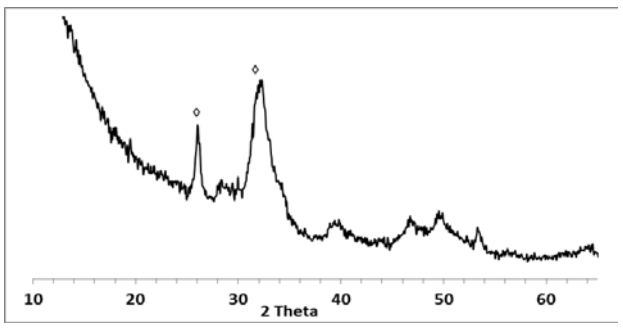

Fig.3. X-Ray diffraction pattern of the cement sample after 3 month immersion in deionized water at 37°C.
The 002 reflection of Ca apatite is indicated by (□).

3.3 FTIR spectroscopic analysis

The result of Infrared Spectrum presented in fig.4 (a) (5 min after mixing) shows the presence of bands characteristic of dicalcium phosphate listed in JCPDS n° 9007. The result presented in fig.4 (b) (60 minutes after mixing) shows the appearance of traces of the bands characteristic of apatitic structure.

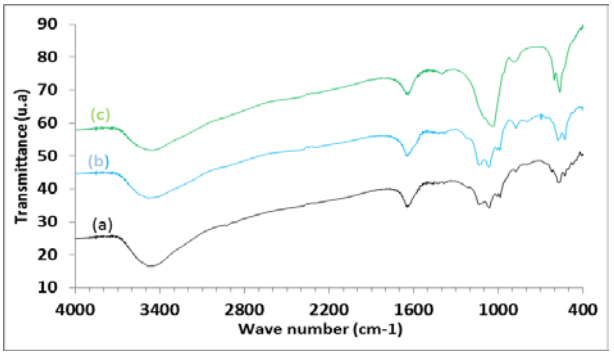

Fig.4. Infrared spectrum of the cement samples after different times of immersion in deionized water at 37°C: (a) 5 min, (b) 60 min, and (c) 24 h.

The result of Infrared Spectrum presented in fig.4 (c) shows the transformation within one day of immersion in water of DCPD into apatite phase identified by ν_1 PO_4 (960 cm^{-1}), ν_2 PO4 (460 cm^{-1}), ν_3 PO_4 (1020-1120 cm^{-1}), and ν_4 PO_4 (563-601 cm^{-1}). Moreover the result shows the formation of poorly crystallized apatite; with ions HPO_4^{2-} group visible at 875 cm^{-1}. We can notice the absence of the characteristic bands of the OH groups (630 and 350 cm^{-1}). The final phase of cement (3 month of maturation) is a poorly crystallized apatite with presence of HPO_4^{2-} group (fig.5).

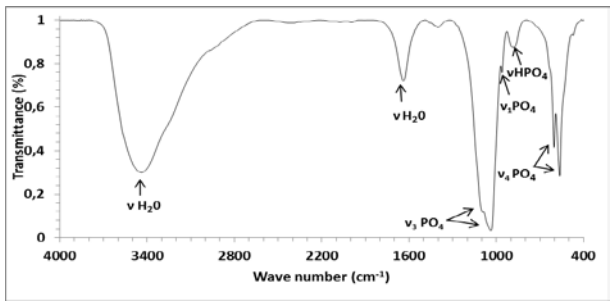

Fig.5. FTIR spectrum of the cement sample after 3 month of maturation in deionized water at 37°C

3.4 Morphological characterization

The morphology of the samples formed during the evolution of the cement are examined under a Transmission Electron Microscopy equipped with an energy dispersive spectroscopy (EDS).
Before the setting of cement (5min after mixing), the morphological analysis of paste displayed the

homogeneous formation of spherical and cohesive nanosized grains (fig.6 (a)). After the setting of paste (60 min after mixing), the cement consists essentially of agglomerates formed of platelets with an average length of 360 nm and an average width of 100 nm. The EDS profile (fig.6 (a and b)) show that analysis phases have a calcium to phosphorus ratio equal Ca/P = 1 which characterises the dicalcium phosphate phase.

The morphology of the crystals of cement after 24h of maturation (fig.6 (c)) shows smaller needle-like with a length between 50 to 100 nm; the entangled form ensures the hardness of the cement. Analyze by EDS (fig.6 (c)) shows a higher calcium concentration than phosphorus which characterises the apatitic phase.

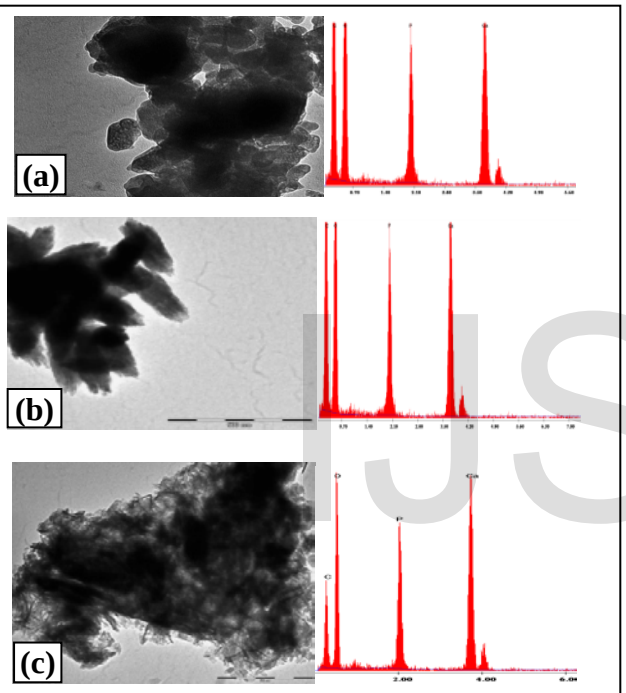

Fig.6. TEM micrographs of the cement samples after immersion in deionized water at 37°C: (a) 5 min, (b) 60 min, (c) 24 h

We can note that during the morphological analysis by MET, of the various samples, the presence of the organic phase (xanthan and glycerophosphate) prevents morphological observations.

4 Discussion

Ca-deficient apatite cement was prepared by mixing the calcium phosphate solid phase and the calcium phosphate liquid phase. After mixing, the components turn into a mouldable paste that sets in approximately 5-22 min and hardness without disintegration in deionized water.

The prepared cement was examined by the composition, the structural and morphological analysis performed on species formed during the evolution of paste. The results indicate that the first stage of hydration of the cement, correspond to the formation of DCPD by reaction between TTCP and H_3PO_4 (Eq 6). At the beginning, the morphological analysis of DCPD shows the formation homogeneous and spherical nanosized grains. This morphology contributes significantly to the good malleability of the paste which can be moulded to the desired shape. The used additives seem to play the glue role leading to the better cohesion between grains particularly suitable for rheological properties of cement without disintegration after immersion in water. For example, Ishikawa and al [13] showed that spherical cement particles resulted in a better injectability than irregularly shaped particles. The evolution of cement continues and the microstructure change can be clearly observed by a transformation of the spherical morphology of DCPD grain to entangled platelets. This step corresponds to the final setting of cement. The hydration of cement continues through free water from the reaction medium which disappears slowly to participate in the formation of another precipated phase more hydrated. This process is accompanied by a disparition of DCPD and TTCP. Consistent with the literature [14], the reaction between the TTCP and the DCPD does not lead directly to the formation of calcium apatite; there is a formation of an intermediate stage. It's the octocalcium phosphate (OCP: $Ca_8(PO_4)_4(HPO_4)_2$), $5H_2O$, with atomic ratio Ca/P = 1.33) (Eq 7). The precipitation of OCP phase allows the beginning of hardening of cement.

The paste loses its malleability and becomes rigid in 30 minutes. OCP phase is then transformed by incorporating free ions in the environment to an apatite phase (Eq 8) which crystallizes in needle-like entangled. This phase ensures the hardness of the cement.

$$Ca_4(PO_4)_2O + 2H_3PO_4 + H_2O \rightarrow 4CaHPO_4, 2H_2O \quad (6)$$

$$2Ca_4(PO_4)_2O + 2CaHPO_4, 2H_2O \rightarrow Ca_8(PO_4)_4(HPO_4)_2, 5H_2O + 2Ca^{2+} \quad (7)$$

$$Ca_8(PO_4)_4(HPO_4)_2, 5H_2O + (2-x)Ca^{2+} \rightarrow Ca_{10-x}(PO_4)_{6-x}(HPO_4)_x(OH)_{2-x} \quad (8)$$

The chemical composition and crystalline structure of the hardened cement did mimic the mineral part of bone. Indeed, the obtained product is mainly a poorly crystallized apatite, rich in HPO_4^{2-} ions with atomic ratio Ca/P less than 1.67 ($Ca_{10-x}(PO_4)_{6-x}(HPO_4)_x(OH)_{2-x}$). The in vivo resorbability rate of this cement can be theoretically predicted to be more interesting compared to the hydroxyapatite (atomic ratio Ca/P = 1.67), in agreement with the comparative extent of dissolution in acidic buffer (amorphous calcium phosphate)>> α-TCP > β-TCP > ca-deficient HA >> HA)

[15],[16],[17], the stoichiometric hydroxyapatite remains the least soluble material in a biological medium.

5 Conclusion

In this paper we present the preparation and the characterisation of ca-deficient apatite cement's new formulation. The cement is prepared by mixing solid phase composed of tetracalcium phosphate powder with an aqueous solution comprising phosphoric acid, and calcium chloride. We added to these basic formulation organic and inorganic additives that improved the homogeneity, plasticity, and setting time adjustment of cement. The obtained paste sets in approximately 5–22 min and hardens without disintegration in deionized water. The study of the evolution of cement allowed us to define the species that occur from the paste state to the finished product. After mixing, we have at first the formation of the cohesive nanosized spherical grains of dicalcium phosphate dihydrate. This morphology contributes significantly to the good malleability of the paste. The setting was then caused by transformation of spherical grains, to entangled platelets of dicalcium phosphate dihydrate. The hardening of paste is caused by formation of octocalcium phosphate and the hardness is caused by formation of Ca-deficient apatite poorly crystallized high in HPO_4^{2-} ions with atomic ratio Ca/P less than 1.67.

A range of products has been prepared successfully with molar ratio of calcium/phosphate adjusted between 1.33 and 1.67. The cement's new formulation developed and characterized in this work could have very good prospects for medical applications.

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