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Phase transformation on bone cement: Monocalcium phosphate monohydrate into calcium-deficient hydroxyapatite during setting

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Abstract

This study evaluated the phase transformation of calcium phosphate cement (CPC) using a mixture of monocalcium phosphate monohydrate (MCPM) and $CaCO_3$ as the solid phase and either water or a sodium phosphate buffer (SPB) solution ($pH=7.0$) as the liquid phase. The synthetic CPC was characterized by X-ray diffraction (XRD) analysis and transmission electron microscopy (TEM). The setting reaction in the SPB solution involved three phase transformations. Firstly, MCPM and $CaCO_3$ reacted with sodium phosphate immediately to form dicalcium phosphate dehydrate (DCPD) which continued to dissolve. Secondly, meanwhile, an intermediate amorphous calcium phosphate (ACP) was formed. Finally, ACP transformed into calcium-deficient hydroxyapatite (CDHA). In contrast, the reaction stopped at the first stage in water. Consequently, the SPB solution not only caused the dissolution of DCPD but also provided the buffering capacity to induce the conversion of the starting materials to CDHA.

Keywords: Calcium phosphate cement; Monocalcium phosphate monohydrate; Phase transformation; Setting reaction

1. Introduction

Calcium phosphate cements (CPCs) have attracted considerable attention in dental and medical applications because they self-set and transform into hydroxyapatite (HA). HA, in turn, is biocompatible and bioactive and can chemically bond with the bone [1]. Self-setting CPCs can be easily molded during surgery and simply implanted or injected into the bone defects, allowing them to be used as an endodontic filling materials [2,3], implant materials for periodontal bone defects [4], and binders for other non-setting calcium phosphate based implant materials [5]. During the self-setting process, the calcium phosphate crystals precipitate, and the CPC hardens by the entanglement of the precipitated crystals, which takes place at around room or body temperature.

Generally, CPC is fabricated using various calcium phosphate powders as starting materials and mixing them with water or an aqueous solution to form a paste. Calcium phosphates can be classified as being acidic or basic calcium phosphate on the basis of their Ca/P ratio. Calcium phosphate is classified as being acidic when its Ca/P is lower than 1.67 and basic when its Ca/P is greater than 1.67.

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Acidic calcium phosphates include monocalcium phosphate monohydrate (MCMP; $Ca(H_2PO_4)_2$), dicalcium phosphate dehydrate (DCPD; $CaHPO_4 \cdot 2H_2O$) or anhydrous (DCPA; $CaHPO_4$), octacalcium phosphate (OCP; $Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O$) and α- or β-tricalcium phosphate (α-TCP or β-TCP; $Ca_3(PO_4)_2$). Some basic calcium phosphates are tetracalcium phosphate (TTCP; $Ca_4(PO_4)_2O$), calcium oxide (CaO), calcium hydroxide ($Ca(OH)_2$) and calcium carbonate ($CaCO_3$). Therefore, ideally an apatitic CPC can be obtained by mixing the acidic and basic calcium phosphates together.

In the late 1990s, a large number of CPC systems were developed to obtain HA [6–8]. Later, studies were conducted to form calcium-deficient hydroxyapatite (CDHA; $Ca_9(HPO_4)(PO_4)_5OH$) since CDHA is considered to be compositionally closer to bone mineral than is stoichiometric HA. Brown et al. proposed a formulation of DCPD and TTCP that reacted with water to form CDHA [9]. Another formulation of CDHA was formed by mixing MCPM, α-TCP, and CaO with water [10]. In addition, Bermudez et al. [11], Miyamoto et al. [12,13] and Fulmer and Brown [14] reported that CDHA could be obtained by mixing α-TCP and CaO or $CaCO_3$ with water.

The setting process for CPCs is complex and involves the dissolutions of the starting materials as well as the precipitation of intermediate or end products. Therefore the setting behaviors (hardening time, compressive strength, and composition) depend on many factors, such as the particle size, components of the solid phase, ratio of powder to liquid, presence (or absence) of a HA seed, reaction temperature, and choice of aqueous liquid. Among these, the choice of the aqueous solution significantly affects the hardening rate and determines the end products. For example, a fast setting CPC (FSCPC) that employs a sodium phosphate buffer (SPB) solution sets six times faster than a conventional CPC that uses water as the liquid phase [15–18].

The purpose of this study was to investigate the role of the SPB solution in the setting process using a CPC consisting of MCPM and $CaCO_3$ mixing with either water or SPB solution. Although X-ray diffraction (XRD) analysis has been frequently utilized in the studies of CPC, as it can provide both qualitative and quantitative information, the accuracy of this technique is limited by the crystallinity and crystal size of products. Furthermore, the overlapping of peaks may result in them being wrongly assigned to be the wrong phase. The present study used transmission electron microscopy (TEM) to evaluate the phase transformation during the setting reaction and to identify the formed intermediate phases.

2. Material and methods

Commercially available MCPM (WAKO) and $CaCO_3$ (WAKO) were ground for 5 h using zirconia in a rotating mill before mixing. The liquid phase used was either pure water or SPB solution prepared from NaH_2PO_4 and Na_2HPO_4. The CPC powder was added into the liquid in a powder-to-liquid ratio of 1:1 and kneaded on a glass slab with a spatula to produce a paste. After mixing for 2 min, the CPC paste was loaded into a mold and stored in an incubator at 37 °C and 100% humidity. In order to stop the cement setting reaction at a specific period, the CPC specimens were removed from the molds at a given time, and were cooled in liquid nitrogen for 10 min and freeze dried. The freeze-dried samples were then ground to powder for following analysis.

The crystallinity and phase of the CPC were identified using powder XRD (PHILIPS X′Pert) analysis at 40 kV and 30 mA and at a scanning speed of 2°/min. The infrared spectra of the samples dispersed in KBr tablets were recorded by means of a Fourier transform infrared spectrophotometer (FTIR; Bio-RAD FTS3500). The surface morphology and microstructure of the CPC were observed by scanning electron microscopy (SEM; JEOL JSM-6500F) and transmitted electron microscopy (TEM; JEOL 2000FX2).

3. Results and discussion

Fig. 1(a) and (b) shows the XRD patterns of CPC mixed with water and SBP solution (pH 7), respectively. XRD analysis of CPC with water revealed that DCPD was formed after 0.5 h of setting and then partly transformed into DCPA. In addition, no HA related phases were found even after 1week. When the SPB solution was used, it was noticed that DCPD formed in this case too, and this was followed by the formation of CDHA with poor

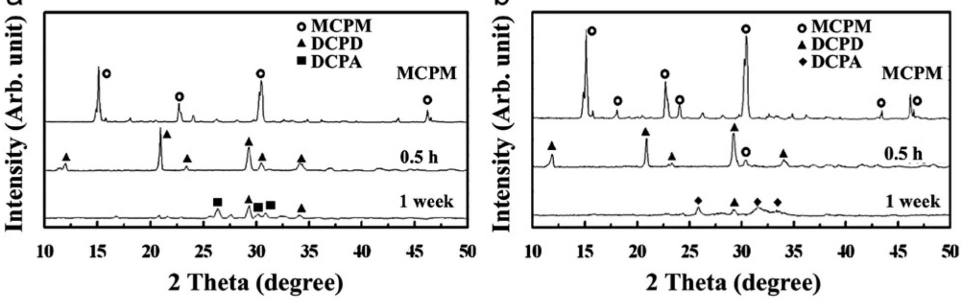

Fig. 1. XRD patterns of CPC formed in (a) water and (b) pH 7 PBS.

crystallinity after 1 week. Furthermore, the crystallinity of CDHA increased with reaction time.

Fig. 2 shows the infrared spectrum of the CPC mixed with the SPB solution; the absorption bands corresponding to the apatite phases were found at 950 cm^{-1} (PO_4^{-3}, v1), 1060 cm^{-1} (PO_4^{-3}, v3), 600 cm^{-1} (PO_4^{-3}, v4) and 560 cm^{-1} (PO_4^{-3}, v4). Furthermore, the absorption band at 870 cm^{-1} corresponding to HPO_4^{-2}, v5 provided evidence that the CPC consisted of CDHA.

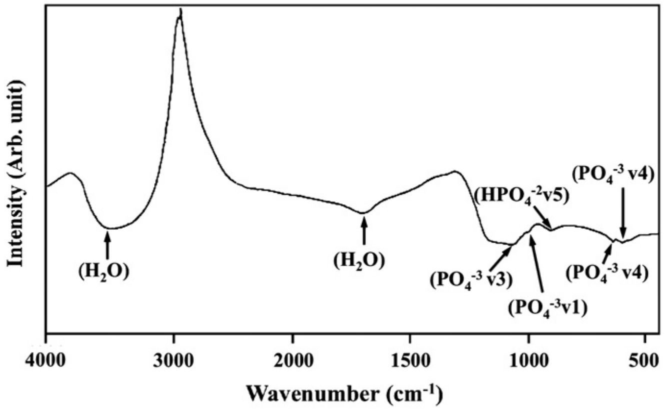

Fig. 2. Infrared spectrum of CPC formed in pH 7 PBS after 1week reaction.

Fig. 3(a) and (b) shows the CPC formed with water after reacting for 12 h and 24 h, respectively. As shown in Fig. 3(b), the CPC in this case exhibits a polygon-like morphology with pits on the crystal surfaces. These crystals were identified as being DCPD by XRD pattern analysis (Fig. 1(a)). DCPD crystals were assumed to exist among the DCPA crystals, but they were hard to be distinguished from morphological observation. The CPC formed with SPB solution exhibited the porous morphology after 12 h of reacting, suggesting the dissolution of DCDP, as shown in Fig. 4(a). After 24 h, a great number of crystals with plate-like shapes were observed, as shown in Fig. 4(b). These plate-like crystals have been previously reported in literatures [15,16] and are postulated to be CDHA. It is clear that the CDHA crystals grow perpendicular to the DCPD surface. This growth mechanism is different from the results in literature [17] where the apatite with shell-like shape formed and covered the starting materials. These apatite shells isolate the starting materials from solution and were expected to slow down the reaction. In this study, the perpendicular orientation of plate-like CDHA crystals appeared to allow ion transport and affected the rate of DCPD dissolution less.

Fig. 5(a) shows the TEM micrograph after 24 h of reacting. A large number of round particles containing bubbles were observed and the selected area diffraction pattern (SADP) shows a diffuse ring, suggesting that the round particle represents an amorphous phase. In addition,

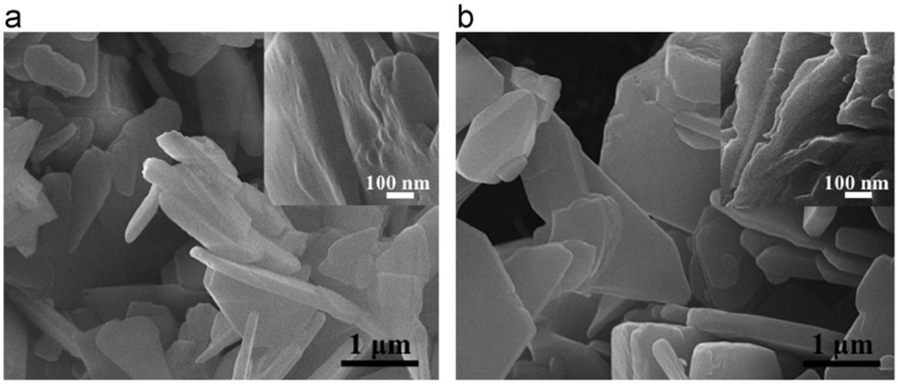

Fig. 3. SEM micrographs of CPC formed in water for (a) 12 h and (b) 24 h.

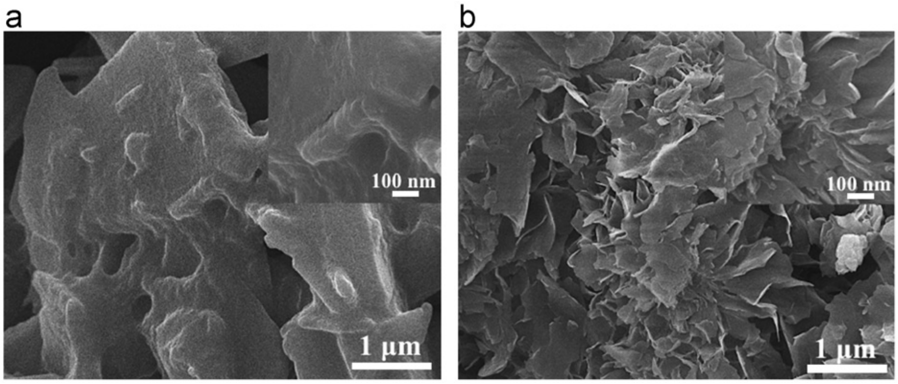

Fig. 4. SEM micrographs of CPC formed in pH 7 PBS for (a) 12 h and (b) 24 h.

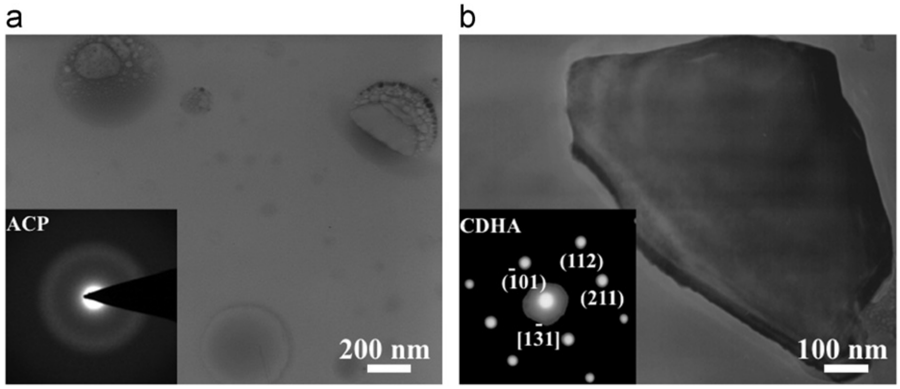

Fig. 5. TEM micrographs of CPC formed in the pH 7 PBS for 24 h: (a) ACP and (b) CDHA.

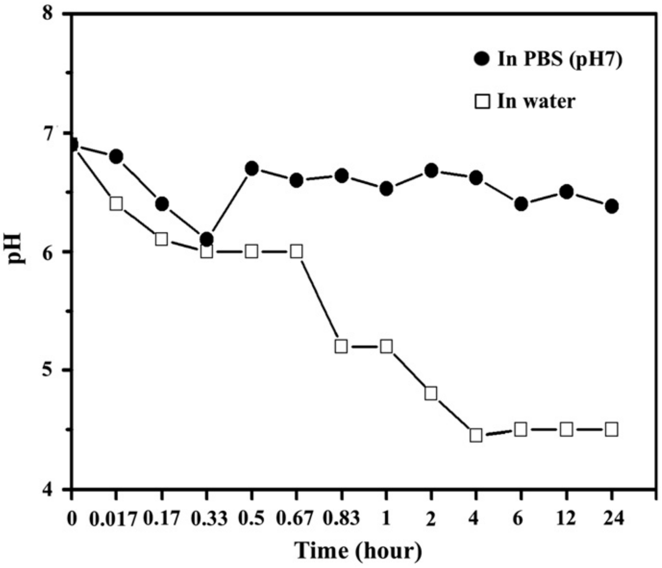

Fig. 6. Variation of pH in CPC slurry with time.

a precipitate with a plate-like shape was observed, which was identified as a single CDHA crystal using SADP, as shown in Fig. 5(b).

Fig. 6 shows that the pH of the CPC in the SPB solution initially decreased with time, then remained constant and finally decreased again after 24 h. However, the pH of the CPC in water dramatically decreased in the beginning to 5.1, then remained almost constant, and finally decreased again to a pH of 4.2. The final pH was below the invariant point (pH 4.7) and that is between those of DCPD and HA.

In general, during the hydration process of CPC, HA formation is dependent on the extent of DCPD dissolution and the properties of the solution used. Flumer et al. have reported that utilizing an SPB solution can enhance the HA formation rate since the DCPD dissolution becomes congruent [14]. In addition, HA nucleation is also influenced by the solution instability, which is determined by the saturation of calcium and phosphate ions as well as the pH value. On the basis of the pH measurements, we postulate that the SPB solution maintained the pH of the CPC constant, which, in turn, allowed the solution to remain highly unstable. Meanwhile, this also delayed the pH of CPC reaching the invariant point between that of DCPD and HA. In the SPB solution with a pH below 7, the formation of HA was also demonstrated, which suggests that the pH was not the only factor involved [12]. Consequently, the concentration of the phosphate ions is also a limiting factor for the formation of HA. In brief, the use of the SPB solution as liquid phase is helpful in establishing an unstable environment, which is necessary to initiate HA formation.

4. Conclusions

This study evaluated the phase transformation of CPCs made using either water or SPB solution as the liquid phase and compared the differences between them. MCPM and $CaCO_3$ were used as the starting materials. The results showed that the phase transformation of the CPC formed in SPB solution comprised of three stages, and ended in CDHA and DCPD. The first stage was the MCPM transformation into DCPD. Then, in the second stage, the DCPD dissolved while an intermediate ACP phase was formed. Finally, the intermediate phase transformed into CDHA and unreacted DCPD was left as residue. On the other hand, in water, MCPM transformed into DCPD initially, and part of DCPD converted into DCPA at the end. The factors causing the transformation of DCPD into CDHA in the SPB solution were also found to support phosphate ions and the pH buffering capability of the PBS.

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