Development of Calcium Phosphate Cement for Rapid Crystallization to Apatite

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The purpose of this study was to develop an α -tricalcium phosphate (α -TCP) cement which transforms to hydroxyapatite (HAP) in a relatively short period. We used calcium and phosphate solutions as the liquid phase for the α -TCP cement. The α -TCP powder was first mixed with CaCl_2 solution, and then mixed with NaH_2PO_4 or Na_2HPO_4 solution for a total powder/liquid ratio of 1.8. The setting time became shorter with the increase in the concentration of calcium and phosphate solutions, reaching 5 min, whereas the setting time was longer than 30 min when distilled water was used as the liquid phase. X-ray diffraction analysis revealed that the cement was mostly transformed to HAP within 24 h when kept in an incubator. We concluded that α -TCP should be mixed with calcium and phosphate solutions since this results in a moderate setting time and fast transformation to HAP even if the method of mixing becomes a little complex.

Key words: Calcium phosphate cement, Tricalcium phosphate, Hydroxyapatite

INTRODUCTION

Cement consisting of calcium phosphates is known to have an excellent tissue response. To give a typical example, cement^{1–4)} powders consisting of tetracalcium phosphate (TTCP) and dicalcium phosphate (DCPA or DCPD) are hardened by mixing with water. Crystal phases in the set cement finally change to hydroxyapatite (HAP: $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$). HAP materials are known to bond with bone directly and thus can be used as bone-replacing materials^{5,6)}. On the other hand, α -tricalcium phosphate (α -TCP: α - $\text{Ca}_3(\text{PO}_4)_2$) also sets to form calcium deficient HAP when mixed with water⁷⁾, and thus is used in dental clinics as a root sealer. The medical applications of α -TCP cement have been investigated in terms of bioactivity and biocompatibility^{8–11)}. The α -TCP phase in the α -TCP cement system takes a long time—several days—for the transformation to HAP. Therefore, α -TCP abundantly exists in the set cement for a long time. This phenomenon has an adverse influence upon biocompatibility, because the α -TCP phase of the set cement has high solubility. The solubility of α -TCP is much higher than those of HAP and β -tricalcium phosphate (β -TCP), and thus it has not been thought suitable for use as a biomaterial.

Moreover, the pH of the set cement surface increases as α -TCP dissolves in the set cement⁷⁾. This increase of pH in the α -TCP cement results in the presence of inflammatory cells in the surrounding set cement until several weeks after implantation^{8,9,11,12)}. However, α -TCP has been reevaluated recently, and the use of an α -TCP-HAP mixture and a functionally gradient α -TCP-HAP ceramic have become topics of interest^{8,9,13,14)}. It is reported that composite materials composed of α -TCP-HAP have better biocompatibility and bioactivity than HAP alone^{14,15)}. These reports suggest that a small quantity of α -TCP in composite calcium phosphate ceramics is better than a large quantity or no α -TCP at all. Although the detailed mechanism of α -TCP bioactivity has not yet been clarified, it seems reasonable that bone formation requires original materials, that is, calcium and phosphate. Thus a small quantity of remaining α -TCP in the set cement may be effective, though a large quantity poses problems.

The aim of this study, therefore, was to develop a calcium phosphate (α -TCP) cement which transformed to HAP in a relatively short period. We used calcium chloride and sodium hydrogen phosphate solutions as the liquid phase, although the mixing liquid of α -TCP cement is usually water. These solutions were selected because the mixture of calcium chloride and potassium hydrogen phosphate solutions precipitated needle-like HAP crystals with a large aspect ratio¹⁶⁾. However, the sodium hydrogen phosphate solution was introduced as a substitute for the potassium hydrogen phosphate solution because of its biocompatibility. We examined the rate of transformation to HAP and the properties of set α -TCP cement mixed using various solutions, and found that this system was affected by the method used and the concentration of the solution employed.

MATERIALS AND METHODS

Preparation of the specimens

α -TCP, the powder phase of cement used in this study, was prepared from a mixed powder consisting of calcium carbonate (CaCO_3) and dicalcium phosphate dihydrate (DCPD: $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$). The mixture, with a Ca/P molar ratio of 1.5, was fired at 1400°C for 5 hours. After heat treatment, x-ray diffraction of the resultant α -TCP revealed no other crystal. The α -TCP was crushed to a fine powder in an alumina ceramic cell by a ball mill (P-7 Planetary Micro Pulverizer, Fritsch Co., Idar Oberstein, Germany). The average particle size of the α -TCP powder measured by a sedimentation method based on Stokes' law with the use of an automatic centrifugal particle analyzer (CAPA300, Horiba, Kyoto, Japan) was 2 μm . Liquid phases for mixing the α -TCP powder were calcium chloride (CaCl_2) and sodium phosphate (either $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ or $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) solutions. Various concentrations of calcium and phosphate solutions were prepared to investigate the crystal phase and properties of the set cement. To combine the powder and liquid phases, the powder phase was first mixed with CaCl_2 solution, and then mixed with the same volume of one of the phosphate solutions ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ or $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) as for calcium solution at

a total liquid ratio (powder/liquid) of 1.8. The total time for mixing of cement was 1.0 minute. Utensils used for the mixing consisted of a plate of glass and a spatula of stainless steel for dental zinc phosphate cement. After mixing, the cement paste was placed in a plastic mold, and the mold was kept in an incubator at a temperature of 37°C and relative humidity of approximately 100%.

Analysis of specimens

The crystal phases in set cement were analyzed with a powder x-ray diffractometer system (XRD; ADG-301, Toshiba Co., Tokyo, Japan). The XRD conditions were Ni monochromatized $\text{CuK}\alpha$ radiation ($\lambda = 0.1540$ nm) generated at 30 kV and 12 mA. The specimen for XRD analysis was prepared by crushing the set cement or paste with an agate mortar. A scanning electron microscope (SEM; S-700, Hitachi Co., Tokyo, Japan) was used to observe the microstructure of set cement. For SEM observation, the set cement was sputtered with gold. The setting time of cement specimens was measured using a Vicat-needle, according to the method of Japanese Industrial Standard JIS T6602 (Correspondence: ISO 1566) for dental zinc phosphate cement. Measurement was carried out at room temperature (approximately 20°C).

Strength

The strength of set cement was evaluated by the wet diametral tensile strength (DTS) test. The cement paste was filled into a split mold (6 mm diameter $\times$ 3 mm height), and the mold with the paste was stored in the incubator for 24 hours. After storage, the wet DTS of 10 pieces for each concentration of mixing liquids was estimated using a Universal Testing Machine (AGS-500A Autograph, Shimadzu Corp., Kyoto, Japan), with a cross-head speed of $1.0 \text{ mm} \cdot \text{min}^{-1}$.

RESULTS

Fig. 1 shows the XRD patterns for the crystal phase in set cement when the α -TCP powder used in this study was mixed with distilled water. The crystal phase of the powder before mixing was completely α -TCP. The crystal phase of set cement was observed to be almost all α -TCP by XRD at 7 days after mixing. After 14 days, the α -TCP in the crystal phase of set cement was decreased by approximately one-half. HAP crystals eventually crystallized in set cement. The crystal phase for a commercial root sealer (Type 1, Sankin Industry, Tochigi, Japan) consisting of α -TCP powder was measured by XRD. After 7 days in an incubator following setting, the crystal phase of α -TCP was also the same as the powder phase. Fig. 2 shows XRD patterns of the changes of the crystal phase in set cement using 1.0 mol/L CaCl_2 solution and 0.6 mol/L NaH_2PO_4 solution as mixing liquids for 24 hours after mixing. One hour after mixing, HAP crystals were slightly crystallized in the set cement. Six hours after mixing, the HAP content in set cement was increased, and α -TCP content was decreased. Compared with distilled water as the mixing liquid, the crystal phase in set cement was almost all transformed from α -TCP to HAP after 24 hours by the

Fig. 1 Powder X-ray diffraction patterns of set cement at 1, 7, and 14 days after mixing, when α -tricalcium phosphate powder produced in this study was mixed with distilled water. After mixing, the paste was kept in an incubator at 37°C and relative humidity 100%. (T=the maximal peak of α -tricalcium phosphate).

Fig. 2 Powder X-ray diffraction patterns of the changes of the crystal phase in set cement using 1.0 mol/L CaCl₂ and 0.6 mol/L NaH₂PO₄ solutions as mixing liquids, for 24 hours. After mixing, the paste was kept in an incubator at 37°C and relative humidity 100%. (T=the maximal peak of α -tricalcium phosphate; α TCP= α -tricalcium phosphate).

use of the calcium and phosphate solutions as mixing liquids. On the other hand, the crystal phase in set cement using 0.6 mol/L Na₂HPO₄ solution as a substitute for 0.6 mol/L NaH₂PO₄ solution was also transformed after 24 hours. However, the crystallinity of HAP in the set cements using NaH₂PO₄ solution was slightly higher than that with Na₂HPO₄ solution. These results suggested that the mixing liquids used in this study were markedly more effective than distilled water for mixing. However, the rate of transformation to HAP in set cement decreased with increased solution concentration. In the case of set cement mixed simultaneously with calcium chloride

and sodium hydrogen phosphate, DCPD was rapidly produced in the crystal phase of set cement. The DCPD in set cement existed for a long time; in addition, the crystallizing rate of HAP was decreased by the existence of DCPD.

When only calcium or phosphate solution was used in the set cement, rapid-crystallization to HAP did not occur. The use of only NaH_2PO_4 or Na_2HPO_4 phosphate solution, in particular, decreased the rate of transformation to HAP. These transformation rates were similar to that obtained with distilled water. However, the use of CaCl_2 solution was more effective than the distilled water and phosphate solutions. The XRD patterns of set cement mixed with CaCl_2 solution for several elapsed times (1, 7, and 14 days) are summarized in Fig. 3. Approximately 30% of HAP was crystallized into α -TCP at 1 day (24 hours) after mixing, though the transformation rate was less than that obtained when using the calcium and phosphate solutions together. Seven days after mixing, the remaining α -TCP in set cement was almost all transformed to HAP.

SEM micrograph of the inside of the set cement made using CaCl_2 (1.0 mol/L) and NaH_2PO_4 (0.6 mol/L) obtained at 24 hours after mixing is shown in Fig. 4. It was

Fig. 3 Powder X-ray diffraction patterns of set cement mixed with 1.0 mol/L CaCl_2 solution for several elapsed times (1, 7, and 14 days). After mixing, the paste was kept in an incubator at 37°C and relative humidity 100%. (T=the maximal peak of α -tricalcium phosphate).

Fig. 4 Inside microstructure of set cement (after 24 h) mixed with 1.0 mol/L CaCl_2 and 0.6 mol/L Na_2HPO_4 solutions, observed by scanning electron microscopy. (Scale bar=5 μm).

Fig. 5 Relation between setting time of cement pastes and concentration of mixing liquids. The calcium and phosphate solutions for mixing liquids were used with the Ca/P ratio of 1.67, at all times.

confirmed by SEM observation that plate-like crystals were crystallized in the set cement. Inside the set cement a porous structure was formed by the entangling of plate-like crystals. The plate-like crystals in set cement were also crystallized with using 0.6 mol/L Na₂HPO₄ solution as a substitute for 0.6 mol/L NaH₂PO₄ solution.

The setting time of cement measured by the Vicat-needle method became shorter with the increase in the CaCl₂ and NaH₂PO₄ concentrations, reaching 5 min when the concentration of the NaH₂PO₄ was 2.2 mol/L, as shown in Fig. 5. The setting time was longer than 30 min when distilled water was used as the liquid phase. In the case of cement paste using Na₂HPO₄ solution as a substitute for NaH₂PO₄ solution, the setting time was also delayed with the decrease in the concentrations of mixing liquids. However, the setting time using Na₂HPO₄ solution was slightly longer than that with NaH₂PO₄ solution. Fig. 6 shows the relation between the Ca/P ratio of the two mixing liquids and the setting time of cement pastes. The influence of the Ca/P ratio on setting time was measured in the range from 0.5 to 5.0 of Ca/P ratio by using 1.4 mol/L NaH₂PO₄ solution and various CaCl₂ solutions. The setting time was delayed with the increase of the Ca/P ratio in the mixing liquids. In the case of a Ca/P ratio of 1.67, the setting time of the cement was 21 min.

The relation between DTS of set cements and concentration of mixing liquids is shown in Fig. 7. The calcium and phosphate solutions for mixing liquids were used with the Ca/P ratio of 1.67, at all times. The DTS of the set cement after 24 hours

Fig. 6 Relation between setting time of cement pastes and Ca/P ratio of the two mixing liquids. The mixing liquids for various Ca/P ratio were used with 1.4 mol/L NaH_2PO_4 solution and various CaCl_2 solutions.

Fig. 7 Relation between diametral tensile strength (DTS) of set cements and concentration of mixing liquids. After setting, the set cement was kept in an incubator at 37°C and relative humidity 100%.

was 1.5 ± 0.2 MPa when α -TCP was mixed with CaCl_2 (1.0 mol/L) solution followed by NaH_2PO_4 (0.6 mol/L) solution. This mixture gave the maximum value of DTS. The bulk density of this set cement was 1.37 ± 0.03 g/cm³ after storage in a desiccator at 80°C for 5 hours.

DISCUSSION

HAP powders prepared by wet synthesis are known to form needle-like crystal, plate-like crystal or grain-like crystal. The α -TCP cement used in this study crystallized plate-like crystals of HAP, as confirmed by SEM observation. Ohgaki *et al.* reported that needle-like crystals of HAP were obtained by dropping CaCl_2 and dipotassium hydrogen phosphate (K_2HPO_4) solutions into 100°C water¹⁶⁾. They explained that the needle-like crystal was produced from the first precipitation of DCPD. Moreover, they demonstrated that the aspect ratio of the needle-like crystal decreased with an increase of the Ca/P ratio between calcium acetate ($\text{Ca}(\text{CH}_3\text{COO})_2$) and K_2HPO_4 solutions¹⁷⁾. These results may suggest that a region of low pH promotes the precipitation of needle-like HAP crystals. The typical calcium phosphate cement consisting of TTCP and DCPA (or DCPD) also forms the needle-like crystals of HAP, and is hardened by entanglement in the needle-like crystals^{2,3,18,19)}. Therefore, it seems that DCPD

participates before the formation of the needle-like crystal of HAP. The cement in this study was also able to crystallize DCPD, depending on the conditions of the mixing liquids and the method employed. However, the crystallization of DCPD in set cement decreases the transformation rate of HAP because the DCPD exists for a long time. From the results of this study, the mixing conditions for rapid-crystallization to HAP caused the plate-like crystals of HAP to be formed (Fig. 4). The presence of plate-like crystals in the set cement suggested that α -TCP was transformed to HAP via an octacalcium phosphate (OCP: $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$) phase because the plate-like crystal originates from a crystal form of OCP. Graham and Brown reported that α -TCP is transformed to HAP through precipitation of OCP under the environmental conditions of a basic region²⁰⁾. Moreover, Fulmer *et al.* demonstrated that calcium deficient HAP in a mixture consisting of TTCP and monocalcium phosphate monohydrate (MCPM) was rapidly precipitated by use of $\text{Ca}(\text{OH})_2$ ²¹⁾. The pH of this cement paste will become basic when α -TCP powder is first mixed with calcium solution. Therefore, a crystalline nucleus of OCP may be produced by the first mixing with calcium solution. However, XRD analysis in this study was not able to detect a peak ($2\theta = 4.5^\circ$) of OCP in set cement. After the first mixing, the cement paste rich in calcium will be offset by the second mixing with phosphate solution. We guess that the rapid crystallization to HAP was due to the precipitation of OCP.

The DTS of set cement was lower than those of the other calcium phosphate cements^{19,22)}. The reason for this low strength may have been the bulk density of plate-like crystals being lower than that of needle-like crystals. The calcium phosphate cements that crystallize HAP needle-like crystals have high strength^{2,19)} compared with the cement in this study. The densities of HAP and α -TCP are 3.16 g/cm^3 and 2.86 g/cm^3 , respectively. On the assumption that the α -TCP of powder phase completely transformed to HAP, the porosity for set cement of the maximum DTS value is approximately 57%, calculated from the bulk density (1.37 g/cm^3). The porosity of calcium phosphate cement consisting of TTCP and DCPA ranged from 40% to 45% using powder/liquid (P/L) ratios from 3.0 to 6.0¹⁹⁾. Therefore, it is possible that the DTS in this study was increased by the increased P/L ratio. The strength of the set cement needs to be investigated before clinical application. However, the use of this α -TCP cement system may be in conflict with the need for strength and rapid-crystallization to HAP.

Generally, the raw materials used for the precipitation of HAP are tetracalcium phosphate (TTCP), octacalcium phosphate (OCP), dicalcium phosphate dihydrate (DCPD), dicalcium phosphate (DCPA), monocalcium phosphate monohydrate (MCPM), α -TCP and β -TCP²²⁾. These calcium phosphates are not stable compounds under environmental conditions around neutral pH, compared with HAP. When these compounds of calcium phosphate in cement dissolve *in vivo*, an increase or decrease of pH occurs on the cement surface because of its dissolution^{22,23)}. One of the important factors for biomaterials is that of biocompatibility. The biocompatibility of a material is governed by the character of the surface layer. The surface of HAP shows excellent biocompatibility because a small quantity of HAP dissolution causes

almost no change in pH at the HAP surface. The pH of biomaterial surfaces should be neutral. Therefore, it is desirable that the crystal phase in cement consisting of calcium phosphate transforms to HAP as fast as possible. We demonstrated that the powder phase (α -TCP) of set cement in this study was transformed to HAP within 24 hours after mixing when kept in an incubator. Though the biocompatibility and bioactivity *in vivo* of the cement have not yet been clarified, the results of this study suggest that the biocompatibility of the cement will be better than that of the conventional α -TCP cement because of its rapid-crystallization to HAP. α -TCP has been known to increase pH when it dissolves in water. The α -TCP powder prepared in this study also showed markedly increased pH. The pH of the NaH_2PO_4 solution used for the liquid phase in this study was approximately 4.5 in 0.6 mol/L solution. Therefore, it seems that the low pH of NaH_2PO_4 solution will offset the increase of pH in the α -TCP cement. If this hypothesis is correct, the pH of cement paste may be nearly neutral. Moreover, this cement could be a better biocompatible material than the conventional α -TCP cement.

CONCLUSION

The rapid crystallization to HAP of calcium phosphate (α -TCP) cement was investigated by using various mixing liquids. The results suggested that the crystal phase in set cement could be changed from α -TCP to HAP within 24 hours by the use of two mixing liquids consisting of calcium chloride (1.0 mol/L) and sodium hydrogen phosphate (0.6 mol/L) solutions. It seems that α -TCP cement improved by using these mixing liquids is more effective in terms of biocompatibility than conventional α -TCP cements.

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