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Hydrolyses of calcium phosphates-allografts composite in physiological solutions

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Abstract Hydrolysis of calcium phosphates cement-allografts composite in calf serum and that in saline were examined in comparison with those of the calcium phosphates cement in both the solutions. The calcium phosphates cement consists of α-tricalcium phosphate (α-TCP), tetracalcium phosphate (TetCP), dicalcium phosphate dihydrate (DCPD), and hydroxyapatite (HAP), which is clinically used as Biopex. In the hydrolyses of Biopex-allografts composite in both the solutions, the calcium phosphates cement was transformed into HAP. On the other hand, in the hydrolyses of Biopex, HAP was formed after 1 day and octacalcium phosphate (OCP) was gradually formed after 7 days. In the presence of allografts, plate-like crystals were deposited and in the absence of allografts, needle-like crystals were deposited in both the solutions. By the addition of allografts, the hydrolysis process of the calcium phosphates cement was significantly changed.

1. Introduction

As bone replacement, autografts, allografts, and calcium phosphate ceramics have been used. Autografts and allografts are substituted for living bone by remodeling. In contrast, the calcium phosphate ceramics are hardly substituted in most cases [1]. As they have good biocompatibility, osteoconductivity, and availability, there have been considerable interests in development of calcium phosphates ceramics.

In 1983 Brown and Chow developed a paste-like calcium phosphate cement which fitted bone defects [2]. Since their paper, there have been many papers dealing with paste-like calcium phosphate cements as a filling material in medical and dental field [2–6]. Kurashina et al. developed a calcium phosphates cement made of α-tricalcium phosphate (α-TCP, α-Ca_3 $(PO_4)_2$), tetracalcium phosphate (TetCP, $Ca_4O(PO_4)_2$), and dicalcium phosphate dihydrate (DCPD, $CaHPO_4 \cdot 2H_2O$) [7–10]. After mixing of the calcium phosphates with aqueous solution of sodium succinate and sodium chodroitin sulfate, the resulting cement paste was transformed into hydroxyapatite (HAP, Ca_{10} $(PO_4)_6(OH)_2$) and hardened. The HAP formation of the cement was proposed by a combination of the following formula [11].

$$2Ca_3(PO_4)_2 + Ca_4O(PO_4)_2 + H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 \quad (1)$$

$$2Ca_4O(PO_4)_2 + 2CaHPO_4 \cdot 2H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 3H_2O \quad (2)$$

$$3Ca_3(PO_4)_2 + 2H_2O \rightarrow Ca_9(HPO_4)_x(PO_4)_{6-x}(OH)_x(H_2O)_y \quad (3)$$

The compressive strength against soaking time in simulated body fluid increased for the first 7 days. The maximal compressive strength was 94.7 MPa [7]. In an *in vivo* study, new bone formation occurred around the cement without intervention of soft tissue [8–10]. However, the cement was remaining in rabbit mandibles for one and half years [8].

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From the osteoconductivity and the sufficient strength, the cement was clinically used as Biopex.

Recently, Haraguchi et al. reported that resorption of Biopex-allografts composite was recognized after implanting in rabbit born after 3 weeks and that most of Biopex-allografts composite was resorped for 24 weeks [12]. On the other hand, they reported that Biopex was hardly resorped for 24 weeks.

In this study, we examined early stage of hydrolyses of Biopex-allografts composite in calf serum and in saline. We found that allografts affected the hydrolysis of the calcium phosphates in allografts-Biopex composite.

2. Experimental

2.1. Materials

Biopex was obtained from Mitsubishi Materials Co., Ltd. Biopex consists of calcium phosphates powder and an aqueous solution. The calcium phosphate powder is a mixture of 75 wt% α-tricalcium phosphate (α-TCP), 18 wt% teracalcium phosphate monoxide (TetCP), 5 wt% dicalcium phosphate dihydrate (DCPD), and 2 wt% hydroxyapaite (HAP). The aqueous solution contains 12 wt% sodium succinate and 5 wt% sodium chondroitin sulfate. α-TCP was obtained from Taihei Chemical Industrial Co., Ltd. TetCP and DCPD were obtained from Wako Chemical Industrial Co., Ltd. Calf serum were obtained from GIBCO and saline were obtained from Otsuka Pharmaceutical Co., Ltd. Allografts were excised from femurs and tibiae of rabbits. The allografts were sterilized in saline at 80°C for 10 minute When the allografts were used, they were thawed and finely divided by mortar and pestle.

2.2. Method

1.5 g of calcium phosphates powder of Biopex was mixed with 1.0 g of allografts by mortar and pestle. The mixture was added to 1.0 cm^3 of an aqueous solution of sodium succinate and sodium chondroitin sulfate to give Biopex-allografts composite paste. The resulting paste was loaded in Teflon cells of 4.0 mm in diameter and 13.5 mm in length. After hardening of the paste at room temperature, two Teflon cells packed with Biopex-allografts composite were soaked in 40 cm^3 of calf serum or saline (0.9 wt% NaCl solution), kept in an incubator at 37.5°C up to 21 days, changing the calf serum weekly. After being soaked for 1 day, 3 days, 7 days, and 21 days, the samples were dried *in vacuo*. For comparison, Biopex, α-TCP, α-TCP/TetCP (2.0 g/0.5 g), and α-TCP/DCPD (2.3 g/0.2 g) were soaked in calf serum and in saline, similarly.

2.3. Measurements and analyses

The crystalline phases of the reaction products were identified by a powder X-ray diffractometer (XRD, RU-200B, Rigaku Co., Ltd.) with Cu Kα radiation generated at 50 kV and 150 mA. FT-IR spectra were recorded on a Fourier transformation infrared spectroscopy (JIR-Winspec100, JEOL). Solid-state ^{31}P-NMR spectra were recorded on a solid-state ^{31}P magic angle spinning NMR spectrometer (GX-270W, JEOL) with 3–4 kHz of a magic angle spinning rate. The morphologies of the products were observed by a scanning electron microscopy (SEM, S-5000, Hitachi).

3. Result

3.1. XRD analysis

Fig. 1 shows XRD patterns of the mixture of the calcium phosphates powder of Biopex and allografts powder and those of Biopex-allografts composite after soaking in calf serum. The peak of (020) plane of DCPD disappeared after 1 day. As the soaking time increased, the intensities of HAP peaks increased, those of α-TCP and TetCP peaks decreased. α-TCP and TetCP peaks were still present after 7 days. When Biopex-allografts composite were soaked in saline, the XRD patterns of Biopex-allografts composite were similar to those in calf serum.

Fig. 2 shows XRD patterns of the calcium phosphates powder of Biopex and Biopex after soaking in calf serum. The peak of (020) plane of DCPD disappeared after 1 day. As the soaking time increased, the intensities of HAP peaks increased, those of α-TCP and TetCP peaks decreased. The peak of (010) plane of octacalcium phosphate (OCP) was shown after 7 days. The peak intensity further increased for 21 days. When Biopex was soaked in saline, the XRD patterns of Biopex were similar to those in calf serum.

The decrease ratios of α-TCP were calculated by the following formula.

Decrease ratio of α-TCP $= (\alpha\text{-TCP}_{132} + \alpha\text{-TCP}_{170})/(\alpha\text{-TCP}_{132} + \alpha\text{-TCP}_{170} + \text{HAP}_{211} + \text{HAP}_{112})$, where α-TCP_{132} and α-TCP_{170} are the intensities of (132) and (170) planes of α-TCP and where HAP_{211} and HAP_{112} are the intensities of (211) and (112) planes of HAP, respectively. Similarly, the decrease ratios of the related composites were calculated [13].

As shown in Fig. 3, the rates of HAP formation of Biopex-allografts composite and Biopex in serum did not decrease compared with those in saline. In contrast, those of HAP formation of α-TCP, α-TCP/TetCP, and α-TCP/DCPD in serum decreased compared with those in saline.

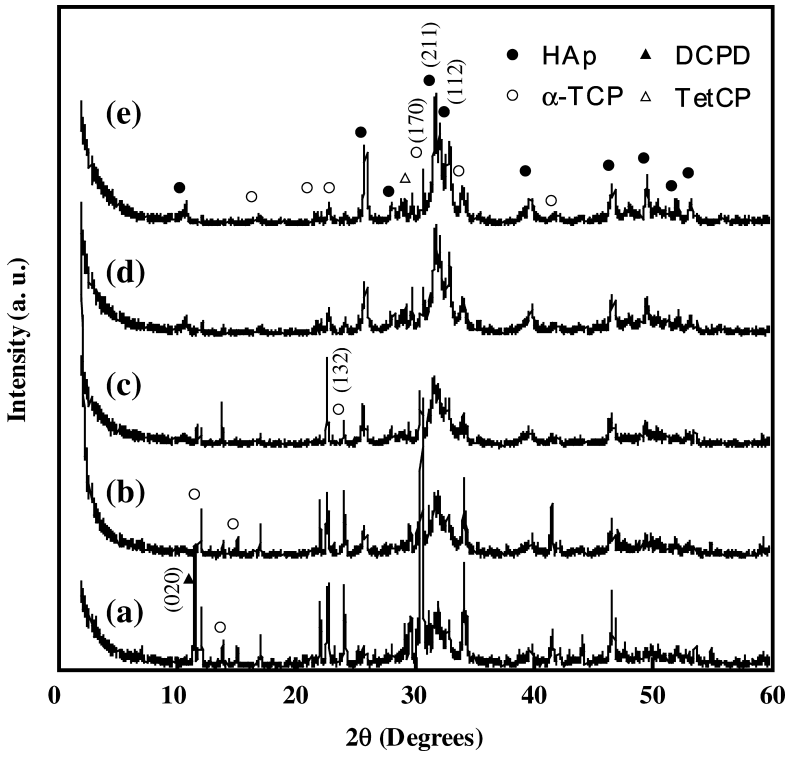

Fig. 1 XRD patterns of (a) a mixture of the calcium phosphates powder of Biopex and allogrfts, Biopex-allografts composite after soaking in calf serum for (b) 1 day, (c) 3 days, (d) 7 days, and (e) 21 days.

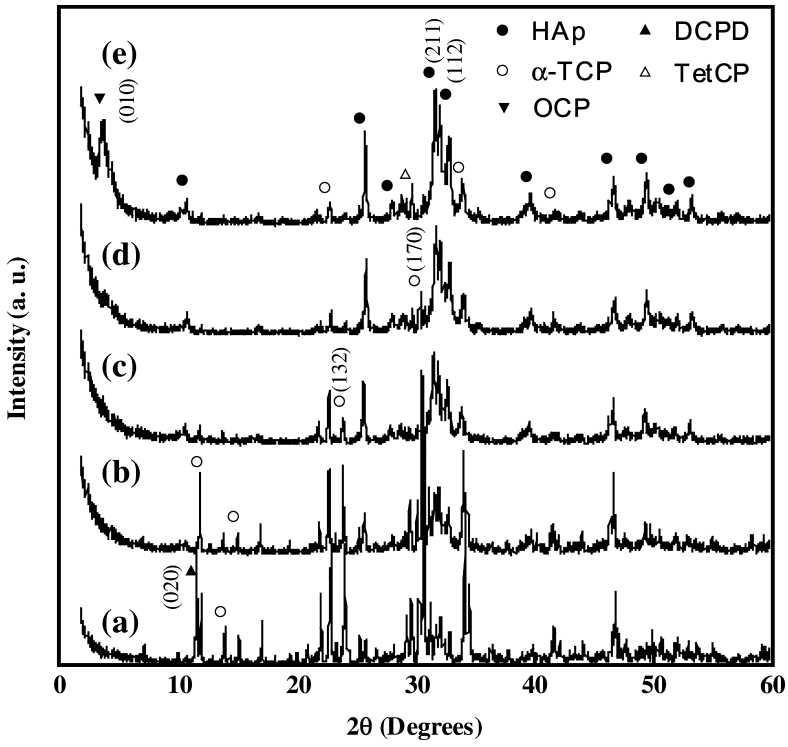

Fig. 2 XRD patterns of (a) the calcium phosphates powder of Biopex, Biopex after soaking in calf serum for (b) 1 day, (c) 3 days, (d) 7 days, and (e) 21 days.

3.2. FT-IR spectra

Fig. 4 shows FT-IR spectra of Biopex-allografts composite after soaking in calf serum and that of the mixture of the calcium phosphates powder of Biopex and allografts. The band at 988 cm^{-1} of DCPD disappeared after 1day. When the soaking time increased, the bands around 600 cm^{-1} derived from α-TCP changed to the characteristic bands of HAP at 602 and 565 cm^{-1}. The absorption bands of CO_3^{2-} group of carbonated HAP at 1454 and 1420 cm^{-1} became sharp.

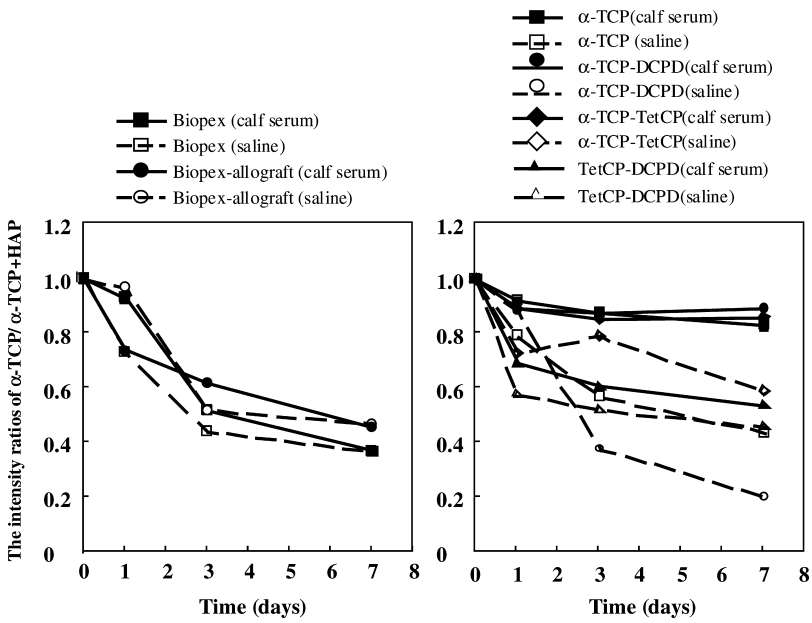

Fig. 3 The intensity ratios of α-TCP/ α-TCP + HAP for soaking time.

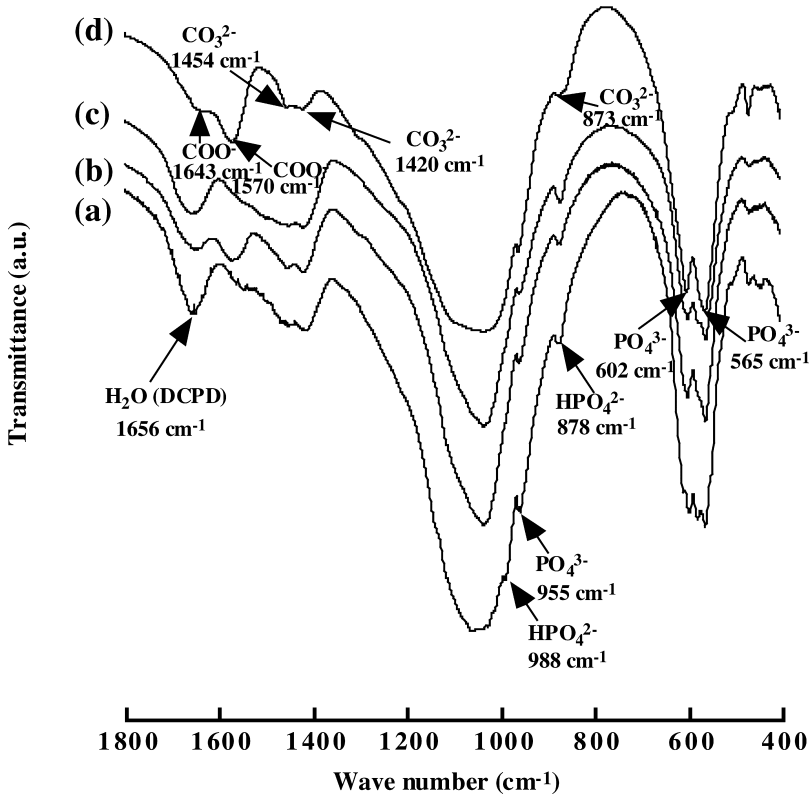

Fig. 4 FT-IR spectra of (a) a mixture of the calcium phosphates powder of Biopex and allografts, Biopex-allografts composite after soaking in calf serum for (b) 1 day, (c) 3 days, and (d) 7 days.

The absorption bands of COO^- groups of sodium chondroitin sulfate at 1643 cm^{-1} and sodium succinate at 1570 cm^{-1} were still observed after soaking for 7 days. When the samples of Biopex-allografts composite were soaked in saline, the obtained FT-IR spectra were similar to those soaked in calf serum.

Fig. 5 shows FT-IR spectra of the calcium phosphates powder of Biopex and Biopex after soaking in calf serum. The bands at 988 and 1652 cm^{-1} of DCPD disappeared after soaking for 1 day. When the soaking time increased, the broad band of PO_4^{3-} of α-TCP around 600 cm^{-1} decreased and the sharp bands of PO_4^{2-} group at 602 and 564 cm^{-1}

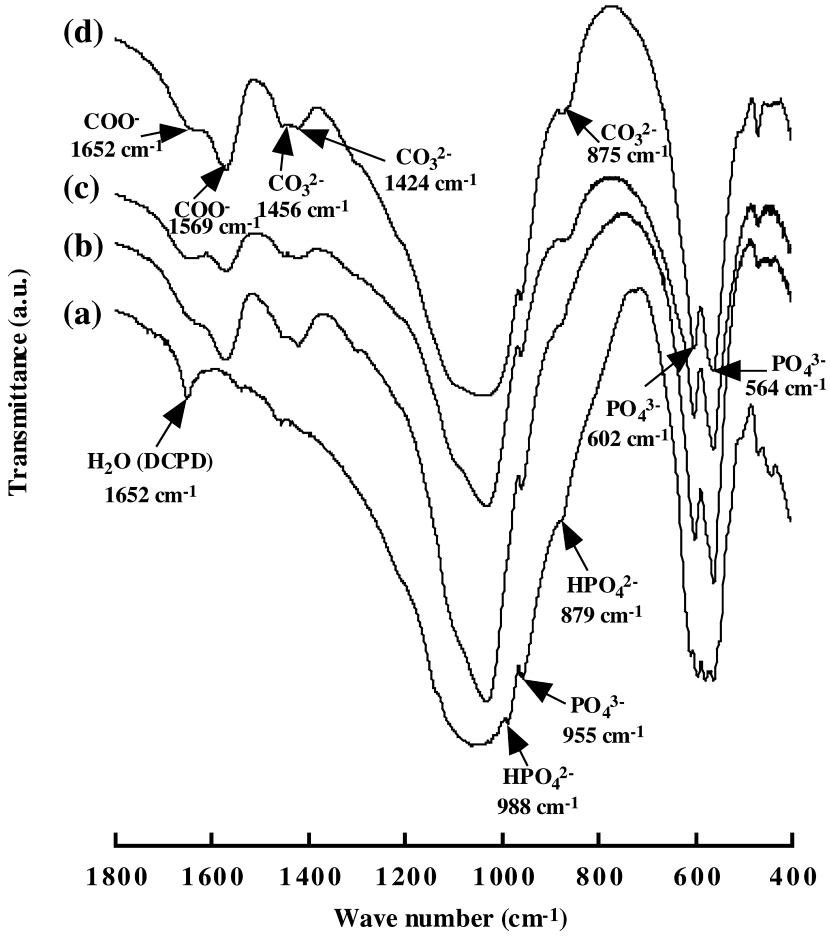

Fig. 5 FT-IR spectra of (a) the calcium phosphates powder of Biopex, Biopex after soaking in calf serum for (b) 1 day, (c) 3 days, and (d) 7 days.

corresponding to HAP appeared. The absorption bands of CO_3^{2-} group at 1456 and 1424 cm^{-1} became sharp. The absorption bands of COO^- groups of sodium succinate and sodium chondroitin sulfate were still observed after 7 days. When the samples of Biopex were soaked in saline, the obtained FT-IR spectra were similar to those soaked in calf serum.

3.3. ^{31}P MAS NMR spectra

Fig. 6 shows ^{31}P MAS NMR spectra of Biopex-allografts powder and Biopex-allografts composite after soaking in calf serum. Figure 7 shows ^{31}P MAS NMR spectra of Biopex and Biopex after soaking in calf serum. As the soaking time increased, intensity of HAP signal increased and those of α-TCP at 0.3 and 1.6 ppm and TetCP signals at 5.1 ppm decreased. α-TCP at 1.6 ppm and TetCP at 5.1 ppm shoulder signals were shown after 7 days in both cases. The spectra of Biopex-allografts composite and Biopex after soaking in saline were similar to those in calf serum, respectively.

3.4. SEM observation

Fig. 8(a) shows SEM photographs of Biopex-allografts composite after soaking in calf serum. Biopex-allografts composite was hydrolyzed to form needle-like crystals after 7 days. In the case of soaking in saline the morphology of crystals were similar to that in serum.

Fig. 8(b) shows SEM photographs of Biopex after soaking in calf serum. Biopex was hydrolyzed to form plate-like crystals and small amount of needle-like crystals after 7 days. Similarly, in saline plate-like crystals small amount of needle-like crystals were formed.

4. Discussions

In each hydrolysis, DCPD almost disappeared after 1day. In the later process, the hydrolysis of Biopex might be attributed to hydrolyses of TetCP/α-TCP and α-TCP. From FT-IR spectra, the formation of carbonate-HAP was observed, and the formation of Ca-def HAP was confirmed by the formation of β-TCP after thermal treatment of each product at 800°C [14].

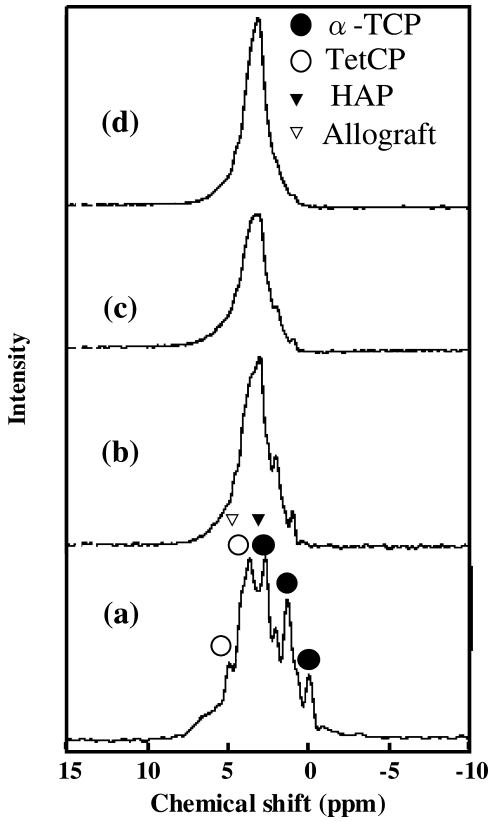

Fig. 6 [31] MSA NMR spectra of (a) a mixture of the calcium phosphates powder and allografts, Biopex-allografts composite after soaking in calf serum for (b) 1 day, (c) 3 days, and (d) 7 days.

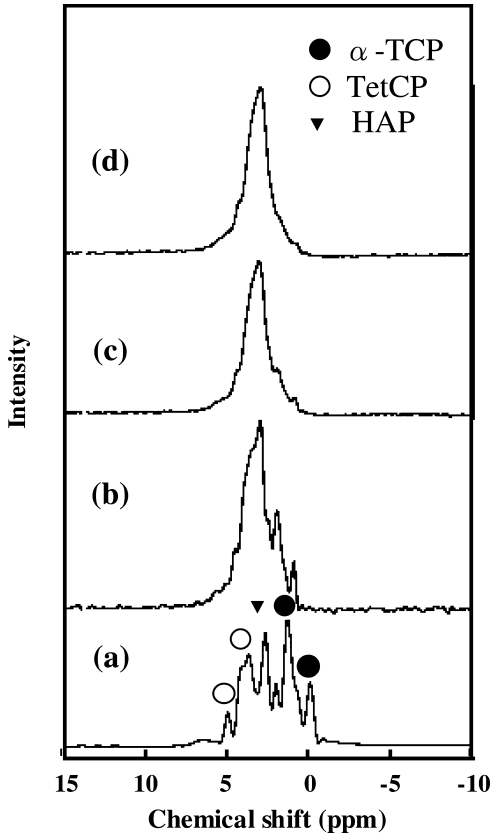

Fig. 7 [31]MSA NMR spectra of (a) the calcium phosphates powder of Biopex, Biopex after soaking in calf serum for (b) 1 day, (c) 3 days, and (d) 7 days.

In the hydrolysis of Biopex, OCP was formed besides HAP from the results of XRD. In the spectra of solid state NMR and FT-IR the formation of OCP was not clearly demonstrated because of the small amount of OCP formation and overlap of the characteristic signals and bands of OCP with those of HAP. It is known that OCP is formed in hydrolysis of α-TCP/DCPD [15]. In fact, under the present condition of the hydrolysis in both the solutions, α-TCP/DCPD was transformed into OCP after 1day. However, the hydrolysis of α-TCP/DCPD may not be involved in the formation of OCP from Biopex because of the early disappearance of DCPD. As another path to OCP, hydrolysis of α-TCP in acidic condition is plausible [16]. However, in the present condition α-TCP was not transformed into OCP. The rates of HAP formation of α-TCP, α-TCP/TetCP, and α-TCP/DCPD in serum decreased compared with those in saline. In contrast, those of Biopex and Biopex-allografts composite in serum did not decrease. The dependence on solution and the late formation of OCP suggest that hydrolyses of Biopex and Biopex-allografts composite were not a simple combination of formula (1), (2), and (3). As a possible reaction path in the hydrolysis of Biopex, ACP as an intermediate may be involved besides direct conversion of α-TCP and/or TetCP/α-TCP to HAP.

In the hydrolyses of Biopex-allografts composite, the similar reaction profile as shown in Fig. 3 and the similar products except OCP were obtained. We repeated the experiments six times and in only one case we found the peak of OCP with weak intensity in XRD pattern after soaking for 7days in calf serum, where Mg^{2+} ion was considered to promote ACP formation [17]. In the hydrolyses of Biopex-allografts composite, hydrolysis process via ACP plays an important role.

ACP does not show spectroscopic features. Therefore, identification of ACP by XRD, FT-IR, and ^{31}P MAS NMR analyses is more difficult, when other calcium phosphates coexist. In a certain case, thermal treatment of ACP above 650°C gives α-TCP and/or β-TCP [18]. Since thermal treatment of each hydrolysis product at 800°C gave βTCP, we did not observe the transformation of ACP to α-TCP below the β- to α-TCP transition temperature. However, these results may not deny the presence of ACP in the process.

It is known that ACP was transformed into OCP under acidic condition [19]. In these experiments, pH of each solution was around 9. In spite of basic condition, Biopex was transformed into OCP. Thus, in these hydrolyses the local pH at crystal growth site should decrease. In contrast with Biopex, OCP was hardly formed in the presence of allografts.

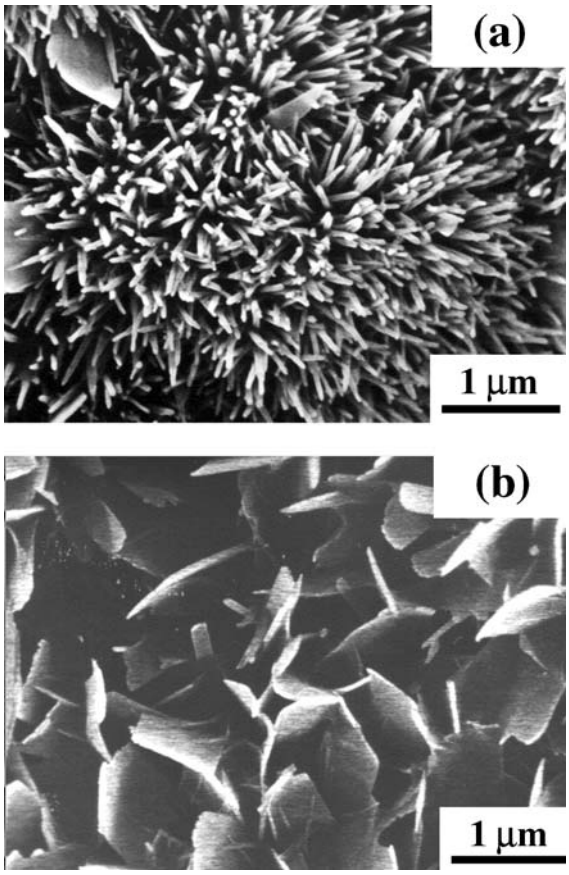

Fig. 8 SEM photographs of (a) Biopex-allografts composite and (b) Biopex after soaking in calf serum for 7 days.

It suggests that the allografts controlled local pH during the hydrolysis of Biopex-allografts composite.

Biopex was hydrolyzed to form plate-like crystals and small amounts of needle-like crystals, whereas Biopex-allografts composite was hydrolyzed to form needle-like crystals. Shape of the crystals depends on local pH during the formation of apatite [20]. This pH-dependence of morphology is consistent with the observed results.

5. Conclusions

In hydrolyses of Biopex in calf serum and in saline, OCP and HAP were formed and plate-like crystals were deposited. In hydrolyses of Biopex-allografts composite in both the solutions, HAP was formed and needle-like crystals were deposited. In the hydrolyses of Biopex-allografts composite and Biopex, the calcium phosphates may be partially transformed into ACP as an intermediate. We clearly demonstrated that allografts affected the hydrolysis of Biopex.

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