# Mineralization of Hydroxyapatite on a Polymer Substrate in a Solution Supersaturated by Polyelectrolyte

**Takashi Iwatsubo,[1] Samuel P. Kusumocahyo,[1] Toshiyuki Kanamori,[1] Toshio Shinbo[2]**

[1]*Research Center of Advanced Bionics, National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki 305-8565, Japan*
[2]*Collaboration Department, National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki 305-8568, Japan*

**ABSTRACT:** In this article, a new method to construct composites of hydroxyapatite (HAP) and polymer material is introduced. A previously developed method for mineralization of $CaCO_3$ on a polymer substrate was applied to HAP. A solution that contained $Ca^{2+}$, $PO_4^{3-}$, and OH- ions was supersaturated with polyacrylic acid (PAA) that, at the same time, formed a polymer complex with the substrate, a polyvinyl alcohol (PVA) film, at the substrate surface. In this thin surface layer, nucleation of HAP took place. Subsequently, the disklike domains of HAP that were generated spread until they covered the PVA film surface. By regulating the pH of the supersaturated solution at around 7.4, the domain size decreased and the quantity of deposited material increased. Approximately 20 mg of HAP coating was obtained on a PVA film of 1 cm radius when the film was soaked in single 200 mL batch of the supersaturated solution

for 21 days. The junction between HAP layer and PVA substrate film was found to be very firm. When a crosslinked PVA/PAA was used as the substrate, the film swelled in the supersaturated solution to form a hydrogel. Then mineralization took place within the gel, and a transparent monolithic composite of HAP and the polymer network was obtained. In 13 days, the weight increase was 29 mg, which corresponded to a 71 wt % HAP mineralization ratio of the composite. By changing crosslinking degree and HAP mineralization ratio, the flexibility of composite will be controlled in a wide range. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 100: 1465–1470, 2006

**Key words:** biomineralization; crosslinking; hydrogels; nucleation; polyelectrolytes

## INTRODUCTION

In vertebrate skeletal tissues, hydroxyapatite (HAP), $Ca_{10}(PO_4)_6(OH)_2$, deposits on collagen fibrils initially in the gap regions (interbands) between individual tropocollagen molecules, and this composite of HAP and collagen fibrils forms bone material that contains ~65 wt % HAP.[1] If HAP could be synthetically deposited on collagen, the biomimetic composite could be used as a stand-in for bone material. For this purpose, the manufacture of organic/inorganic composites between organic polymers and HAP has been investigated extensively.[2–9] For example, composites of small particles of HAP covalently bonded to a silicone rubber substrate have excellent elasticity and thus overcome the brittleness intrinsic to HAP.[4] The coprecipitation method of synthesis produces good yields of bulk collagen/HAP composites.[5,6] Numerous investigations of methods for manufacturing composites have led to the development of HAP mineralization processes whereby HAP is preferentially deposited on

polymer substrates without precipitating from the solution phase. Akashi et al. developed the alternate soaking process in which a polymer substrate bearing ionic groups was alternately soaked in $Ca^{2+}$ and $PO_4^{3-}$ containing solutions and rinsed with water after each soaking period.[7] This cycle results in the mineralization of HAP. In a method developed by Kokubo et al., nucleation is conducted in simulated body fluid (SBF) in the presence of Ca glass; then HAP is mineralized in 1.5 SBF, which has ion concentrations 1.5 times those of SBF.[8,9] To construct another type of polymer/biomineral composite, Kato et al. developed a powerful method for mineralizing $CaCO_3$ on a polymer substrate.[10,11] In this method, a supersaturated solution is prepared by Kitano's technique,[12] with $CO_2$ gas. Then a polyelectrolyte, such as polyacrylic acid (PAA) was dissolved in the supersaturated solution to suppress the precipitation of $CaCO_3$ from the liquid phase. The dissolved PAA combined with a substrate polymer at the surface as revealed from X-ray photoelectron spectroscopy (XPS) analysis.[13] The —COOH in the polymer complex promotes the heterogeneous nucleation of $CaCO_3$. At the same time, PAA contribute, to some extent, to an increase of dissolved ions in the supersaturated solution (suppress the nucleation), because

they normally decrease the activities of small ions in aqueous solution.[14]

On the basis of their method, we proposed in a previous report a simple method for obtaining composites of polymer substrate and $CaCO_3$, in which the supersaturation was accomplished by the PAA alone and heterogeneous nucleation was promoted by the PAA in a same mechanism as that of Kato's method.[15] Our method will have an advantage in that it will have many application fields because the mineralization is not restricted within carbonate salts. In this article, we show the applicability of our method for obtaining composites of polymer substrate and HAP.

## EXPERIMENTAL

### Materials

Polyvinyl alcohol (PVA) film (thickness, 50 $\mu$m) was obtained from Kuraray Co., Ltd. (Osaka, Japan). This film was insoluble in water at 30°C. PVA (degree of polymerization, ~2000), calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), sodium chloride (NaCl), diammonium hydrogen phosphate (($NH_4)_2HPO_4$), and 1$M$ tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) buffer were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Poly(vinyl acetate) (PVAc) with a molecular weight (MW) of about 90,000 was purchased from Polysciences, Inc. (Warrington, PA, USA). Two types of PAA (MW, ~2000; average MW, 250,000) were purchased from Sigma-Aldrich Corp. (St. Louis, MO).

### Mineralization conditions

The polymer substrates adopted were the purchased PVA, PVAc, and crosslinked PVA/PAA films. The PVAc film was cast from its 1 wt % dioxane solution and had a thickness of 60 $\mu$m. The PVA/PAA complex film was cast from an aqueous solution containing 0.5 wt % of PVA (degree of polymerization 2000) and 0.5 wt % of PAA (MW 250,000). Then, the dried film was heated at 110°C in an oven for 1 h to introduce crosslinking between the PVA and PAA polymer chains.[16] The crosslinked PVA/PAA film had a thickness of 30 $\mu$m. The effective substrate surface for mineralization was a circle with a radius of 1 cm in dry state.

Two kinds of supersaturated solutions, $S1$ and $S2$, were used. $S1$ was 5 m$M$ in $CaCl_2$ and 3 m$M$ in ($NH_4)_2HPO_4$, the source materials for HAP, and 0.139 m$M$ in the repeating unit of PAA (MW 2000) as a polyelectrolyte. The pH of $S1$ was 6.86. $S2$ was 140 m$M$ in NaCl and 45 m$M$ in Tris-HCl, and the concentrations of $CaCl_2$, ($NH_4)_2HPO_4$, and PAA were the same as in $S1$. The pH of $S2$ was 7.45. The pH and NaCl concentrations were chosen to be the same as in SBF.

**TABLE I**
**Conditions in Four Kinds of Experiments**

| Experiment No. | Polymer substrate | Solution |
|---|---|---|
| (1) | PVA | $S1^a$ |
| (2) | PVA | $S2^b$ |
| (3) | PVAc | $S1$ |
| (4) | PVA/PAA | $S2$ |

[a] $S1$: 5 m$M$ in $CaCl_2$, 3 m$M$ in ($NH_4)_2HPO_4$, and 0.139 m$M$ in the repeating unit of PAA (MW 2000) (pH = 6.86).
[b] $S2$: 5 m$M$ in $CaCl_2$, 3 m$M$ in ($NH_4)_2HPO_4$, 0.139 m$M$ in the repeating unit of PAA (MW 2000), 140 m$M$ in NaCl, and 45 m$M$ in Tris-HCl (pH = 7.45).

The supersaturation was certainly accomplished by the existence of PAA, because the solution not containing the PAA generated precipitation, whereas $S1$ and $S2$ kept their transparency for more than 2 weeks. The polymer substrate film was soaked in 200 mL of supersaturated solution maintained at 30°C.

Four kinds of experiments were conducted: (1) PVA film was soaked in $S1$, (2) PVA film was soaked in $S2$, (3) PVAc film was soaked in $S1$, and (4) crosslinked PVA/PAA film was soaked in $S2$. These are listed in Table I.

### Evaluation of mineralized materials

The weight increase, $\Delta M$ (mg), was calculated as $M - M_0$, where $M$ and $M_0$ are the weights of the sample dried in vacuum for 24 h after and before mineralization, respectively. The amount of products was not given in milligram per square centimeter unit, because the polymer substrates swelled up to some extent when immersed in supersaturated solution. Especially, PVA/PAA substrate swelled largely and mineralization took place inside the substrate. Moreover, the yield of mineralization process can be easily estimated from $\Delta M$. Since the volume of solution was 200 mL, the concentration of HAP corresponded to 0.5 m$M$ and the formula weight of HAP was 1004, the yield of mineralization is given by (1000/1004) $\Delta M$ (%) that is nearly equal to $\Delta M$ (%). In addition to $\Delta M$, the mineralization ratio, $W$ (%), was defined as $W = 100(\Delta M/M)$ to express the HAP relative content in the composite of HAP and PVA/PAA.

The pH of the solution was measured several times during the mineralization process in experiments (1) and (2). The surface and cross section of the obtained composites were observed by scanning electron microscopy (SEM; DS-720, Topcon Corp., Tokyo, Japan). The sample surface was platinum-coated before SEM observation.

The obtained composites were slurried in a solvent (hot water for PVA, dioxane for PVAc) to remove excess substrate, and the insoluble materials were collected. After being dried, they were crushed to pow-
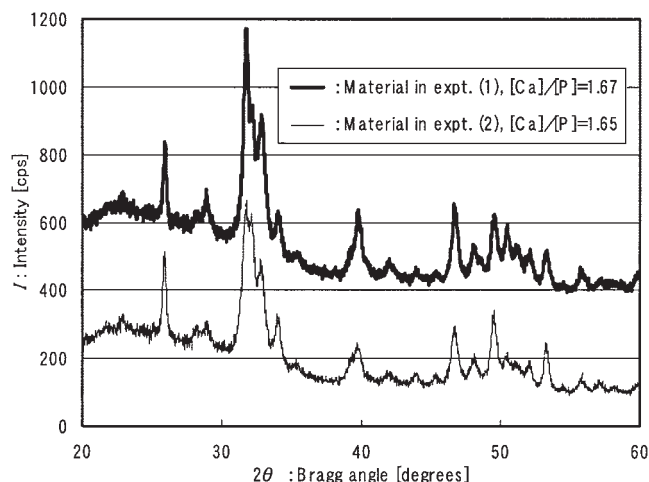
**Figure 1** X-ray powder diffraction patterns of the materials mineralized in expts. (1) and. (2). The thick line is shifted toward upper side by 300 cps from measured curve.

der in a mortar. The composite generated from the crosslinked PVA/PAA substrate was directly crushed without removal of excess substrate that was crosslinked and therefore insoluble. Then the powders were analyzed by X-ray diffraction (XRD; Geiger-Flex RAX01, Rigaku Co., Ltd., Tokyo, Japan).

The powders that had been used for XRD were then dissolved in HCl solution. After filtration of the insoluble gel-like materials, the filtrate was analyzed by inductively coupled plasma atomic emission spectroscopy (ICPS-7500, Shimadzu Corp., Kyoto, Japan) to determine the [Ca]/[P] ratio.

## RESULTS AND DISCUSSION

### Mineralization on substrate

In all four kinds of experiments, mineralized materials were obtained without any precipitation from the solution. The XRD patterns of the mineralized materials in experiments (1) and (2) coincided with the pattern for HAP[17] (Fig. 1). Since the lattice of calcium hydrogen phosphate hydroxide (CHPH), $Ca_9HPO_4(PO_4)_5OH$, gives an XRD pattern similar to that of HAP,[18] confirmation of HAP deposition required analysis of the [Ca]/[P] values. The measured values, 1.67 in expt. (1) and 1.65 in expt. (2), confirmed the mineralized materials as HAP.

SEM images of HAP in expt. (1) revealed that at the early stage of mineralization after nucleation on the substrate surface, concentric disklike domains that were similar to $CaCO_3$ domains[10,11,13,15] had grown on the surface [Fig. 2(a)]. At the stage after the surface had been covered with HAP domains [Fig. 2(b)], the HAP layer increased the thickness in the direction perpendicular to the surface. In the cross section im-

age, layers of HAP ~10 $\mu$m thick were observed with clear interfaces on both sides [Fig. 2(c)].

SEM also revealed that the surface domains of HAP in expt. (2) [Fig. 2(d)] were smaller than those in expt. (1) [Fig. 2(a)]. This difference can be attributed to the difference in the initial pH values between the two solutions. The pH of $S2$ in expt. (2) (7.4) was higher than that of $S1$ in expt. (1) (6.86), and so nucleation took place more easily in the former, and the domains became smaller as the number of domains increased.

Using soaking processes with different time intervals, we determined the time course of the mineralization process (Fig. 3). The mineralization of HAP proceeded in a manner similar to that for $CaCO_3$. In expt. (1), the pH of the supersaturated solution decreased as HAP formation proceeded, because HAP tends to mineralize in weak alkali solutions. At the early stage of the process, the HAP mineralization rate was large because the growth of HAP took place in the thin surface layer of the polymer complex, which had closer affinity to HAP. There are three reasons for the decline in HAP formation: (1) the decrease in pH, (2) the decrease in $Ca^{2+}$ and $PO_4^{3-}$ concentrations, and (3) the fact that the growth rate in the direction perpendicular to the surface was smaller than that in the plane of the surface. After 15 days, mineralization terminated with 10 mg of weight increase consuming 10% of source ions, $Ca^{2+}$ and $PO_4^{3-}$ (10% yield). At
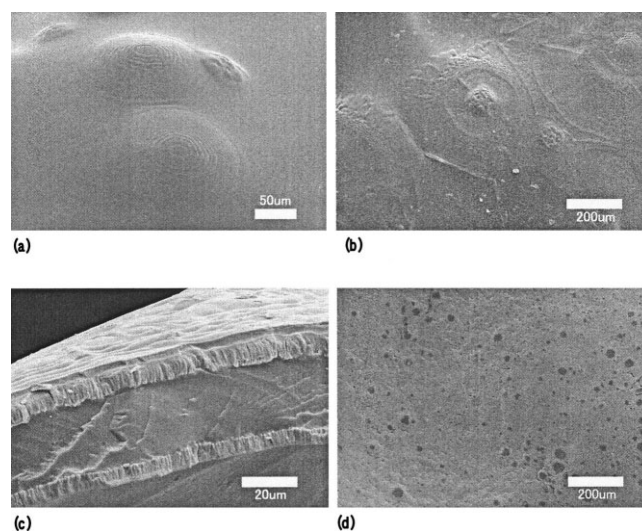


**Figure 2** SEM images of HAP and PVA composites. (a) Surface image of composite at an early stage of mineralization in expt. (1) using $S1$. The film became lumpy because each domain has a half-lens configuration. (b) Surface SEM image of composite at a thickening stage in expt. (1) using $S1$. By renewing the supersaturated solution every 10 days, we obtained a weight increase of 26.5 mg in 49 days on both sides of the substrate (a circle of radius 1 cm). (c) Cross section SEM image of composite at a thickening stage in expt. (1) using $S1$. The sample is the same as that shown in (b). (d) Surface SEM image of composite at a thickening stage in expt. (2) using $S2$.
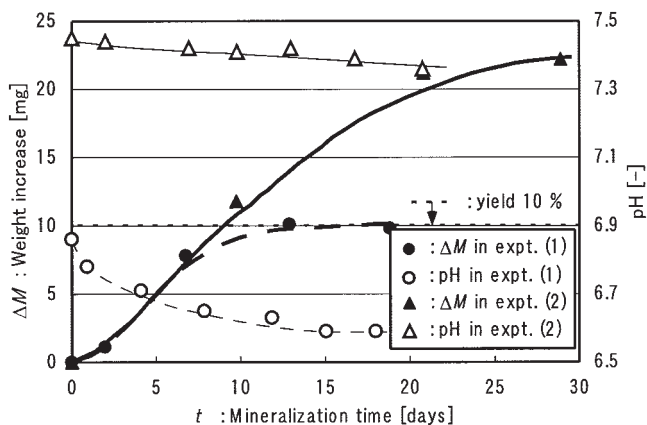
**Figure 3** Time courses of HAP mineralization on PVA substrate in expts. (1) and (2) were the following: closed symbols, weight increase; open symbols, pH changes; and dotted line, 10% yield level, which was plotted for evaluation of this mineralization method. The pH values were measured during the longest soaking period. Yield of mineralized HAP, is given by $(1000/1004)\Delta M$ %, where 1004 is the formula weight of HAP.

this stage, the solution is not supersaturated. In expt. (2), the pH was maintained at around 7.4 by Tris-HCl buffer, and therefore the yield was higher than in expt. (1). A weight increase of more than 20 mg was obtained in about 21 days and the solution seemed still supersaturated.

When we initially applied our method to the mineralization of $CaCO_3$, the presence of PAA in the solution allowed us to increase the concentration of dissolved ions in the supersaturated solution and, at the same time, combined with the substrate polymer to form thin surface layer of polymer complex.[13,19] In this thin polymer complex layer, the —COOH promoted the heterogeneous nucleation of $CaCO_3$. Like the nucleation of $CaCO_3$, the heterogeneous nucleation of HAP will take place in the thin surface layer of polymer complex between PAA and the PVA substrate. But formation of a complex between PAA and the substrate polymer is not adequate condition for the nucleation of HAP, because the mineralized material obtained from supersaturated solution $S1$ is not always HAP. The XRD pattern and the [Ca]/[P] value of the material obtained from expt. (3) (Fig. 4) revealed it to be calcium hydrogen phosphate hydrate, $Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O$ (octacalcium phosphate, OCP; characteristic peak at $2\theta = 4.7$),[20] although the [Ca]/[P] value of 1.46 deviated somewhat from the ideal value of 1.33. (We are investigating this discrepancy.) It has been proposed that OCP is a precursor of HAP, that is, OCP mineralizes first and is then converted to HAP by hydrolysis in vivo.[21] In this way, the mineralized Ca salt varies depending on the polymer complex. In the mineralization of $CaCO_3$, the polymorphism changed depending on the species of poly-

electrolyte in the solution phase.[10] The conformation of polyelectrolyte combined with the substrate surface was expected to have an influence on the crystallization process.[10] In our case of HAP, the conformation of PAA adsorbed will be different on the substrate between PVA and PVAc.

When subjected to ultrasonic treatment at 38 kHz with 120 W for 15 min, a hybrid of HAP and a PVA film immersed in water maintained its structure, which indicated the firm junction between HAP and the PVA film were formed. However, a hybrid of OCP and PVAc was crumbled under the same conditions, probably owing to the weak mechanical strength of PVAc.

## Mineralization within substrate

In expt. (4), the crosslinked PVA/PAA film swelled like a soft gel when it was soaked in the supersaturated solution. Then mineralization took place within the hydrogel. The gel hardened as the mineralization proceeded. The XRD pattern indicated that the mineralized material could be either HAP or CHPH (Fig. 5). The [Ca]/[P] value of 1.72 suggested that the material will likely be HAP, although this value was somewhat larger than the theoretical value of 1.67 for HAP. The discrepancy is probably due to absorption of $Ca^{2+}$ by the carboxylic groups of PAA in the crosslinked PVA/PAA complex.

A weight increase of more than 20 mg was obtained in ~7 days (Fig. 6), which means that the mineralization rate was almost three times than that in expt. (2) in which heterogeneous nucleation took place only at the substrate surface. The mineralization ratio, $W$, reached nearly 70% at 13 days. With this substrate that was the complex of PVA and PAA, heterogeneous nucleation and the subsequent growth of small HAP
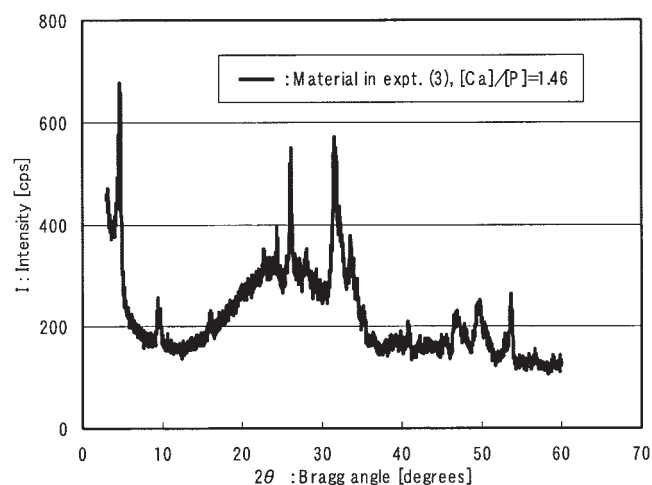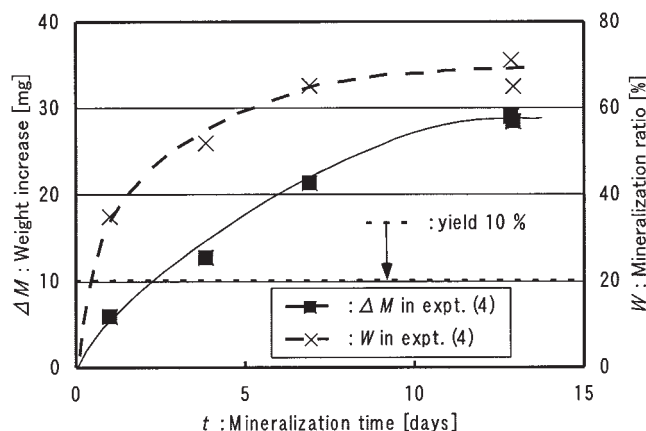


**Figure 4** X-ray powder diffraction pattern of the material mineralized in expt. (3).
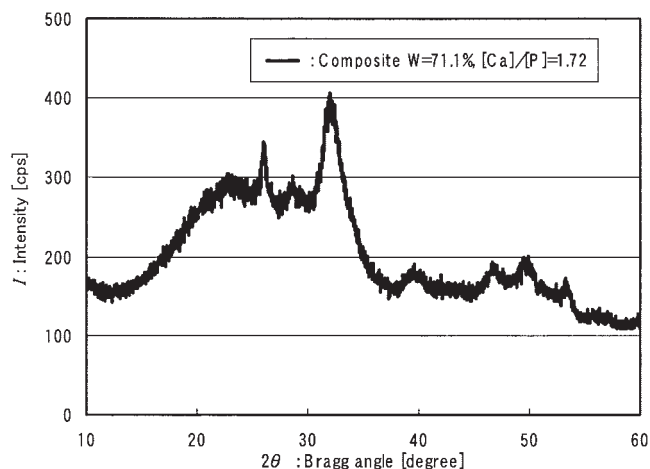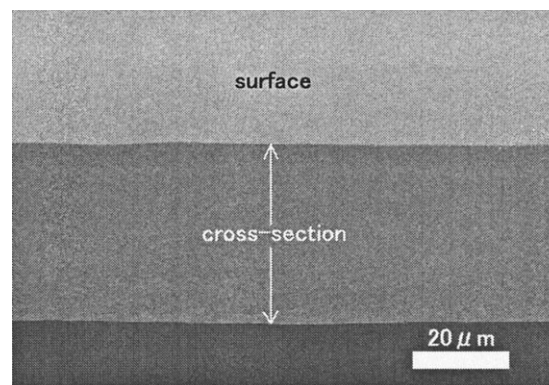
**Figure 5** X-ray powder diffraction pattern of the material mineralized in expt. (4).



**Figure 7** Cross section SEM image of monolithic composite with $W = 71.1\%$ obtained in expt. (4). Thickness of the composite is about 40 $\mu$m.

particles took place in a three-dimensional gel medium that had close affinity to HAP. Furthermore, the loose network of polymer chains enabled the diffusion of $Ca^{2+}$, $PO_4^{3-}$, and $OH^-$ from the outside to the inside of the gel. These factors led to the high mineralization rate. Composites with low $W$ values were found to be flexible in water. Composites having high $W$ values (~70%) were brittle in dry state but pliable in wet state. In SEM photograph (Fig. 7), we can recognize that the monolithic composite has no apparent interfaces in cross section or domains at the surface and is quite homogeneous in this magnification ratio. Owing to the dispersed distribution of HAP in three-dimensional medium, the polymer network can allow flexibility of the composite. After the inside of the gel became filled with HAP, the mineralization can take place only at the bulk gel surface, and the growth rate then will turn to be slow. The low density of the polymer chains in swollen gel membrane provided enough space for the growing HAP particles without disrupting the gel network. As can be seen in Figure 8, the monolithic composite obtained ($W = 70.2\%$), (b), was transparent and the size of it is larger than that of PVA/PAA film, (a), before mineralization. The roles of PAA (degree of polymerization 2000) in the solution, between mineralization on PVA and that within PVA/PAA will be different. In both mineralization, PAA in the solution contribute to elevate supersaturated concentration of ions (suppress the nucleation). However, in the former PAA combines with PVA at the surface and heterogeneous nucleation takes place in this thin layer, whereas in the latter PAA does not need to combine with substrate polymer. Since PAA was preincorporated into the polymer complex substrate, the only role of the PAA in the solution was to increase the concentration of dissolved ions.
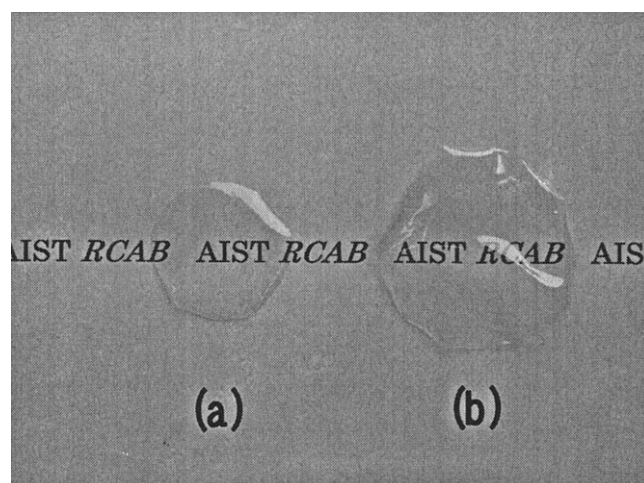


**Figure 6** Time course of mineralization in expt. (4) were the following: solid curve, weight increase; dashed curve, mineralization ratio; and dotted line, 10% yield level.



**Figure 8** Photograph of (a) crosslinked PVA/PAA film before mineralization and (b) crosslinked PVA/PAA film with HAP after mineralization ($W = 70.2$ wt %). Both films are in dry states.

## CONCLUSIONS

By using a method that we had previously applied to the mineralization of CaCO$_3$, we accomplished the mineralization of a HAP layer on a PVA substrate film from a solution supersaturated with PAA without any solution precipitation. The heterogeneous nucleation took place at a thin surface layer of PVA/PAA complex formed on the substrate. Controlling the pH of the solution enabled us to obtain a large weight increase. We adopted a crosslinked film of a miscible blend of PVA/PAA as a substrate medium, and this substrate swelled in the supersaturated solution. The mineralization took place inside the swollen gel medium, and the growth rate was three times that on the PVA film. The resulting monolithic HAP/polymer composite was transparent, and bended in the wet state. Changing crosslinking degree and HAP mineralization ratio, it will be possible to construct a variety of composites having different elastic and swelling properties. If biocompatible polymer can be applicable to this method, the resulting composite will be used as a stand-in for bone material. Further investigation is being held along this line by the authors.

## References

1. Frankel, R. B.; Mann, S. In Encyclopedia of Inorganic Chemistry; King, R. B., Ed.; Wiley: Chichester, 1994; Vol. 1, p 269.
2. Tretinnikov, O. N.; Kato, K.; Ikada, Y. J Biomed Mater Res 1994, 28, 1365.
3. Goissis, G.; Maginador, S. V. S.; Martins, V. C. A. Artif Organs 2003, 27, 437.
4. Furuzono, T.; Wang, P. L.; Korematsu, A.; Miyazaki, K.; Oido-Mori, M.; Kowashi, Y.; Ohura, K.; Tanaka, J.; Kishida, A. J Biomed Mater Res 2003, 65B, 217.
5. Kikuchi, M.; Itho, S.; Ichinose, S.; Shinomiya, K. Tanaka, J. Biomaterials 2001, 22, 1705.
6. Itoh, S.; Kikuchi, M.; Koyama, Y.; Takakuda, K.; Shinomiya, K.; Tanaka, J. Biomaterials 2002, 23, 3919.
7. Taguchi, T.; Muraoka, Y.; Matsuyama, H.; Kishida, A.; Akashi, M. Biomaterials 2001, 22, 53.
8. Kokubo, T. J Non-Cryst Solids 1990, 120, 138.
9. Oyane, A.; Kim, H. M.; Furuya, T.; Kokubo, T.; Miyazaki, T.; Nakamura, T. J Biomed Mater Res 2003, 65A, 188.
10. Kato, T.; Suzuki, T.; Amamiya, T.; Irie, T.; Komiyama, M. Supramolecular Sci 1998, 5, 411.
11. Kato, T.; Amamiya, T. Chem Lett 1999, 28, 199.
12. Kitano, Y. Bull Chem Soc Jpn 1980, 1962, 35.
13. Zhang, S.; Gonsalves, K. E. Langmuir 1998, 14, 6761.
14. Nagasawa, M.; Kagawa, I. J Polym Sci 1957, XXV, 61.
15. Iwatubo, T.; Sumaru, K.; Kanamori, T.; Yamaguchi, T.; Shinbo, T. J Appl Polym Sci 2004, 91, 3627.
16. Kuhn, W.; Hargitay, B.; Katchalsky, A.; Eisenberg, H. Nature 1950, 165, 514.
17. JCPDS No. 09–0432. International Center for Diffraction Data: Newton Square, PA, 1998.
18. JCPDS No. 46–0905. International Center for Diffraction Data: Newton Square, PA, 1995.
19. Iwatsubo, T.; Kusmocahyo, S. P.; Sinbo, T. J Appl Polym Sci 2002, 86, 265.
20. JCPDS No. 26–1056. International Center for Diffraction Data: Newton Square, PA, 1976.
21. Brown, W. B. Clin Orthop 1996, 44, 205.