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Growth of fibrous hydroxyapatite in the gel system

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Hydroxyapatite (HAP) was prepared in the agar gel system, where Ca^{2+} ions were incorporated in the gel and PO_4^{3-} solution was layered over the gel. When the concentration of Ca^{2+} was lower than 1.0 M and the initial solution Ca/P molar ratio was lower than about unity, fibrous HAP several centimetres to several tens of centimetres in length was grown upwards in the PO_4^{3-} solution from the gel phase, while at the larger Ca^{2+} concentration and initial Ca/P molar ratio $CaHPO_4 \cdot 2H_2O$ (DCPD) in the form of gelatinous precipitate, particulate precipitates or needle-like crystals were preferred to HAP. The fibrous HAP was calcium deficient and composed of small elongated hollow ovals linked in a zigzag row. This was considered to be formed in the following manner. First, Ca^{2+} ions supplied through pores in the gel reacted with PO_4^{3-} ions to form a small spherulite at the pore exit, then the spherulite was bloated and elongated by the osmotic pressure or capillary force until a part of the oval was broken for Ca^{2+} ions to be gushed out into the PO_4^{3-} solution. The above two processes were repeated to form elongated hollow ovals linked to the preceding ones.

1. Introduction

Hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ (HAP) is an important constituent of bones and teeth. In the technological field, it has been an attractive material for chromatographic separation, catalysis, ion-exchange and bone and tooth implants [1]. HAP has been synthesized by a solid-state reaction between Ca^{2+}- and PO_4^{3-}-bearing compounds and/or under solution conditions in the form of powders [2] which are eventually sintered to a dense polycrystalline body by firing [3]. A single crystal of HAP has been grown under hydrothermal conditions or from the melt [1].

The present paper describes the process for making HAP from Ca^{2+} and PO_4^{3-}-solutions using the gel system. The gel system has been used as one of the techniques for producing single crystals, where ions slowly diffuse into the gel containing other ions, or ions counter-diffuse through the gel to form crystals of the prescribed compositions in the gel. The detail of the crystal growth in gels is thoroughly reviewed by Henisch [4].

The growth of HAP and other calcium phosphate crystals using the gel system, has been reported by Hunter *et al.* [5] and LeGeros *et al.* [6], who used low concentrations of Ca^{2+} and PO_4^{3-} ions such as 0.005 and 0.01 M, and found that HAP was formed in the agar, silica or collagen gel, dicalcium phosphate dihydrate $CaHPO_4 \cdot 2H_2O$ (DCPD) coexisting with HAP. In the present study, much higher concentrations of the corresponding ions were employed and it was found that the long fibrous HAP was grown upwards from the gel phase when the PO_4^{3-} solution was layered over the agar gel containing Ca^{2+} ions. The qualitative explanation of the growth of the fibrous HAP was attempted.

2. Experimental techniques

The 0.1 to 2.0 M $Ca(NO_3)_2$ solutions and 0.1 to 2.0 M $(NH_4)_2HPO_4$ solutions were prepared. The pH of the Ca^{2+} and PO_4^{3-} solutions were in the range 4.0 to 5.5, and 8.3 to 8.7, respectively.

Agar was dissolved in the Ca^{2+} solutions at the boiling temperature. The concentration of agar was changed from 1.0 to 10.0 g/100 ml of the solution, but the concentration used mainly below will be 5.0 g/100 ml solution. After the agar containing Ca^{2+} solution was transferred to a test tube of i.d. 30 mm, cooled to room temperature and the gel set, 40 ml PO_4^{3-} solution was carefully layered over the gel phase. The test tube thus set up was kept standing in the room. A set-up consisting of the agar gel containing PO_4^{3-} and overlying Ca^{2+} solution was not available because agar-containing PO_4^{3-} solution did not solidify at room temperature.

Fibrous products, gelatinous or particulate precipitates were formed in the PO_4^{3-} solution over the gel phase after some duration, depending on the concentration of the solutions, as will be seen below. They were carefully removed from the system, thoroughly washed with distilled water and then dried in the room for several days. For comparison, the Ca^{2+} solution was added to the PO_4^{3-} solution under a vigorous stirring at an initial solution Ca/P molar ratio of 1.0 or 2.0. The precipitates were filtered, washed with distilled water and dried. Those products obtained in the gel system and by the solution-mixing method were

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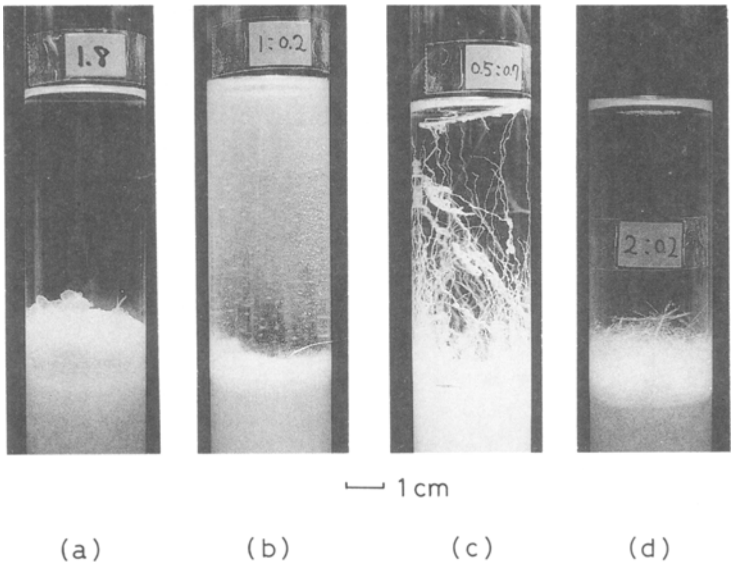


Figure 1 Morphology of calcium phosphate products in the agar gel system; (a) gelatinous precipitate, (b) particulate precipitate, (c) fibrous product and (d) needle-like crystal. The numbers attached to test tubes denote the concentrations of Ca^{2+} and PO_4^{3-} ions, respectively.

subjected to X-ray diffraction (XRD) analysis, microscopic observation and chemical analysis.

Crystalline species in the as-prepared products and those heated to different temperatures up to 1000° C were identified by the conventional XRD technique using the nickel-filtered Cu*Kα* radiation. Lattice constants of HAP were determined from *d*-values for (300), (222), (213) and (004) reflections. For that purpose, silicon powder of extra high purity was mixed with specimens as an internal standard.

Fibrous products were embedded in epoxy resin, which were cut and polished for optical microscopic observation.

Chemical analysis of fibrous products was performed by a spectrophotometric phosphovanadomolybdate method for phosphorus and an atomic absorption method for calcium. An HITACHI 200-20 spectrophotometer and a Nippon Jarrell-Ash AA-855 atomic absorption and flame emission spectrophotometer were used, respectively.

3. Results

3.1. Products produced in the gel system

The precipitate produced by mixing the Ca^{2+} solution with the PO_4^{3-} solution under stirring was dicalcium phosphate dihydrate $CaHPO_4 \cdot 2H_2O$ (DCPD), irrespective of the concentration of the starting solution used in the present work. No other crystalline species was obtained by the solution-mixing method. It has been reported that DCPD precipitates on mixing the Ca^{2+} solution with the PO_4^{3-} solution under stirring if the "solution concentration product" $[Ca^{2+}][PO_4^{3-}]$ is larger than about $10^6\,mM^2$ [7]. The $[Ca^{2+}][PO_4^{3-}]$ of the starting solutions in the present case was in the range of 1 to $4 \times 10^6\,mM^2$, thus the present result is consistent with the previous one mentioned above.

On the other hand, in the gel system, HAP and/or DCPD began to form at a few minutes to several hours after the PO_4^{3-} solution was poured on to the Ca^{2+}-containing agar gel, as given in Table I, in the different forms such as the gelatinous precipitate (Fig. 1a), particulate precipitate (Fig. 1b), fibrous product (Fig. 1c) and needle-like crystals (Fig. 1d).

Figs 2a and b show some examples of XRD patterns of the precipitates. Fig. 2a presents the change of XRD pattern with the concentration of Ca^{2+} and PO_4^{3-} solutions at an initial solution Ca/P molar ratio of unity, and Fig. 2b is for the change with the initial solution Ca/P molar ratio at a Ca^{2+} concentration of 1.0 M. It is seen that HAP is formed at a

TABLE I Crystalline products obtained in the agar gel system

Sample	Ca(M)	P(M)	Products	Sample	Ca(M)	P(M)	Products
2	0.2	0.2	HAP	22	1.0	0.7	DCPD + HAP
3	0.3	0.3	HAP	23	1.0	0.8	HAP
4	0.4	0.4	HAP	24	1.0	0.9	HAP
5	0.5	0.5	HAP	25	1.0	1.0	HAP
6	0.5	0.25	DCPD + HAP	26	1.0	1.2	HAP
7	0.5	0.3	DCPD + HAP	27	1.0	1.4	HAP
8	0.5	0.35	DCPD + HAP	28	1.0	1.6	HAP
9	0.5	0.4	HAP	29	1.0	1.8	HAP
10	0.5	0.45	HAP	31	2.0	0.2	DCPD
11	0.5	0.6	HAP	32	2.0	0.4	DCPD
12	0.5	0.7	HAP	33	2.0	0.6	DCPD + HAP
13	0.5	0.8	HAP	34	2.0	0.8	DCPD + HAP
16	1.0	0.1	DCPD	35	2.0	1.0	DCPD + HAP
17	1.0	0.2	DCPD	36	2.0	1.2	DCPD + HAP
18	1.0	0.3	DCPD + HAP	37	2.0	1.4	DCPD + HAP
19	1.0	0.4	DCPD + HAP	38	2.0	1.6	DCPD + HAP
20	1.0	0.5	DCPD + HAP	39	2.0	1.8	DCPD + HAP
21	1.0	0.6	DCPD + HAP	40	2.0	2.0	DCPD + HAP

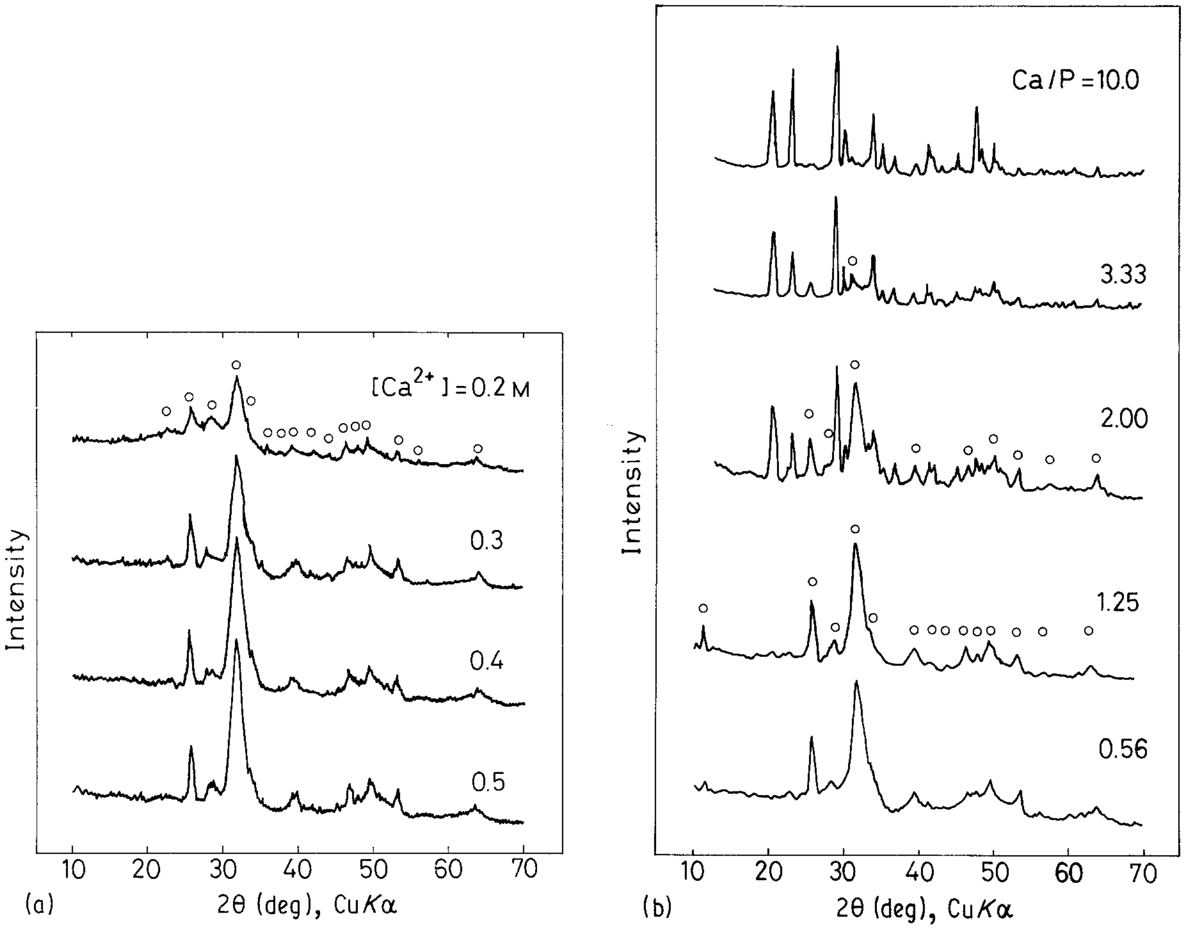


Figure 2 X-ray diffraction patterns of calcium phosphate products in the agar gel system: (a) change with Ca^{2+} concentration at an initial solution Ca/P molar ratio of unity, and (b) change with initial solution Ca/P molar ratio at a Ca^{2+} concentration of 1.0 M. (○) Hydroxyapatite HAP, other peaks are for dicalcium phosphate dihydrate (DCPD).

relatively low Ca/P molar ratio and a low concentration of Ca^{2+}, and the crystallinity of HAP obtained is not so high.

In Fig. 3, crystalline species and morphology of the precipitate in the agar gel system are summarized as functions of the initial solution Ca/P molar ratio and the concentration of Ca^{2+} solution. It is more clearly seen from Fig. 3 that the concentration of Ca^{2+} solution lower than 1.0 M and the initial Ca/P molar ratio lower than 1.0 are favoured for the formation of fibrous HAP (Fig. 1c). At a higher concentration of Ca^{2+} and a larger initial Ca/P molar ratio, gelatinous or particulate DCPD is preferred to HAP. At an initial Ca/P molar ratio as high as 10.0, needle-

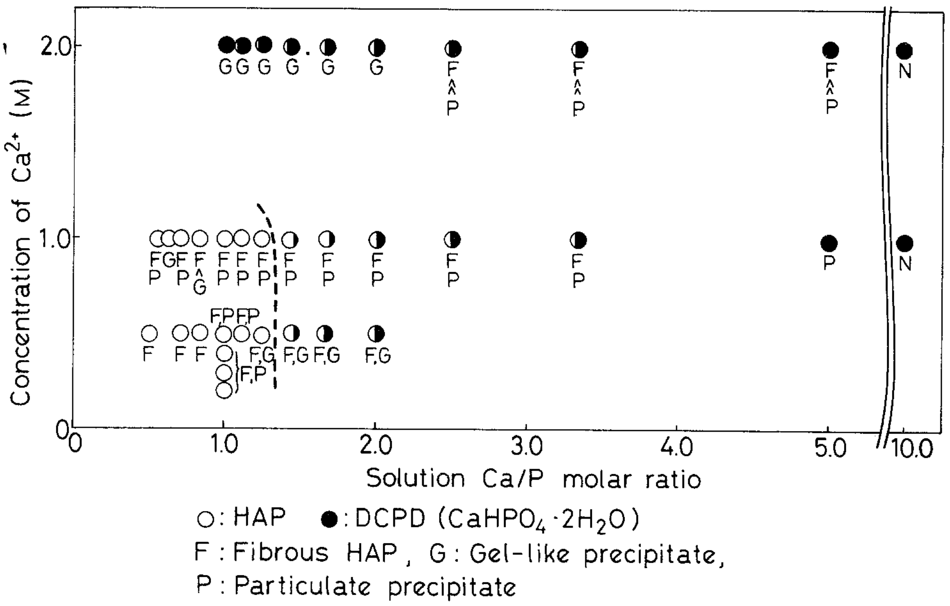
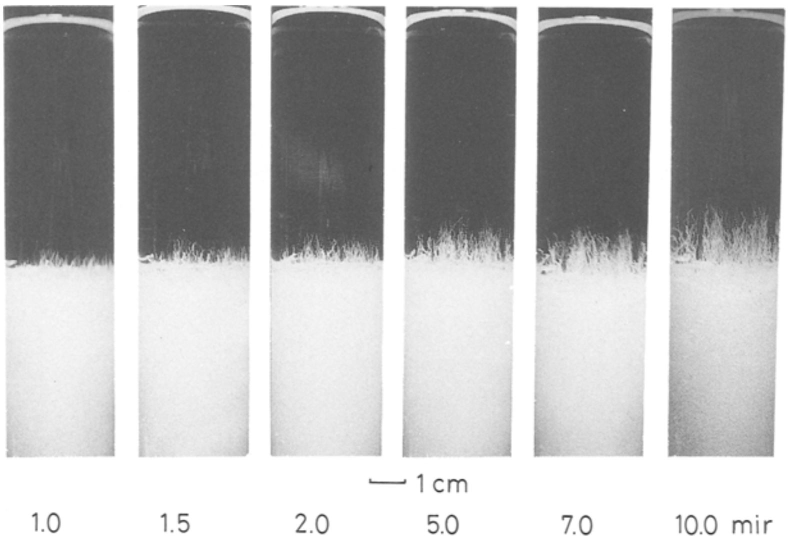
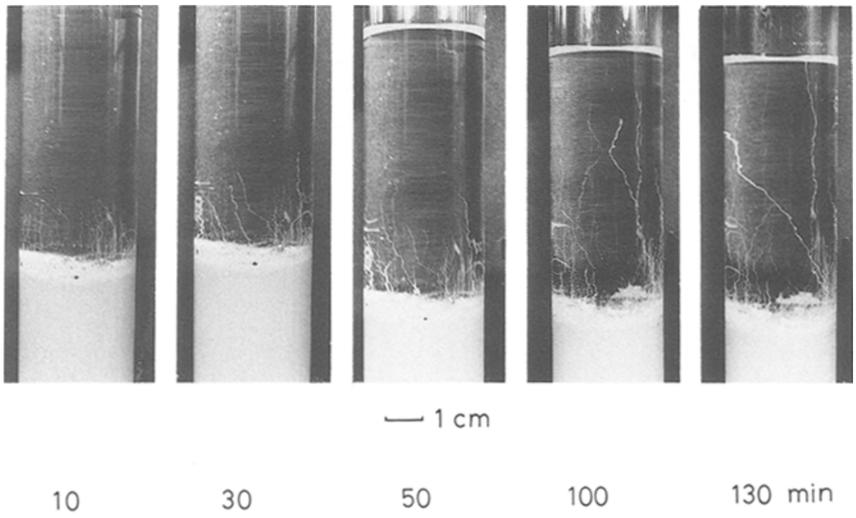


Figure 3 Species of calcium phosphate formed in the gel system and its morphology as functions of the initial solution Ca/P molar ratio and the Ca^{2+} concentration.



(a) Ca/P = 1M/1M, agar 5g/100ml solution



(b) Ca/P = 1M/1M, agar 5g/100ml solution

Figure 4 Examples of the growth of HAP in the agar gel system.

like DCPD crystals (Fig. 1d) are formed in the PO_4^{3-}-solution. Although it is not shown in Fig. 3, precipitation occurred in the gel when the 0.1 M Ca^{2+} solution and 0.1 M PO_4^{3-} solution were used.

The analysed Ca/P molar ratio of the fibrous HAP produced at low Ca^{2+} concentrations and low initial solution Ca/P molar ratios was 1.3 to 1.4. Lattice constants a_0 and c_0 of the fibrous HAP were 0.9439 and 0.6871 nm, respectively. a_0 is slightly larger than that of the stoichiometric HAP (a_0 = 0.9423 nm).

The HAP was transformed to β-tricalcium phosphate, β-$Ca_3(PO_4)_2$ (β-TCP) around 800° C.

3.2. Growth of fibrous HAP

In Figs 4a and b, two typical examples of the growth of fibrous HAP in the agar gel system are shown. It is seen that fibrous HAPs grow at a considerably higher rate. The growth rate is about 0.1 $cm\,min^{-1}$ for those two cases. However, the growth rate of fibrous HAP was not uniquely determined, but differed from run to run even though experimental conditions were kept unchanged. No considerable and systematic effects of the concentration of the starting Ca^{2+} and PO_4^{3-} solutions and the concentration of agar in the gel on the growth rate of the fibrous HAP were observed in our experiments.

It is observed that zigzag-featured HAP fibres do not move upwards as they grow, but the growth occurred at the tip of the fibres. Furthermore, as can be seen in Fig. 4b, some fibrous products reached the top of the PO_4^{3-} solution in 130 min. Then, a transluscent film precipitate was developed over the solution surface, being centred at the tip of the fibrous HAP.

The microphotograph of the section of the fibrous HAP is shown in Fig. 5. The hollow structure of the fibres is clearly noted. And it seems that a fibre is composed of elongated ovals linked in a row.

4. Discussion

4.1. Composition of the fibrous HAP

Stoichiometric HAP has a Ca/P molar ratio of 1.67. On the other hand, it is known that HAP precipitated under the solution conditions is often calcium-deficient and gives a lower Ca/P molar ratio [8]. In

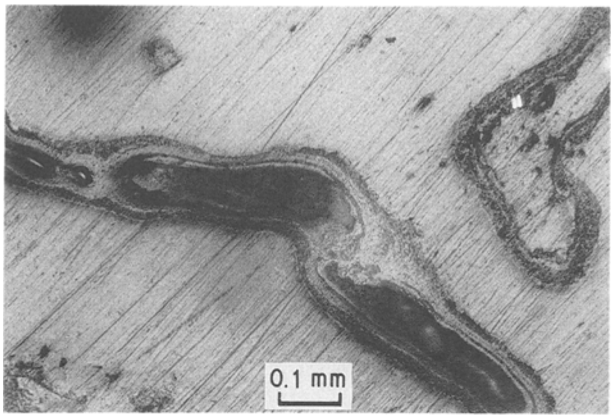


Figure 5 Microphotograph of the section of fibrous HAP embedded in epoxy resin.

general, the calcium-deficient HAP expressed by formula $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x} \cdot nH_2O$ has a Ca/P molar ratio ranging from 1.5 to 1.67. As mentioned above, the Ca/P molar ratio of the fibrous HAP obtained at a low Ca^{2+} concentration and a low initial solution Ca/P molar ratio in the present work was 1.3 to 1.4, which is considerably lower than the reported lowest value for the calcium-deficient HAP.

The crystallinity of our fibrous HAP is not high as seen in Fig. 2. In addition, the "concentration product" $[Ca^{2+}][PO_4^{3-}]$ of 10^4 to $10^5\,mM^2$ in the fibrous HAP formation region of Fig. 3 is also favourable for the formation of amorphous calcium phosphate [7], suggesting that it may possibly coexist with the fibrous HAP. These facts may be responsible for too a low Ca/P molar ratio of the present fibrous HAP. It has been reported that calcium-deficient HAP transforms to β-TCP, around 800° C [9], while the stoichiometric HAP does not. The present fibrous HAP was transformed completely to β-TCP around 800° C. Accordingly, our fibrous HAP may be concluded to be calcium-deficient although the exact Ca/P molar ratio remains to be clarified.

4.2. Growth mechanism of the fibrous HAP

The fibrous HAP obtained in the present work has a hollow structure and is composed of small elongated ovals linked in a zigzag row. The occurrence of growth at the tip of the fibre and the HAP film precipitate developed over the surface of the PO_4^{3-} solution in the gel system, centring at the tip of the HAP fibre, suggest that Ca^{2+} ions are supplied through the tubing of the fibre.

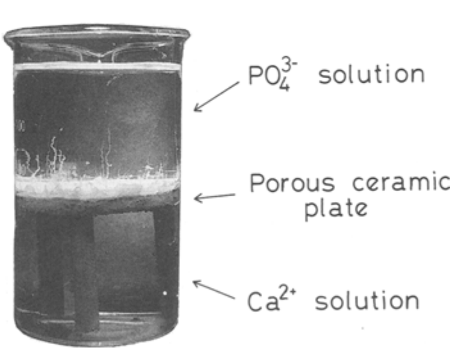


Figure 6 The growth of HAP in the system using a porous ceramic plate partition.

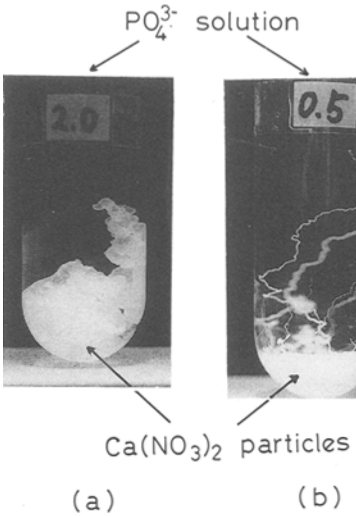


Figure 7 Morphology of the calcium phosphate grown on the crystalline $Ca(NO_3)_2$ particles dipped in the PO_4^{3-} solution. The numbers attached to the test tubes represent the PO_4^{3-} concentration of the overlying solution.

In order to confirm such a speculation, two supplementary experiments were performed. First, Ca^{2+} and PO_4^{3-} solutions were separated by a porous ceramic plate as shown in Fig. 6. Then, fibrous HAP was grown upwards in the PO_4^{3-} solution, suggesting that pores in the ceramic plate provide the path for Ca^{2+} ions. In the second, PO_4^{3-} solution was carefully layered on the $Ca(NO_3)_2$ crystalline particles. The gelatinous film precipitate was immediately formed to envelope the $Ca(NO_3)_2$ particles, and became bloated with the elapse of time. Only inside the gelatinous film precipitate, were Ca^{2+} ions found. Once the bloated gelatinous film was broken to form small holes or fissures, it was observed that semispherules newly developed from there. In such a manner, the gelatinous precipitate grew in the manner cumulus in which clouds mount, as shown in Fig. 7a. The precipitate removed from the solution and dried was HAP. Sometimes, fibrous HAPs were grown on $Ca(NO_3)_2$ crystalline particles as shown in Fig. 7b, just as in the gel system.

The results obtained in the latter supplementary experiment seem similar to those in the long-established so-called "Chemical Garden". That is, when the aqueous water glass solution (specific gravity 1.07 to 1.08) is carefully layered on crystalline salts such as $MnCl_2$, $CoCl_2$ and $MnSO_4$ in test tubes, coloured fibrous matter grows upwards in the water glass solution. This phenomenon has been explained as follows. A metal salt is dissolved in water and reacts with silicate ions to form a semipermeable metal silicate film, through which water is continuously supplied to the remaining metal salts. As the amount of salt dissolved is increased, the osmotic pressure is increased until some parts of the gelatinous film are broken and the gel film is newly developed from there. Accordingly, a gel precipitate grows up.

In the case of the gel system or the system using a porous ceramic partition, the gelatinous film formation at the interface between Ca^{2+} and PO_4^{3-} solutions

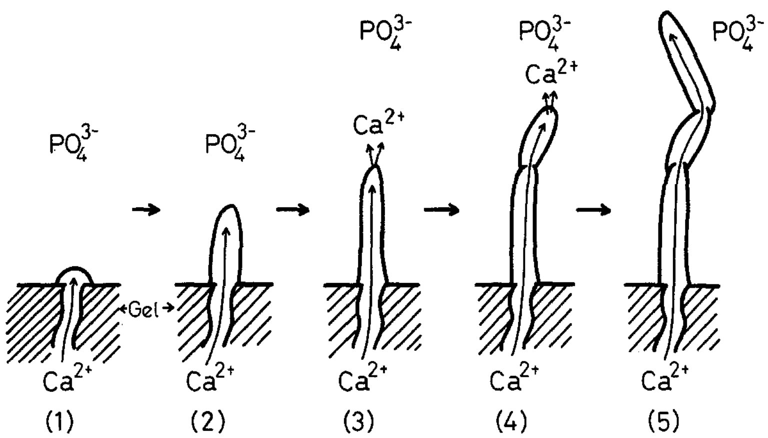


Figure 8 Schematic presentation of the growth of fibrous HAP from the gel phase in the PO$_4^{3-}$ solution.

is considered to occur at relatively small-scaled places, that is, at small pore exits faced with the PO$_4^{3-}$ solution. The thin fibrous HAP grows at the pore exit of the gel or ceramic plate as shown schematically in Fig. 8. If the size of the originally formed gelatinous spherulites at the pore-exit is large, spherulites are coalesced to make a film precipitate covering the whole gel surface, which is considered to depend on the concentrations of Ca^{2+} and PO$_4^{3-}$ ions and the initial solution Ca/P molar ratio.

The growth of the fibrous HAP seems step-like as shown in Fig. 8. Namely, the formation of an elongated hollow oval, the breakage of the oval at the weakest part and the formation of a succeeding oval by the reaction of PO$_4^{3-}$ ions with Ca^{2+} ions gushing out of the broken part, successively occur. During this process, Ca^{2+} ions are continuously supplied to the tip of the fibrous products by the osmotic pressure or capillary force. The row of elongated ovals are, thus, resulted. Because the weakest part of the individual oval is not always at the tip, Ca^{2+} ions do not necessarily gush out upwards in the solution, making the resultant zigzag fibres.

The resulting fibrous HAP was, thus, not monolithic but composed of many elongated ovals linked in a zigzag row, so that its mechanical strength is not so high. One possible approach to strengthen the fibre may be the application of a sintering technique. Unfortunately, as mentioned above, our fibre is calcium-deficient and not sinterable, due to the transformation to β-TCP around 800° C. Therefore, it is necessary to prepare the fibrous HAP of stoichiometric Ca/P molar ratio to strengthen it. Furthermore, the problem of whether the present fibrous matter is originally HAP or amorphous calcium phosphate which may be transformed to HAP in the solution remains to be solved. Work on the problems is continuing in our laboratory.

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