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Synthesis of fluoride-releasing carbonate apatites for bone substitutes

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Abstract Fluoride (F^-)-substituted B-type carbonate hydroxyapatite (CHAP) powders were prepared for application as bone substitute materials having the ability to enhance bone formation and to suppress bone resorption due to the therapeutic effect of F^-. F^- was adsorbed on CHAP in a sodium fluoride solution followed by heating at 700°C in carbon dioxide flow to substitute F^- for the hydroxyl ion in the CHAP structure. The F^- contents in the F-substituted CHAP powders were 16–22 times greater than that in normal adult human bones. The carbonate ion contents in the F-substituted CHAP powders corresponded to or were higher than that in normal adult human bones. F-substituted CHAP powder with CO_3^{2-} and F^- contents of 11.03 and 0.66 wt%, respectively, slowly released F^- in a physiological salt solution to a sufficiently high F^- level. The F^- concentration slowly increased and reached 67.20 ± 4.81 μg l^{-1}, which was 1.5–9.3 times higher than that in the body fluid of normal adult humans, near the therapeutic window of F^-, and far lower than the estimated toxic level. Therefore, the F-substituted CHAP can promote bone formation. The present F-substituted CHAP has the advantage of slow F^- release over sodium fluoride and sodium monofluorophosphate which are highly soluble salts and cannot be sintered into a ceramic body.

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1 Introduction

The fluoride ion (F^-) has attracted attention due to its therapeutic ability of osteoporosis healing since the bone mass is increased by F^- administration [1]. F^- is known to stimulate osteoblast activity both in vitro and in vivo. Sodium fluoride (NaF) directly increases the proliferation rate and the alkaline phosphatase activity of osteoblastic cells, resulting in the enhancement of new bone tissue formation [2]. The mechanism by which F^- may influence the proliferation and differentiation of osteoblastic cells strongly suggests the alteration of one or several G-protein-dependent tyrosine phosphorylation processes, activation of the extracellular signal-regulated kinase, and possibly other signaling pathways [3, 4]. Hence, sodium fluoride (NaF) therapy has been studied as one of the treatments for osteoporosis [5–7]. The bones after NaF therapy are more resistant to resorption by osteoclastic activity than before the therapy. When F^- substitutes for the hydroxyl ion (OH^-) group of bone mineral, solubility of the bone mineral decreases because fluoroapatite and partially F-substituted hydroxyapatite are less soluble than pure hydroxyapatite at pH 5–7 [8, 9].

Supplemental F^- upon implantation of bone substitute promoted bone formation around the implant and accelerated the recovery process in aged human. Supplemental F^- should be locally administered by slow release until bone regeneration. For the slow release of F^-, NaF is an inadequate material due to its highly soluble nature. On the other hand, type-B carbonate-containing hydroxyapatite (CHAP), in which carbonate groups partially substitute for phosphate groups, can be a suitable material as a host of F^- for slow release; F^- substitutes for the OH^- group in CHAPs. CHAP in a ceramic form is highly biocompatible and resorbable in bone tissue [10]. Therefore, a F-substituted CHAP can release F^- when CHAP is resorbed. In previous studies, several kinds of F-substituted CHAPs were prepared and examined, but only for their dissolution property in terms of Ca release [11–13].

The F-releasing property of CHAP depends on its morphology, particle size and chemical composition. Therefore, the purpose of the present study is to fabricate F-substituted CHAPs containing various amounts of F^- with nearly identical morphology and particle size. In previous studies, F-adsorbed hydroxyapatite and F-substituted CHAPs were prepared separately; F-adsorbed hydroxyapatite was prepared by immersing hydroxyapatite in a NaF solution [14–16] and F-substituted CHAPs were prepared by precipitation or by heating a mixture of CHAP with calcium fluoride in dry CO_2 at 900°C [12, 13, 17]. Adsorption of different amounts of F^- to a F-free CHAP powder followed by heating in dry CO_2 could lead to the formation of F-substituted CHAPs with analogous morphology and particle size regardless of their F^- content. In the present study, the F-releasing property of thus-prepared F-substituted CHAPs was examined by direct measurement of F^- concentration in a fluid in which the CHAPs were immersed, to reveal whether the CHAPs can sufficiently increase the F concentration in a fluid at the implanted site to promote bone formation.

2 Materials and methods

2.1 CHAP powders

Two kinds of F-free CHAP powders, C6.80F0.00 and C12.25F0.00, were obtained from STK Ceramics Laboratory (Japan). C6.80F0.00 and C12.25F0.00 powders contain 6.80 and 12.25 wt% CO_3^{2-}, respectively. These powders were prepared by the method reported previously [18]. The powders consisted of a CHAP phase without any other phases, which was revealed by X-ray diffractometry (patterns not shown). The particle sizes of the CHAP powders were adjusted to 20–38 μm before fluoride solution treatment.

2.2 Immersion in NaF solution

NaF solutions with F^- concentrations ranging from 45.2 F mg $l^{-1}$to 4525 F mg l^{-1} were prepared using reagent-grade NaF (Wako pure chemical, Japan).

The C6.80F0.00 and C12.25F0.00 CHAP powders were immersed in the NaF solutions at the powder-to-solution ratio of 5 mg ml^{-1} with stirring for 1 day at room temperature. After stirring, the NaF solution was filtered using a membrane with a pore size of 0.22 μm to separate the powder from the solution. The filtered powders were well washed with ultrapure water and freeze-dried for use as the as-immersed CHAP.

2.3 Heating of as-immersed CHAP powders

Ten milligrams of the as-immersed CHAP powder was heated at 700°C for 3 h in 500 ml min^{-1} CO_2 flow. The heating conditions of the as-immersed powders were determined on the basis of the results of thermogravimetric and X-ray diffractometric analyses of the as-immersed powders, as described in the results section (Figs. 1, 2).

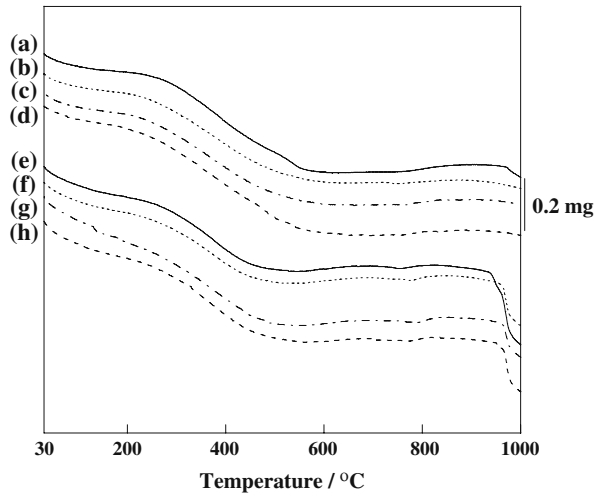

Fig. 1 TG curves of the as-immersed CHAP powders that are to be converted into (**a**) C5.81F0.48, (**b**) C4.99F0.30, (**c**) C4.95F0.19, (**d**) C4.81 F0.27, (**e**) C11.03F0.66, (**f**) C11.16F0.32, (**g**) C10.33F0.23, and (**h**) C10.45F0.17 by heated in 500 ml min^{-1} of CO_2 flow

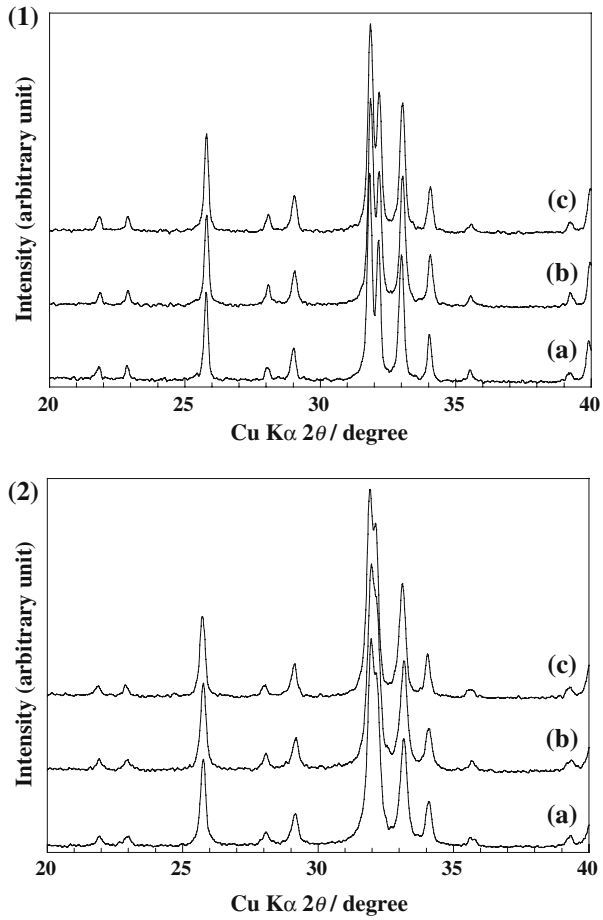

Fig. 2 (**1**) X-ray diffraction patterns of (**a**) C6.80F0.00, (**b**) the as-immersed CHAP powder that is to be converted into C4.95F0.19 and (**c**) the final product C4.95F0.19. (**2**) X-ray diffraction patterns of (**a**) C12.25F0.00, (**b**) the as-immersed CHAP powder that is to be converted into C10.33F0.23 and (**c**) the final product C10.33F0.23

2.4 Characterization

To determine the heating conditions for the as-immersed CHAP powders, simultaneous thermogravimetry and differential thermal analyses (TG-DTA) of the as-immersed powders were carried out in carbon dioxide flow using a TG-DTA apparatus (Thermo plus 2, Rigaku, Japan).

The as-immersed and the heated powders were examined using a powder X-ray diffractometer (Rint 2450; Rigaku, Japan). In addition, infrared spectra of these powders were also recorded by the KBr method using a Fourier transform infrared spectrometer (FTIR: FT/IR-350; JASCO, Japan) to prove that F^- substituted for OH^- groups in the CHAP structure, and to determine the CO_3^{2-} content in the powders quantitatively.

The determination of the CO_3^{2-} content in the powders was carried out as follows. Starting material, either C6.80F0.00 or C12.25F0.00, was mixed with pure hydroxyapatite powder to make a series of CHAP powders with CO_3^{2-} contents from 0.16 wt% to 12.25 wt% as standards for working curve. The standard powders were mixed with KBr powder of 50 times greater weight. Four milligrams of the mixed powders were pressed in a 3-mm-diameter micro-pellet holder at 1,400 MPa for 1 min. The pellet, together with the holder, was dried at 120°C for 1 h followed by pressing again under the same pressure. From the IR spectra of standard pellets, a working curve was drawn for the relationship between the CO_3^{2-} content and the intensity ratio of the carbonate mode at 1,417 cm^{-1} to the v_4 phosphate mode at 570 cm^{-1}. The working curve used was in the form of $y = ax^b + c$, where a, b and c were calculated by the non-linear least-squares method.

The F^- content in the F-substituted CHAP powder was chemically analyzed. A 100 mg sample of the heated powders were dissolved completely with 5 ml of 1 mol l^{-1} hydrochloric acid solution. The solutions were then diluted with ultrapure water to a volume of 100 ml in volumetric flasks. Concentrations of F^- in the solutions were measured using a fluoride ion meter (D-53, HORIBA, Japan). During the measurement, the pH of the solution was adjusted in the range from 4 to 10 by adding a 1 mol l^{-1} sodium hydroxide solution.

The morphology and microstructure of the heated CHAP powders were observed using a scanning electron microscope (SEM: JSM-6360, JEOL, Japan).

2.5 Fluoride ion release test

Forty milligrams of the heated CHAP powder was dispersed in 15 ml of physiological salt solution and allowed to stand at 37°C. After certain period of times, the physiological salt solution was filtered using a membrane with a pore size of 0.22 μm, and the pH and the F^- concentration of the solution were measured.

3 Results

The as-immersed powders were thermally stable even when the powders were heated at 700°C. When as-immersed CHAP powders with various amounts of F^- were heated up to 1,000°C in a carbon dioxide flow, the primary weight loss was mainly attributed to the desorption of water molecules up to 700°C (Fig. 1). The weight loss is also attributed to the partial release of carbon dioxide from the as-immersed powders. Indeed, the CO_3^{2-} content of the heated CHAP powders is lower than that of the starting materials (Table 1). The decrease in CO_3^{2-} content was at most

Table 1 CO_3^{2-} and F^- contents in the CHAP powders after heating at 700°C for 3 h in 500 ml min^{-1} of CO_2 flow

Product name	CO_3^{2-} content (wt%)	F^- content (wt%)	Starting material	F^- concentration of NaF solution/mg l^{-1}
C5.30F0.00	5.30	0.00	C6.80F0.00	–
C5.81F0.48	5.81	0.48	C6.80F0.00	4,525
C4.99F0.30	4.99	0.30	C6.80F0.00	452
C4.95F0.19	4.95	0.19	C6.80F0.00	226
C4.81F0.27	4.81	0.27	C6.80F0.00	45.2
C10.89F0.00	10.89	0.00	C12.25F0.00	–
C11.03F0.66	11.03	0.66	C12.25F0.00	4,525
C11.16F0.32	11.16	0.32	C12.25F0.00	452
C10.33F0.23	10.33	0.23	C12.25F0.00	226
C10.45F0.17	10.45	0.17	C12.25F0.00	45.2

31.4% for C6.80F0.00 and 16.7% for C12.25F0.00. Hence it was considered that carbon dioxide was partially released from the CHAP powders together with the desorption of water upon heating. No impurity phase was detected by XRD in the heated CHAPs, although the CO_3^{2-} content was decreased (Fig. 2). Additionally, weight loss was negligible during the heating of as-immersed powders at 700°C for 5 h (data not shown). The same results were obtained for all as-immersed CHAP powders regardless of the amount of F^-. Therefore, it was confirmed that the CHAPs did not decompose markedly upon heating at 700°C.

F^- was adsorbed on the CHAP powder only by immersion in NaF solution. Except for the case of C6.80F0.00 immersed in the 45.2 F mg l^{-1} NaF solution, the F^- content in the heated CHAPs increased with increasing initial F^- concentration of the NaF solution (Fig. 3). This result showed that the F^- content in the heated CHAP powder was controllable by changing the concentration of the NaF solution. Under the conditions of the present study, the F^- content in the heated CHAP powders reached 0.48 ± 0.00 wt% for products prepared from C6.80F0.00 and 0.66 ± 0.00 wt% for products prepared from C12.25F0.00 (Table 1). The F^- contents in the heated CHAPs were 16–22 times higher than that in normal adult human bones, 0.03 wt% [19].

F^- substituted for the OH^- group in the crystal structure of CHAP not by immersion in a NaF solution but by heating. Only one absorption band attributed to OH stretching was observed at 3573 cm^{-1} in IR spectra of as-immersed CHAPs immersed in the 4525 F mg l^{-1} NaF solution (Fig. 4, 1a, 2a). This result showed that the OH^- group in the CHAP structure was negligibly affected by immersion of the CHAP powder in the NaF solution, as supported by the results of XRD. In the case of heated CHAPs, the absorption band

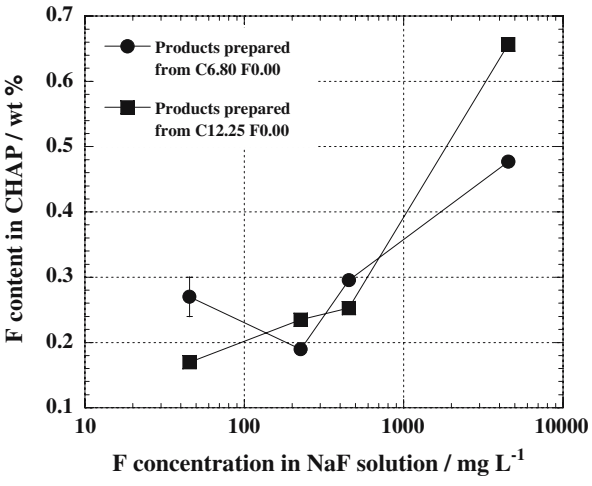

Fig. 3 The F^- content in the heated CHAP powders as a function of the concentration in NaF solution used in the preparation

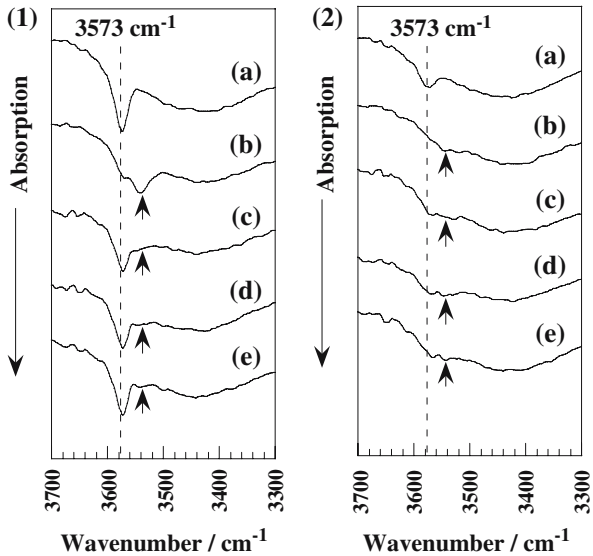

Fig. 4 (**1**) FT-IR spectra (**a**) of the as-immersed CHAP powder that is to be converted into C4.99F0.30 and of (**b**) C5.81F0.48, (**c**) C4.99F0.30, (**d**) C4.95F0.19, and (**e**) C4.81F0.27. (**2**) FT-IR spectra (**a**) of the as-immersed CHAP powder that is to be converted into C11.16F0.32 and of (**b**) C11.03F0.66, (**c**) C11.16F0.32, (**d**) C10.33F0.23, and (**e**) C10.45F0.17

attributed to OH stretching splits into two bands, a relatively strong one at 3573 cm^{-1} and a very weak one at 3541 cm^{-1} for products prepared from C6.80F0.00 and at 3546 cm^{-1} for products prepared from C12.25F0.00 (denoted by an arrow in Fig. 4). When the OH^- sites in the crystal structure of HAP are partially occupied by F^-, an additional OH stretching band attributed to a weak hydrogen bond between a OH^- group and its neighbor F^- appears in the lower frequency range from 3,545 cm^{-1} to 3,537 cm^{-1} [20]. Hence, the new weak OH stretching band in the spectrum of the heated CHAP was assigned to the stretching mode of the OH^- group bound to F^-. These results showed that F^- substituted for the OH^- group when as-immersed CHAP powder was heated at 700°C for 3 h. The same results were obtained for all the heated CHAP powders (Fig. 4). Moreover, the position of the (3 0 0) reflection shifted slightly towards a lower angle when the F^- content in the CHAP increased (Fig. 5). The reflection consisted of one peak that did not split. These results showed that F^- was homogeneously incorporated into the crystal structure of the heated CHAP. Therefore, the heated CHAPs were F-substituted CHAPs.

No appreciable differences in particle size or morphology were observed among the F-substituted CHAPs although the particles in C11.03F0.66 appeared to be slightly larger than other F-substituted CHAPs (Fig. 6). For all the F-substituted CHAPs, small particles less than 1 μm in size aggregated and formed secondary particles showing analogous morphology. Hence the morphology of the CHAP had little influence on its F-releasing property.

F^- was released from the F-substituted CHAPs in vitro. In the initial 3 h of the immersion of the F-substituted CHAPs, the F^- concentration in the physiological salt solution increased to the same level as in the body fluid of a normal adult human (Fig. 7). The F^- concentration maximized within 48 h under the present conditions. The maximum F^- concentrations were maintained at 39.03 ± 7.04 μg l^{-1} for C5.81F0.48 and 67.20 ± 4.81 μg l^{-1} for C11.03F0.66 for at least 192 h.

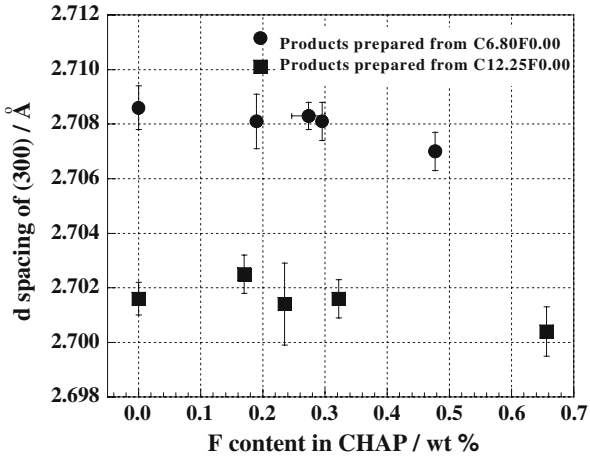

Fig. 5 Change in *d* spacing of (3 0 0) reflection of heated CHAP powder as a function of F^- content

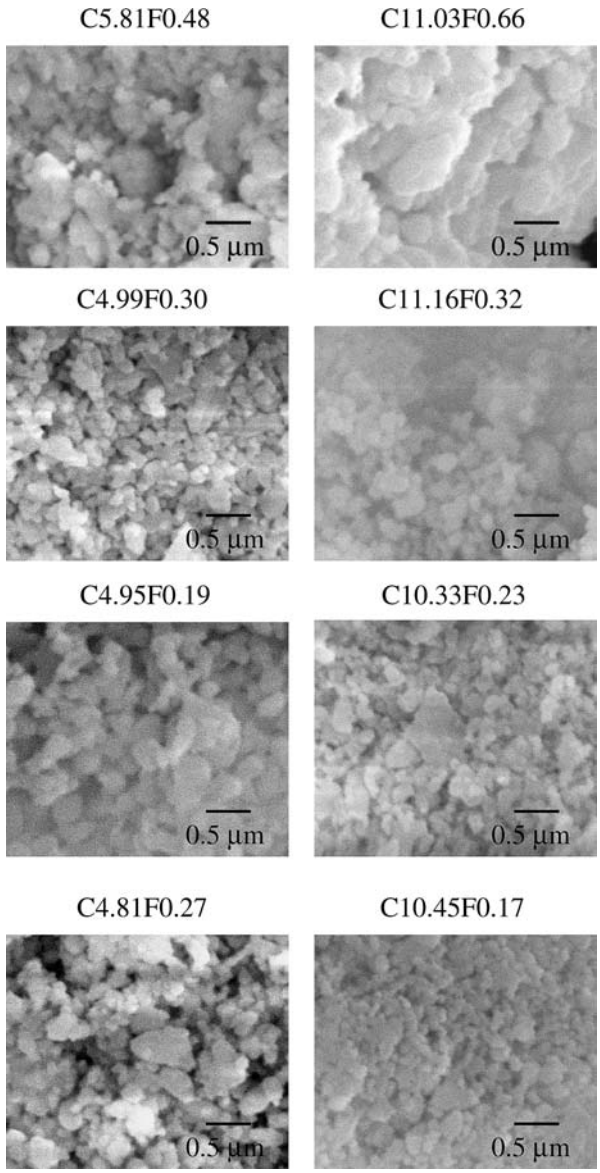

Fig. 6 SEM micrographs of the heated CHAP powders

4 Discussion

The F-substituted CHAP can promote bone formation in vivo because the F-substituted CHAP sufficiently increased the F^- concentration in the surrounding solution. F^- is one of the trace elements needed

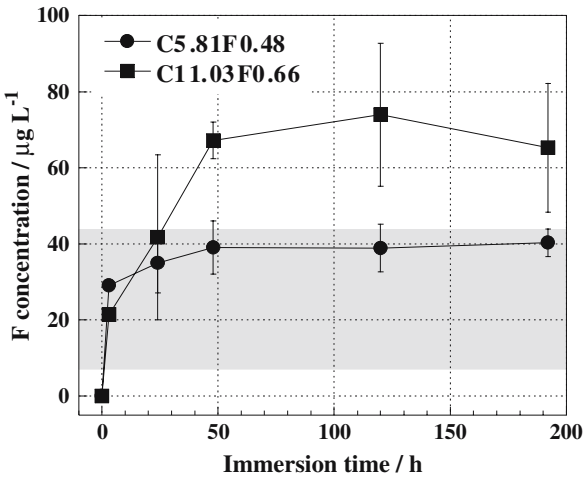

Fig. 7 F^- release curves of the F-substituted CHAP powders in a physiological salt solution. The shadowed area shows the mean F^- concentration in body fluid of normal adult human

for bone formation. Each trace element has an acceptable concentration range in which the element functions to maintain the rates and extent of the element-dependent metabolic reactions within normal limits. When a trace element concentration of the body fluid is as high as that near the upper limit of the acceptable range, it is expected that the element-dependent biological reactions will be enhanced compared with the normal state without evoking any harmful effect of the element. For example, zinc has been demonstrated to improve various osteoblastic cell activities at a concentration 3.0–5.5 times higher than the normal level, which led to the enhancement of new bone formation [21–24]. Intramascular injection of a high-zinc-content calcium phosphate powder increased bone mineral density in the vicinity of the injected site without evoking any harmful effect of the element [25]. In the present study, the heated CHAP powder increased F^- concentration in a physiological salt solution to 67.20 ± 4.81 μg l^{-1}, which is 1.5–9.3 times higher than that in body fluid of normal adult humans (7.2–43.7 μg l^{-1}) [1, 26, 27]. The F^- concentration reached a level near the estimated therapeutic window, that is, the dose range within which a medical substance is effective; the therapeutic window of serum F^- is believed to be 95–190 μg l^{-1} on the basis of limited evidence [1, 28]. The F^- concentration measured in the present study was much lower than the estimated toxic level of the serum F concentration of 950 μg l^{-1} [29, 30]. The F^- contents in the F-substituted CHAPs were also 16 times higher for C5.81F0.48 and 22 times higher for C11.03F0.66 than that in human bone, 0.03 wt% [19].

CHAP is of crucial importance as a carrier and slow releases of F^-. F^- is not incorporated into tricalcium phosphate, which has been demonstrated to be an adequate carrier for many essential trace elements. Although F^- decreases the solubility of apatite, F^- substituted for the OH^- group in a CHAP is released when the CHAP contains a sufficient amount of CO_3^{2-} to increase the extent of CHAP dissolution [11, 12]. There was a linear fall of the negative logarithm of the ion activity product with an increase in the number of CO_3^{2-} ions per unit cell of the apatite crystal structure [31]. Hence, the solubility of the F-substituted CHAP increases with increasing CO_3^{2-} content. In the present study, the CO_3^{2-} contents of the F-substituted CHAPs were 4.81–5.81 wt% in the products prepared from C6.80F0.00 and 10.45–11.03 wt% in the products prepared from C12.25F0.00 after the heat treatment. The CO_3^{2-} contents corresponded to or were higher than that of normal adult human bone, 5.8–7.4 wt% [19, 32]. This result showed that the solubility of the F-substituted CHAPs prepared in the present study was at a level similar to or higher than that of human bone. Hence, it is considered that the F-substituted CHAPs are resorbed by osteoclastic activity and release a greater amount of F^- than that in living bone tissue.

F^- was slowly released from the F-substituted CHAP since most of the F^- is present in its crystal structure. The result of IR spectrometry confirmed that F^- partially substituted for OH^- sites in the CHAP crystal structure. An increase in F-for-OH substitution in the apatite decreases the *a*-axis dimensions or causes the shift of the (3 0 0) reflection in the XRD pattern toward a higher angle [20, 33]. Hence, the slight increase in the (3 0 0) reflection angle with increasing F^- content in the present study also shows that F^- partially substitutes for the OH^- group in the CHAP since the carbonate contents were almost the same in all samples (Table 1).

These results suggest that F^- in the F-substituted CHAP powder is released only by dissolution of CHAP. The dissolution rate can be further reduced by sintering the heated CHAP powders into a ceramic body or granule by the method utilized for sintering carbonate apatite [10]. The present F-substituted CHAP has the advantage of slow F release over the sodium fluoride and sodium monofluorophosphate (Na_2PO_3F) which are highly soluble salts. Unlike those fluoride-containing reagents, the present F-substituted CHAP can also be used to supply calcium.

5 Conclusion

F-substituted CHAP powders with F-releasing ability were prepared. The F-substituted CHAP powder with

CO_3^{2-} and F^- contents of 11.03 and 0.66 wt%, respectively, slowly increases the F^- concentration in a physiological salt solution to a level higher than that in the body fluid of a normal adult human without exceeding the toxic threshold. Therefore, the F-substituted CHAP can promote bone formation. The present F-substituted CHAP has the advantage of slow F^- release over the sodium fluoride and sodium monofluorophosphate, which are highly soluble salts that cannot be sintered into ceramic bodies.

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