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Hydroxyapatite Formation on $CaSiO_3$ Ceramics in Simulated Body Fluid

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We have investigated the formation of hydroxyapatite (HAp) on $CaSiO_3$ ceramics in simulated body fluid (SBF) under various conditions. The effects of $CaSiO_3$ ceramics prepared by various methods and under different reaction conditions with SBF on the formation behavior of HAp in SBF are summarized in this review paper. To determine the effect of microstructure, $CaSiO_3$ ceramics were prepared by sintering powders co-precipitated with NaOH and NH_4OH and also by sintering glass powder. The microstructure consisting of $CaSiO_3$ crystalline grains with thick SiO_2-rich glassy grain boundaries was favorable for fast HAp formation. Release of Ca^{2+} and precipitation of amorphous SiO_2 by rapid dissolution of the crystalline grains are the main reasons for very fast HAp formation in SBF. The flow rate of SBF exerted a considerable influence on the microstructure of the product HAp layer. The rough layer surfaces formed by deposition of agglomerated HAp particles under static conditions changed to smooth surfaces resulting from preferred HAp orientation under slowly flowing condition and changed further to a terraced microstructure under moderately flowing condition. The HAp formation mechanisms on $CaSiO_3$ ceramics is discussed in the light of these experimental results.

Key words: $CaSiO_3$ ceramics, simulated body fluid, hydroxyapatite, bioactivity, soft solution process

1. INTRODUCTION

Since the discovery by Hench et al.[1] of materials that can bond with living bone through the *in vivo* formation of hydroxyapatite (HAp) in human body, many studies have been undertaken to develop similar functional materials, i.e. bioactive materials. Various bioactive materials such as glasses [1,2], sintered hydroxyapatite [3], glass ceramics [4,5], composite materials [6-8], etc, have been synthesized and developed for medical applications. The development of those bioactive materials has involved the use of simulated body fluid (SBF) which has ion concentrations similar to human blood plasma first proposed by Kokubo [4]. This has played a very important role because *in vitro* experiments using SBF are much easier for screening the bioactive properties of materials, even if some exceptions exist, than in *vivo* experiments. These SBF experiments have greatly progressed the studies on the mechanism of HAp formation. The proposed mechanism involves the dissolution of Ca^{2+} from the bioactive materials into the body fluid, with simultaneous formation of an amorphous SiO_2 on the material surfaces. The dissolution of Ca^{2+} increases the degree of supersaturation of the surrounding fluid with respect to HAp while Si-OH bonds on the surface of the amorphous SiO_2 provide favorable sites for HAp nucleation by enhancing the adsorption of Ca^{2+} and the subsequent adsorption of phosphate ions [9].

From a consideration of the chemical components involved in the above bioactive materials and also from the proposed mechanism of HAp formation in body fluid, we concluded that $CaSiO_3$ ceramics are candidate bioactive materials because they consist of CaO and SiO_2, and $CaSiO_3$ has a considerable solubility under weakly alkali pH conditions. $CaSiO_3$ ceramics prepared from coprecipitated powder using NaOH as precipitant showed HAp formation in SBF and the formation rate of HAp evaluated from the thickness provides to be much faster than the other reported bioactive materials [10,11]. We

therefore investigated the effects of powder preparation [12], sintering process [13], soaking conditions in SBF [14], proteins in SBF [15,16], etc on HAp formation in $CaSiO_3$ ceramics.

In this paper, we summarize the performance of $CaSiO_3$ ceramics prepared by various methods and under various reaction conditions on HAp formation in SBF.

2. EFFECTS OF $CaSiO_3$ POWDERS AND CERAMICS

The $CaSiO_3$ powders were prepared by three methods, i.e. coprecipitated powders using NaOH and NH_4OH as precipitants, and glass powder obtained by melting and quenching of the NH_4OH coprecipitated powder. The starting materials for preparation of the $CaSiO_3$ powders were $Si(OC_2H_5)_4$ (TEOS) and $Ca(NO_3)_2 4H_2O$. In the NaOH coprecipitation method, these reagents were dissolved in ethanol with concentrations of 0.2 mol/l and precipitated by adding 0.33 mol/l NaOH solution to the solution. The precipitated gel was filtered and washed to remove residual Na. With increasing amounts of washing water, however, the chemical composition of the gels became depleted in CaO. The washed gels were calcined at 600°C and further heated at 1000°C to obtain crystalline $CaSiO_3$ powder. In the NH_4OH coprecipitation method, gel was dried without washing and calcined at 600°C to remove the residual NH_4-salt, nitrate and ethanol. The calcined gels were then heated at 1000°C to obtain crystalline $CaSiO_3$ powder. The glass powder was obtained by melting the coprecipitated NH_4OH powder at 1600°C and quenching in water. The resultant powders were ground in a planetary ball mill under various conditions. The ground powders were pressed uniaxially to form green pellets (0.25 g) 1 cm in diameter and fired at 900° to 1400°C to produce ceramic samples with a range of bulk densities. Various data for these samples are listed in Table 1. The microstructures of the samples varied mainly according to the Ca/Si ratios and firing temperatures. The samples with Ca/Si<1 fired at 1400°C consisted of elongated pesudowollastonite (PW: the high temperature phase of $CaSiO_3$) grains with glassy grain boundaries due to liquid phase sintering. The sample with Ca/Si=1 contained PW grains with little grain boundary phase. The glass powder sample fired at 1400°C showed a similar microstructure to the coprecipitated powders but at 900°C a different microstructure is seen. This GC1 sample had the highest bulk density with a microstructure consisting of very fine wollastonite (the low temperature phase of $CaSiO_3$) grains dispersed in a glassy matrix.

Table 1 Various data for the $CaSiO_3$ samples

	NaOH precipitated			NH_4OH precipitated				Glass	
	NA1	NA2	NA3	NH1	NH2	NH3	NH4	GC1	GC2
CaO (mass%)	41.7	46.2	45.8	48.3	45.9	45.9	45.9	45.9	45.9
SiO_2 (mass%)	58.3	53.4	53.0	51.7	54.1	54.1	54.1	54.1	54.1
Na_2O (mass%)	0.02	0.4	1.2	-	-	-	-	-	-
CaO/SiO_2	0.76	0.91	0.93	1.00	0.91	0.91	0.91	0.91	0.91
Firing temp.(°C)	1400	1400	1400	1400	1400	1400	1400	900	1400
Cryst. phase*	PW,C	PW,C	PW,N	PW	PW,C	PW,C	PW,C	W	PW,C
Bulk density (g/cm^3)	2.44	2.60	2.35	2.20	2.41	2.14	0.28	2.71	2.57
Water absorption(%)	0.7	0.2	1.0	10.5	1.0	7.9	270	0.5	0.4
Grain size (μm)	7.6	10	28.5	9.3	-	-	-	-	-
HAp formation	1day	1	5	1	3	3	No	No	3
Thickness(5 days)	60 μm	60	30	8	30	30	0	0	30

* PW: pseudowollastonite($CaSiO_3$), C: cristobalite(SiO_2), N: $Na_4Ca_8Si_5O_{20}$, W: wollastonite($CaSiO_3$).

Soaking experiments were preformed using C-SBF [4,5] at 36.5°C for various times and solid/solution (S/S) ratios ranging from 1 to 20 mg/ml. As listed in Table 1, the formation of HAp varies greatly among the samples (reacted at S/S≅10 mg/ml). HAp formation was observed within 1 day in samples NA1, NA2 and NH1 while it was not observed in samples NH4 and GC1 after soaking for up to 30 days. The large difference in HAp formation is

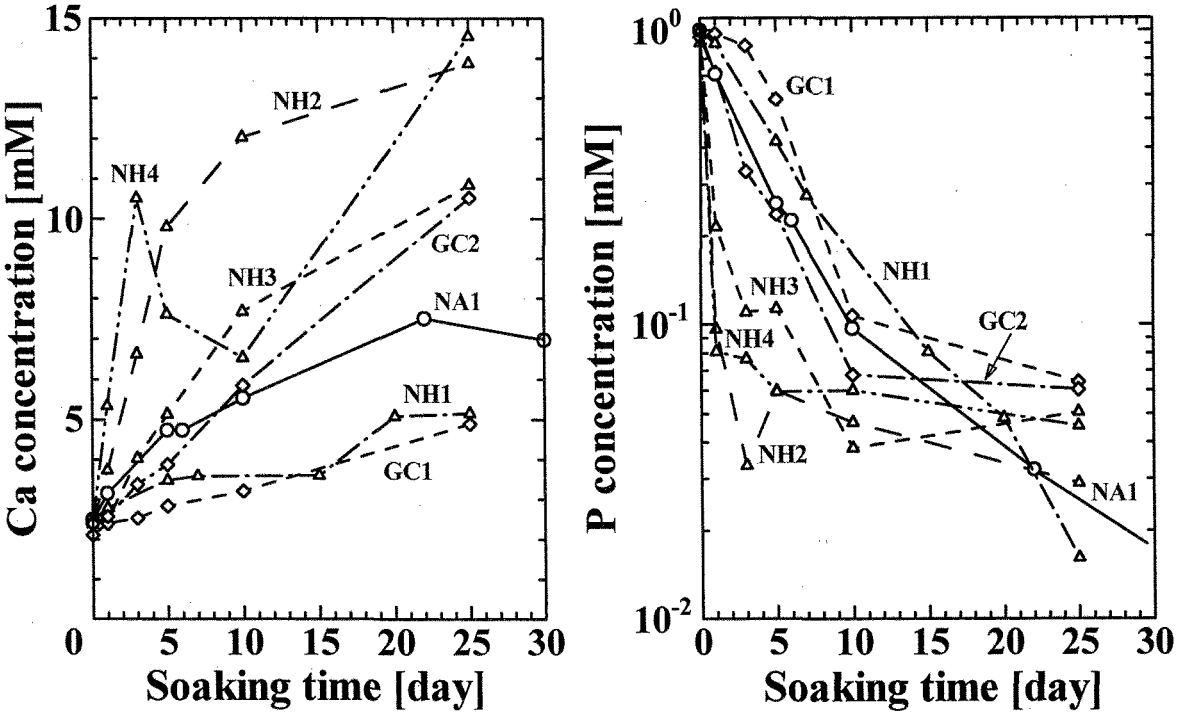

Fig. 1 Changes of Ca and P concentrations by reactions of various samples with SBF.

thought to relate to differences in the solution behavior of the soaked samples in SBF and also the adsorption by the soaked samples of P from the SBF. Fig. 1 shows changes of the Ca and P components in the SBF solutions in which the various samples were soaked as a function of soaking time. Increased Ca concentration in the reacted SBF occurs by dissolution of the soaked sample while a decrease in the P concentration is due to adsorption by the soaked sample. The rates of Ca concentration increase are fastest in sample NH4 only up to 5 days soaking time but slowest in sample GC1. Also, changes in P concentration are fastest in sample NH4 but slowest in sample GC1. Neither of these samples showed HAp formation but sample NH4 showed formation of calcite, corresponding to a lowering of the Ca concentration after 3 days soaking while sample GC1 showed no crystalline phase formation. Since the formation rate of HAp was found to be fastest in sample NA1, moderate rates of increase of Ca and decrease of P concentrations in the reacted SBF are thought to provide more suitable conditions for HAp formation. It is interesting that the faster adsorption of P in sample NH4 compared with NA1 does not result in HAp formation as in NA1. The concentration changes of Ca, P and Si components in reacted SBF were used to evaluate the

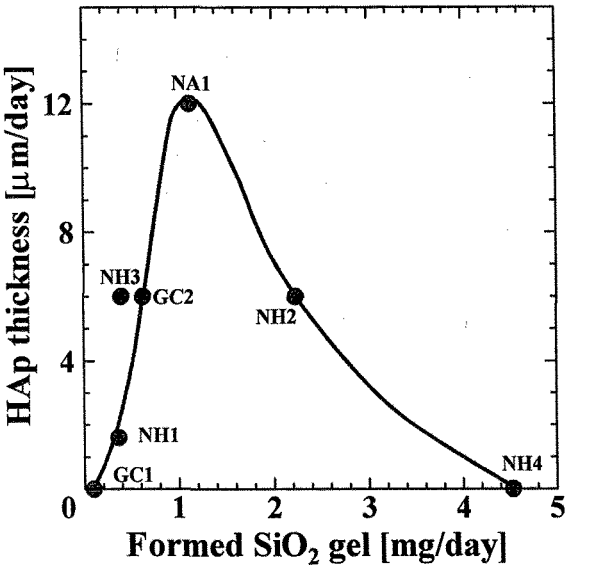

Fig. 2 Relationship between formation rate of amorphous silica by reaction of $CaSiO_3$ ceramics and SBF and growth rate of HAp layer thickness.

amount of precipitated amorphous silica by assuming congruent dissolution of the soaked samples and formation of calcium phosphate. Fig. 2 shows the relationship between the formation rate of amorphous silica and the growth rate of the HAp layer in the various $CaSiO_3$ ceramics listed in Table 1. The growth rate of the

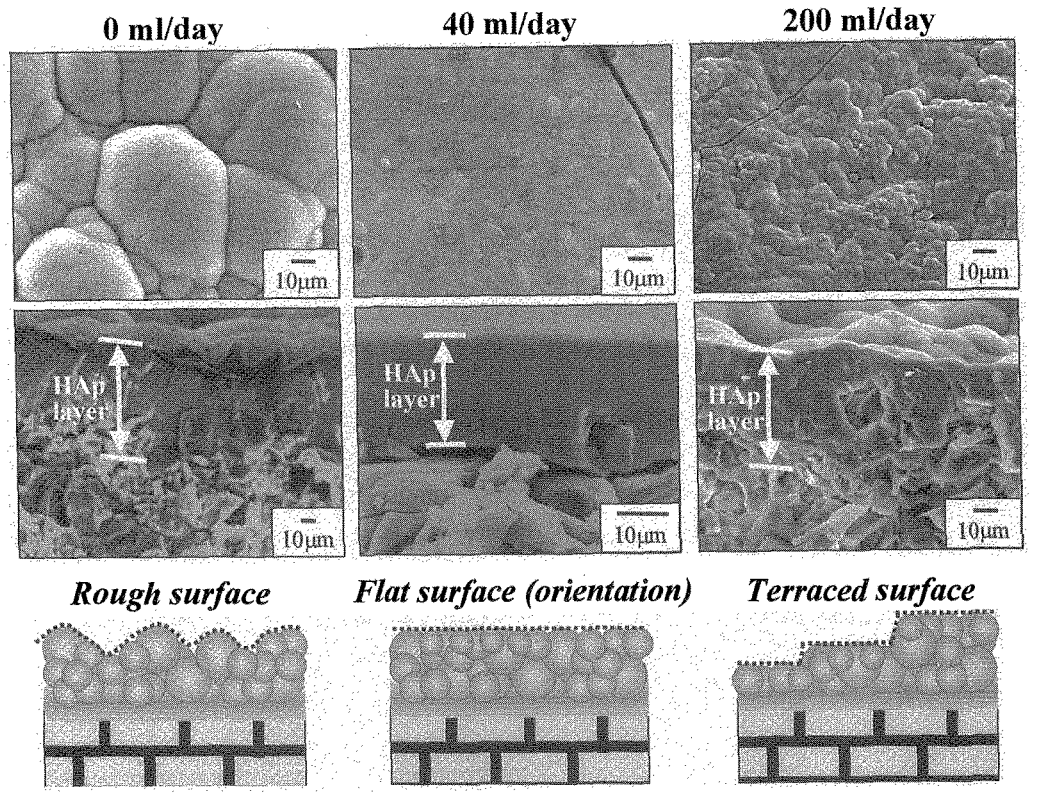

Fig. 3 SEM micrographs and schematically illustrated models of formed HAp layers under various flowing rates.

HAp layer shows a steep increase with increasing formation rate of amorphous silica up to about 1 mg/day, but decreases as the formation rate increases further. This result clearly shows that HAp formation occurs only after a considerable amount of amorphous silica has formed on the surfaces of the soaked samples. The decreasing HAp growth rate at higher formation rates of amorphous silica corresponds to a very steep adsorption rate of the P component from the reacted SBF, which causes no HAp formation but the formation of calcite resulting from the increased pH. This supports the proposed mechanism of HAp formation in SBF [9]. From the results obtained, we consider that the surface formation of amorphous silica by dissolution-precipitation through reaction with SBF is important for HAp formation and that a moderate formation rate is important to promote a high growth rate of the HAp layer in SBF. This can be achieved by controlling the porosity and microstructure of the $CaSiO_3$ ceramic.

3. EFFECT OF REACTION CONDITIONS OF $CaSiO_3$ CERAMICS WITH SBF

In the case of a living body, the blood plasma is continually flowing. It is therefore interesting and important to obtain information regarding HAp formation in flowing SBF rather than under static conditions. We have investigated the effect of circulating and flowing SBF on HAp formation in our $CaSiO_3$ ceramics. HAp formation on the $CaSiO_3$ ceramics was confirmed even under flowing SBF conditions [14]. Some differences are, however, observed in the growth rate of the HAp layer and also in the microstructure upon changing the reaction conditions with SBF. In circulating conditions, the growth rate of the HAp layer was lower than under static and slow flowing conditions. This may be due to the very fast circulating speed of the SBF. On the other hand, the microstructure of the HAp layer obtained under slow flowing conditions was different, showing preferred orientation to (00*l*) of the platy crystals of tiny HAp.

We further examined the effect of the SBF flow rate on the microstructures of the resulting HAp by reacting at flow rates of 0, 40 and 200 ml/day. SEM micrographs of the surfaces and cross-sections of the samples soaked for 25 days are shown in Fig. 3 together with schematic

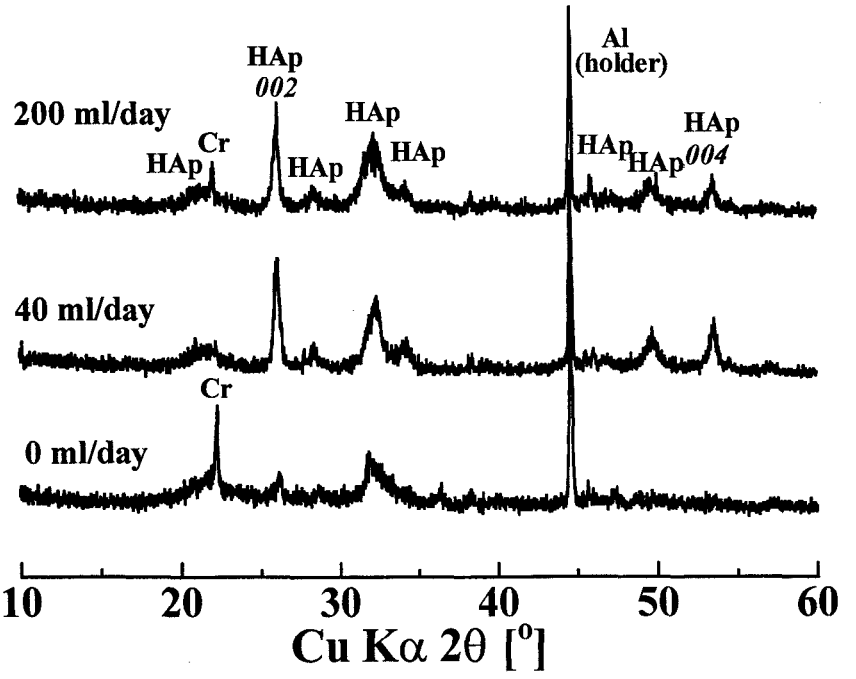

Fig. 4 XRD patterns of $CaSiO_3$ ceramics soaked with SBF under various flowing rates.

diagrams of the microstructures of the product HAp layers. Under static conditions (0 ml/day), the HAp layer consists of spherical particles several tens μm in size, formed by agglomeration of very fine platy HAp particles. By contrast, the microstructure shows flat and smooth surfaces in the sample soaked under slow SBF flow rates (40 ml/day). This microstructure further changes to a terraced surface on soaking at a moderate flow rate (200 ml/day) though the surfaces are not as smooth as those observed at slow SBF flow rates. The XRD patterns of these three samples are shown in Fig. 4. The samples soaked under flowing SBF conditions clearly show strengthening X-ray peak intensities of the (002) and (004) reflections in these XRD patterns. Thus, preferred orientation to (00*l*) is found to be similar in these two samples despite the difference in their microstructures. Flowing SBF causes orientation of HAp crystals in the HAp layers, giving different microstructures. These results may indicate the expected microstructure of HAp formed on $CaSiO_3$ ceramics *in vivo*.

4. SUMMARY

The formation behavior of hydroxyapatite (HAp) on $CaSiO_3$ ceramics in simulated body fluid (SBF) under various conditions was reviewed in this paper.

The HAp formation was found to be strongly influenced by differences in microstructures of $CaSiO_3$ ceramics and they depend on dissolution rate of $CaSiO_3$ ceramics in SBF. The microstructures of $CaSiO_3$ ceramics were dependent on preparation methods and chemical compositions of starting powders, processing conditions of $CaSiO_3$ ceramics, etc. For fast formation of HAp in SBF, it is necessary form amorphous SiO_2 layer on the surface of $CaSiO_3$ ceramics by its partial dissolution and selective precipitation of Si component. The formation rate of amorphous SiO_2 showed a certain correlation with the growth rate of HAp. This means that there is an optimum formation rate of amorphous SiO_2, resulting from a favorable dissolution rate of $CaSiO_3$ ceramics. If the dissolution rate is too fast, no HAp formation but formation of calcite occurred due to rapid adsorption of P component in SBF by the $CaSiO_3$ ceramics.

The flow rate of SBF was found to exert considerable influence on the microstructure of the product HAp layer. The rough layer surfaces formed by deposition of agglomerated HAp particles under static conditions changed to smooth and flat surfaces resulting from preferred HAp orientation to (00*l*) under slowly flowing condition and changed further to a terraced microstructure under moderately flowing condition. This type of orientated HAp microstructure can be suggested to be formed *in vivo* conditions to as considering continual flowing of human blood plasma.

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6. REFERENCES

[1] L.L.Hench, R.J.Splinter, W.C.Allen and T.K.Greenlee, J. Biomed. Mat. Res. Symp. **2**, 117(1971).

[2] L.L.Hench, J. Am. Ceram. Soc. **74**, 1487(1991).

[3] H.Aoki, Medical Applications of Hydroxyapatite. Ishiyaku Euro America, Tokyo, 1994.

[4] T.Kokubo, M.Shigematsu, Y.Nagashima, M.Tashiro, T.Nakamura, T.Yamamuro and S.Higashi, Bull. Inst. Chem. Res. **60**, 260(1982).

[5] T.Kokubo, J. Non-Cryst. Solids, **120**, 138(1990).

[6] P.Ducheyne, W.V.Raemdonck, J.C.Heughebaert and M.Heughebaert, Biomaterials **7**, 97(1986).

[7] J.D.Santos, L.J.Jha and F.J.Monteiro, J. Mat. Sci. Mat. Med. **7**, 181(1996).

[8] S.Falaize, S.Radin and P.Ducheyne, J. Am. Ceram. Soc. **82**, 969(1999).

[9] T.Kokubo, Biomaterials **12**, 155(1991).

[10] P.Siriphannon, S.Hayashi, A.Yasumori and K.Okada, J. Mat. Res. **14**, 529(1999).

[11] P.Siriphannon, Y.Kameshima, A.Yasumori, K.Okada and S.Hayashi, J. Biomed. Mat. Res. **52**, 30(2000).

[12] P.Siriphannon, Y.Kameshima, A.Yasumori, K.Okada and S.Hayashi, Bioceramics. **12**, 145(1999).

[13] Y.Iimori, Y.Kameshima, A.Yasumori and K.Okada, Key Eng. Mat.(2003) in press.

[14] P.Siriphannon, Y.Kameshima, A.Yasumori, K.Okada and S.Hayashi, J. Biomed. Mat. Res. **60**, 175(2002).

[15] K.Okada, P.Siriphannon, Y.Kameshima, A.Yasumori, and S.Hayashi, Key Eng. Mat. **206-213**, 1551(2002).

[16] K.Takakuwa, Y.Kameshima, A.Nakajima and K.Okada, Abstracts of 19th Ceramic Conference of Kanto Branch, p. 46(2003)

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