In Vitro Reaction for Calcium Phosphate Ceramics

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(Received February 24, 2000)

Hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) are bio-compatible materials with bones and teeth. HA
has been widely applied as bone substitutes because of chemical stability *in vivo*, while β-TCP has higher resorb-
ability than HA when the material is implanted in a bone defect. In the present study, both HA and β-TCP
porous ceramics were soaked in the simulated body fluid in order to investigate the reaction between the mate-
rials and the fluid. After the soaking test, carbonate hydroxyapatite was formed on HA surface at 1 week, and
then the amount of precipitates increased with increasing period of the soaking test. While β-TCP was not dis-
solved in the fluid, carbonate hydroxyapatite was also formed on β-TCP surface after 12 weeks, and the amount
of precipitates was less than that on HA. *In vitro* behavior of HA was similar to that *in vivo*, but *in vitro* behav-
ior of β-TCP was not similar to that *in vivo*.

Key words: Bioceramics, Hydroxyapatite, β-TCP, Calcium phosphate

I. Introduction

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$; HA) is inorganic prin-
cipal constituent of hard tissue such as bones or teeth.
There are reports that HA is the most bio-compatible mate-
rials because of chemical stability *in vivo* and the new bone
is formed in direct contact with the HA surface.[1,2] While β-
tricalcium phosphate (β-$Ca_3(PO_4)_2$; β-TCP) is also the bio-
compatible material with bones and teeth, however, β-TCP
show the different behavior in comparison with HA *in vivo*.
When they are implanted into bones, the resorption of HA is
hardly noticed. In the contrast, β-TCP has higher resorb-
ability than HA.[3] The authors have reported quantitative
analysis of the new bone formation for HA and β-TCP in the
distal femurs of rabbits and the resorption of HA and β-TCP
in the bones and muscles of rabbits.[4-8] In the present study,
in order to appreciate inorganic chemical reaction *in vivo*,
HA and β-TCP were analyzed after soaking test in the simu-
lated body fluid with the mineral constituent of human
blood plasma without organic matter.

II. Experimental Method

2.1. Materials

The porous HA used in this study was prepared by sinter-
ing at 900°C, provided by Mitsubishi Materials Co., Ltd.,
Japan. This porous HA is the bone substitute material
which has been already used for clinical applications. The
Ca/P molar ratio of this HA is 1.67 with stoichiometric com-
position of HA. The content of impurities is listed in Table 1.
The porous β-TCP used in this study was prepared from the
HA. In order to obtain fully crystallized pure β-TCP with

the same pore structure as the HA, the porous HA was
soaked in diammonium hydrogen phosphate (($NH_4)_2HPO_4$)
aqueous solution and then sintered at 900°C for 3 h.[4]
Porous materials of HA and β-TCP had the cylindrical
shape of 5 mm in diameter and 5 mm in length. Both mate-
rials had the porosity of 60±5%, pore size from 150 to 400
μm with interconnecting pore structure.

2.2. Soaking test with simulated body fluid

The ion concentrations and pH of the simulated body fluid
are almost equal to those of the human blood plasma, i. e.,
Na^+ 142.0, K^+ 5.0, Mg^{2+} 1.5, Ca^{2+} 2.5, Cl^- 148.8, HCO_3^- 4.2
and HPO_4^{2-} 1.0 mM and buffered at pH 7.25 with the
trishydroxymethylaminomethane. The solution was pre-
pared according to previous method by Kokubo *et al.*[9] This
solution was used to investigate the chemical reaction
between materials and the solution.

The materials were soaked in 100 cm³ of the simulated
body fluid at 37°C for 1-24 weeks. When the soaking test
was carried out over 4 weeks, the solution was exchanged
every for 4 weeks. After the soaking test, the specimens
were taken out from the solutions, washed with very dilute
ammonia solution, and dried at 105°C.

2.3. Analysis

The weight of samples was measured before and after

Table 1. Impurity Content of the Porous Hydroxyapatite used
in the Present Study

						(mass %)
MgO	SiO_2	Al_2O_3	Fe_2O_3	SO_3	Na_2O	K_2O
0.270	<0.001	0.020	0.010	0.010	<0.001	<0.001

soaking test. The produced phases were identified by powder X-ray diffractometry with graphite monochromated CuKα radiation, operating at 40 kV and 20 mA (XRD; Mac Science MXP³, Japan). In order to analyze the deposit on samples, the precise angle of 2θ and full width of half maximum intensity (FWHM) were measured by accurate XRD measurement with using the internal standard of Si (99.99% pure; Kinzoku Kagaku Co. Ltd., Japan). In addition, the samples after soaking test were subjected to Fourier transform infrared spectroscopy (FT-IR; Perkin-Elmer Spectrum 2000, USA), and thermogravimetry and differential thermal analysis were carried out at a heating rate of 10°C/min (TG-DTA; Seiko Instruments TG/DTA32, Japan). The morphology of samples was observed by scanning electron microscopy (SEM; JEOL JSM25S, Japan).

III. Results and Discussion

3.1. Soaking test with simulated body fluid for HA

Weight change of porous HA after soaking test with the simulated body fluid is shown in Fig. 1. The weight of porous HA increased obviously at 1 week, and then the weight increased linearly with soaked period up to 12 weeks, and slightly increased between 12 and 24 weeks. The weight of the materials was about 15 mass% increase at 24 weeks.

No other phases than hydroxyapatite were revealed by XRD in any products studied. The line broadening was observed for the samples after soaking test over 1 week. Full width at harf maximum of intensity for the HA line of 2θ=31.8° increased with increasing soaking period (Fig. 2). The weight increase mentioned above and the line broadening of XRD mean that the precipitation on the porous HA must be hydroxyapatite with small crystallite size and/or with defective lattice structure. The ions of Ca^{2+}, PO_4^{3-} and OH^- of the solution are supersaturated with respect to the hydroxyapatite, therefore growth of the hydroxyapatite was achieved on the porous HA.

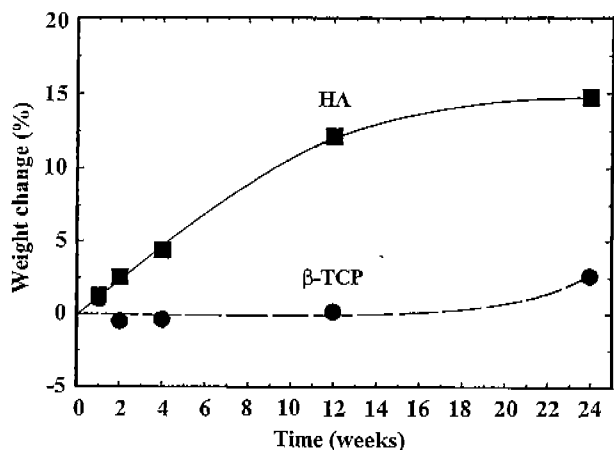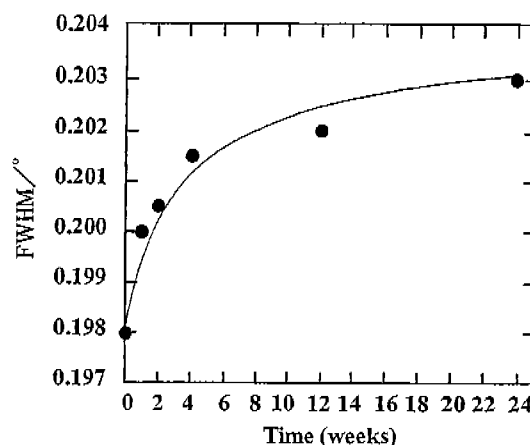Fig. 3 shows infrared spectra of HA after soaking test with

Fig. 2. Full width of half maximum intensity (FWHM) of XRD for HA after soaking test with simulated body fluid.
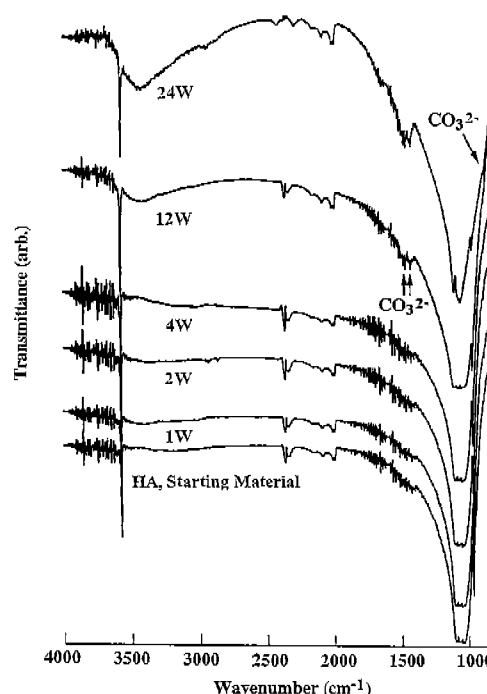

Fig. 3. Infrared spectra of HA after soaking test for 1, 2, 4, 12 and 24 weeks.

the simulated body fluid. The main spectra were assigned as the typical spectra of hydroxyapatite. In the spectra of specimens after soaking at 4 and 12 weeks, carbonate ions in hydroxyapatite were recognized at 1455, 1415 and 880 cm^{-1}, and they were more clearly observed at 24 weeks. According to the previous investigations for carbonate hydroxyapatite,[4,10] the apatite formed on the starting HA must be OH^- and PO_4^{3-} carbonated hydroxyapatite. Thus the increase in weight of HA after soaking test was due to the carbonate-containing hydroxyapatite.

Thermogravimetric curves of the porous HA before and after soaking test are shown in Fig. 4. The weight of samples after soaking test decreased more than that before
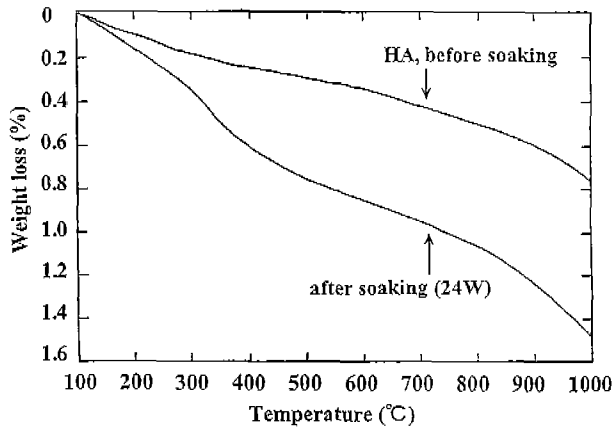

Fig. 1. Weight change of porous HA and porous β-TCP after soaking test with simulated body fluid.

Fig. 4. TG curves of HA before soaking test and after soaking test for 24 weeks.

Fig. 5. Infrared spectra of HA; (a) after soaking test for 24 weeks, and (b) after heating of sample (a) at 1000°C in air.

soaking test. From FT-IR measurement, the spectra of CO_3^{2-} in the sample at 24 weeks was disappeared by heating at about 1000°C (Fig. 5) because of elimination of CO_2 from carbonated hydroxyapatite. This result was corresponded to the report of Monma *et al.*[11] According to XRD, β-TCP was recognized from the heated sample after the thermal analysis. It is known that stoichiometric hydroxyapatite with Ca/P molar ratio of 1.67 and β-TCP with Ca/P=1.50 are formed by decomposition of calcium deficient hydroxyapatite with Ca/P molar ratio less than 1.67 with dehydration on heating above 800°C.[12)-11)] Porous HA of

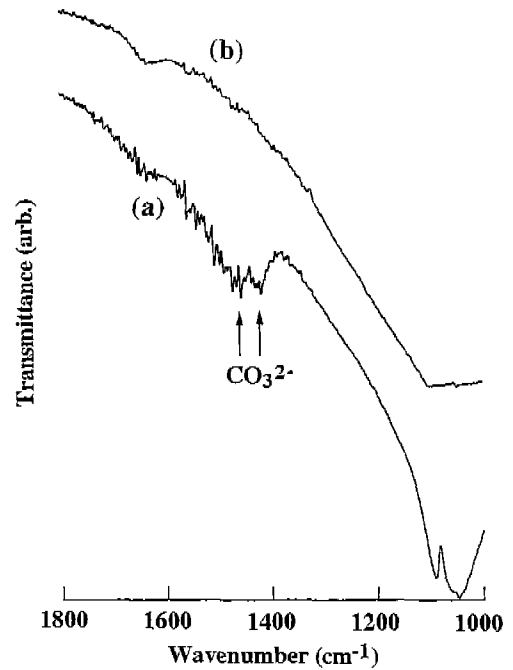starting material was stoichiometric hydroxyapatite, so it was thought that precipitates on the surface of porous HA after soaking test were calcium deficient hydroxyapatite. From these results, it was considered that carbonate-con-
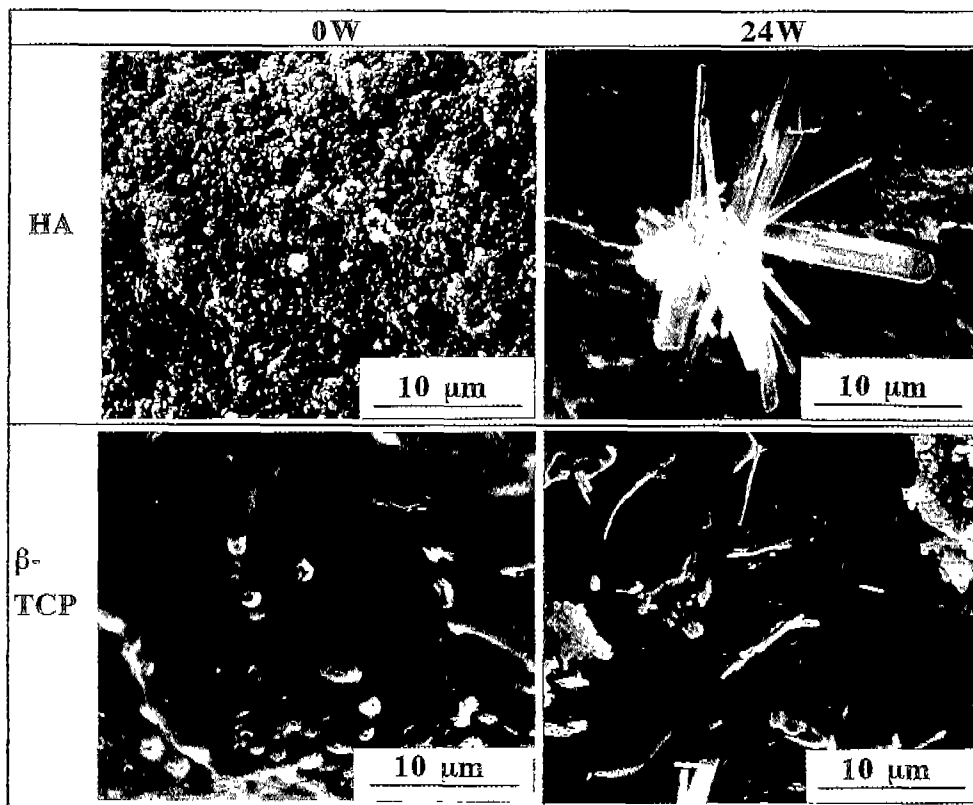

Fig. 6. SEM photographs of porous HA and porous β-TCP before soaking test and after soaking test for 24 weeks.

taining calcium deficient hydroxyapatite was precipitated on porous HA in the simulated body fluid.

After soaking test, formed apatite on the surface of porous HA was observed by SEM. At 2 weeks, rod-like particles were observed on some part of the surfaces and they were more clearly observed at 24 weeks (Fig. 6). The gradual increase of the weight after soaking in the simulated body fluid was due to the carbonate-containing hydroxyapatite precipitation.

When porous HA was implanted in muscles of rabbits, the weight of samples increased because of formation of carbonate-containing hydroxyapatite.[4] Therefore, the results from the experiment *in vitro* using simulated body fluid was similar to the results from implantation tests in the muscle.

3.2. Soaking test with simulated body fluid for β-TCP

The weight of porous β-TCP was scarcely changed up to 12 weeks and slightly increased at 24 weeks (Fig. 1). X-ray diffraction patterns were a little affected by soaking in the simulated body fluid (Fig. 7), and a little changes of infrared spectra of the samples after soaking test were observed. The spectrum of hydroxyl stretch was confirmed faintly at about 3570 cm^{-1}, and the spectrum of carbonate ions was observed slightly in the region of 1400 to 1550 cm^{-1}, because carbonate ions occupied two different sites, that is, PO_4 site and OH site in hydroxyapatite.[15,16] It was suggested that carbonate-containing hydroxyapatite was deposited on the surface of porous β-TCP. At 12 weeks, some fine rod-like particles were observed by SEM on the surfaces of porous β-TCP and they were more clearly observed at 24 weeks (Fig. 6). The morphology of particles on β-TCP was extremely similar to the carbonate-containing hydroxyapatite precipitated on HA. Thus these results indicate that the carbonate-containing hydroxyapatite is deposited on the surface of

porous β-TCP, as well as porous HA. Precipitation of carbonate-containing hydroxyapatite on porous β-TCP was later than that on porous HA, because porous HA must be the nuclear for carbonate-containing hydroxyapatite but β-TCP must not be the apatite nuclear.

When porous β-TCP was implanted in muscles of rabbits, resorption of the material observed.[3-8] In this study, however, β-TCP not dissolved by chemical reaction with the solution. On the contrary, the carbonate-containing hydroxyapatite is deposited on the surface of porous β-TCP, as well as porous HA. From these result, it was thought that β-TCP was bio-degraded by the lowering in pH value by a function of cells such as osteoclast and macrophage *in vivo*.

IV. Summary

Porous ceramics of HA and β-TCP were soaked in the simulated body fluid with pH 7.25 at 37°C for 1-24 weeks. On the surface of porous HA, the rod-like carbonate-containing calcium deficient hydroxyapatite precipitated at 1 week and the amount of precipitates increased with increasing soaking period. This result was similar to that from implantation tests in muscles of rabbits. While β-TCP was not dissolved in the solution and the rod-like carbonate-containing hydroxyapatite also precipitated on the surface of porous β-TCP as well as porous HA. The amount of precipitates on porous β-TCP was less than that on porous HA. The soaking test with simulated body fluid for β-TCP was not similar to the results from implantation tests in muscles of rabbits.

Acknowledgement

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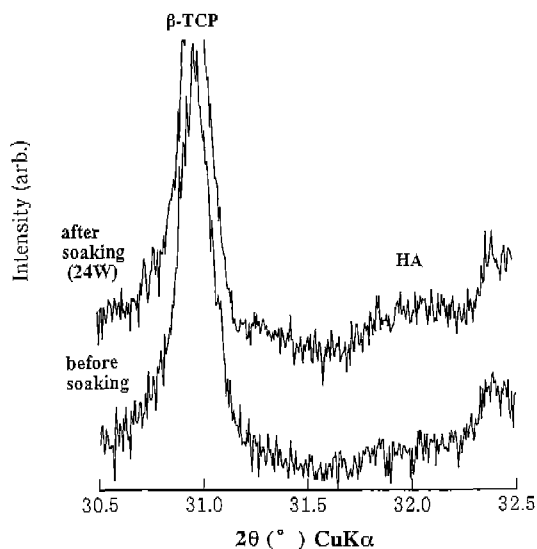**Fig. 7.** XRD patterns of β-TCP after soaking test for 24 weeks.

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