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In vitro estimation of calcium phosphate with pH-controlled simulated body fluid

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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 resorbability 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 pH controlled simulated body fluid with the value from 4.0 to 6.0 in order to appreciate resorption *in vivo*. After the soaking test, the porous HA hardly dissolved in the simulated body fluid at pH 5.2 and slightly dissolved in the simulated body fluid at pH 5.0. On the other hand, the porous β-TCP considerably dissolved in the simulated body fluid at pH 5.2 and slightly dissolved at pH 6.0. From the calculation of ion activity products (K_{IAP}) of simulated body fluid for HA and β-TCP, it was estimated that HA and β-TCP were dissolved less than pH 5.1 and pH 6.0, respectively. Thus, the results of soaking test corresponded to the results of calculation of K_{IAP}. These facts can imply that the difference of behavior of HA and β-TCP *in vivo* can explain the difference of solubility of HA and β-TCP.

Key word: Hydroxyapatite, β-tricalcium phosphate, pH, dissolution, solubility

1. INTRODUCTION

Hydroxyapatite($Ca_{10}(PO_4)_6(OH)_2$;HA)is inorganic principal constituent of hard tissue such as bones or teeth. There are reports that HA is the most bio-compatible materials 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 resorbability than HA[3)]. The results in past research *in vivo*, however, are confused quantitatively, because characters of used materials and experimental condition were not controlled. Ioku and Kurosawa et al. have reported quantitative analysis of the new bone formation for HA and β-TCP in the distal femurs of rabbits, the resorption of HA and β-TCP in the bones and muscles of rabbits and the precipitate of carbonated hydroxyapatite on the surface of HA[4)-8)]. In addition, Toya and Ioku et al. have reported precipitate of bone-like apatite on the surface of HA and β-TCP in the simulated body fluid with pH 7.25[9)-10)]

In the present study, in order to appreciate resorption *in vivo*, HA and β-TCP were analyzed after soaking test in the pH-controlled simulated body fluid with the mineral constituent of human blood plasma without organic matter.

2. EXPERIMENTS

The porous HA used in this study was prepared

by sintering at 900℃, 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 composition of HA. 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 structure as the HA, the porous HA was soaked in diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) aqueous solution and then sintered at 900 ℃ for 3 h[4]. Porous materials of HA and β-TCP had the cylindrical shape of 5mm in diameter and 5mm in length. Both materials had the porosity of 60±5%, diameter of macro-pore: 150-400 μm and diameter of micro-pore: 0.1-0.5 μm.

The ion concentrations of the simulated body fluid are similar to those of the human blood plasma, i. e., Na^+ 142.0, K^+ 5.0, Mg^{2+} 1.5, Ca^{2+} 2.5 and HPO_4^{2-} 1.0 mM. The pH of the solution was adjusted to the value from 4.0 to 6.0 with 4N HCl. The trishydroxymethylaminomethane was added to the simulated body fluid to compared with past soaking test with pH 7.25 [9), 10)]. The solution was prepared referring to previous method by Kokubo et al [11)]. This solution was used to investigate the chemical reaction between materials and the solution.

In this study, all of soaking test was carried out with a flowing solution. The velocity of solution for sample room was 3.6×10^{-2} mm·min^{-1} postulated the velocity of tissular flow. The materials which was admitted into net made from sature were soaked in the simulated body fluid at 37 ℃ for 1 day to 4 weeks. After the soaking test, the specimens were taken out from the solutions, washed with very dilute ammonia solution, and dried at 105 ℃.

The weight of samples was measured before and after soaking test. The samples after soaking test were appreciated by powder X-ray diffractometry with graphite monochromated CuK α radiation, operating at 40kV and 20 mA (XRD; Mac Science MXP^3, Japan) and Fourier transform infrared spectroscopy (FT-IR; Perkin-Elmer Spectrum 2000, USA). The morphology of samples was observed by scanning electron microscopy (SEM; JEOL JSM25S, Japan).

The ion activity product (K_{IAP}) of HA and β-TCP was expressed using abbreviations summarized in Table 1 as, [12), 13)]

$$K_{IAP,HA} = \left(Ca^{2+}\right)^{10}\left(PO_4^{3-}\right)^6\left(OH^-\right)^2$$
$$= \left\{f_{Ca}\left[Ca-T-U\right]\right\}^{10}\cdot\frac{\left\{K_{P3}\left[P-T-2S\right]\right\}^6}{\left\{J\left(H^+\right)\right\}^6}\cdot\frac{K_W{}^2}{\left(H^+\right)^2} \quad (1)$$

$$K_{IAP,TCP} = \left(Ca^{2+}\right)^3\left(PO_4^{3-}\right)^2$$
$$= \left\{f_{Ca}\left[Ca-T-U\right]\right\}^3\cdot\frac{\left\{K_{P3}\left[P-T-2S\right]\right\}^2}{\left\{J\left(H^+\right)\right\}^2}. \quad (2)$$

The K_{IAP} value could beestimated from phosphate concentration, calcium concentration, pH, the values of constants listed in Table 1, and activity coefficients (f_i) calculated from Debye-Huckel limiting law,

$$-\log f_i = \frac{Az_i^2\sqrt{m}}{1+Ba_i\sqrt{m}} \quad (3)$$

where all the abbreviations and their values are listed in Table 1[14)].

Table 1 Summary of abbreviations and equilibrium constants used for the calculation of K_{IAP}[14)]

Symbol	Significance
[i]	Concentration of species i (mol·dm^{-3})
(i)	Activity of i (mol·dm^{-3})
P	Total phosphate concentration (mol·dm^{-3})
J	P/(HPO_4^{2-})
K_{p3}	Third dissociation constant of phosphoric acid
K_W	Ionic product for water
T	Concentration of ion pairs $CaHPO_4^0$ and $CaH_2PO_4^+$
U	Concentration of ion pairs $CaHCO_3^+$ and $CaCO_3^0$
f_i	Activity coefficient for species i
A	Parameter A for Debye-Huckel limiting low
B	Parameter B for Debye-Huckel limiting low
z_i	Valence of ion i
m	Total ionic strength of the solution
a_i	Diameter of ion i

3. RESULTS and DISCUSSION

3-1 Soaking test for porous HA

No other phases than apatite were revealed by XRD and FT-IR in any specimens. Weight change of porous HA after soaking test with the pH-controlled simulated body fluid is shown in Fig. 1. The weight of porous HA hardly decreased for all period in the simulated body fluid with pH value above 5.2 and slightly decreased with increasing period in the simulated body fluid with pH 5.0. From these results, it was assumed that simulated body fluid was saturated for HA at about pH 5.2.

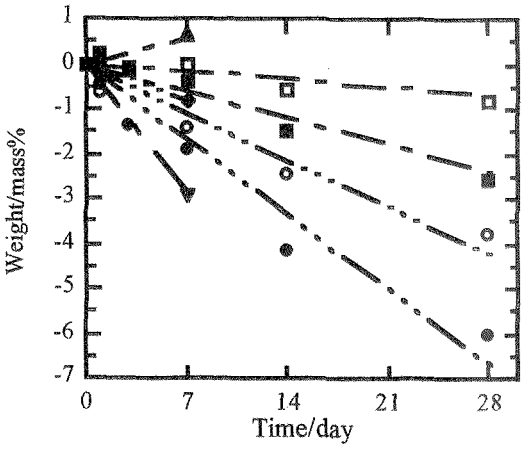

Fig. 1 Changes in weight of porous HA (■, □, ▲, ▼) and porous β-TCP (●, ○, ◆) soaked in the simulated body fluid with pH 4.0 (▼) , 5.0 (■, ●), 5.2 (□, ○) and 6.0 (▲, ◆).

The negative logarithm of ion activity product (pK_{IAP}) for HA decreased linearly with an increase of pH value. From the relationship between the pK_{IAP} and the negative logarithm of solubility product (pK_{sp}=118.65) for HA reported previously[14], it was estimated that the simulated body fluid was saturated for HA at about pH 5.1. When pH value was less than 5.1, the solution was undersaturation for HA. From these results, it was assumed that HA was hardly dissolved in the simulated body fluid with pH value above 5.2 and was slightly dissolved in the simulated body fluid with pH 5.0. Thus, the result of soaking test in the simulated body fluid with pH value from 4.0 to 6.0 corresponded to the result of estimation for HA solubility.

After soaking test, the surface of porous HA was observed by the SEM (Fig. 2). A traces of dissolution was slightly observed on the surface of porous HA after soaking test with pH 5.2, and the traces was more clearly observed after soaking test with pH 5.0 than after soaking test with pH 5.2. These results corresponded to the result of weight change by these soaking test and estimation of solubility.

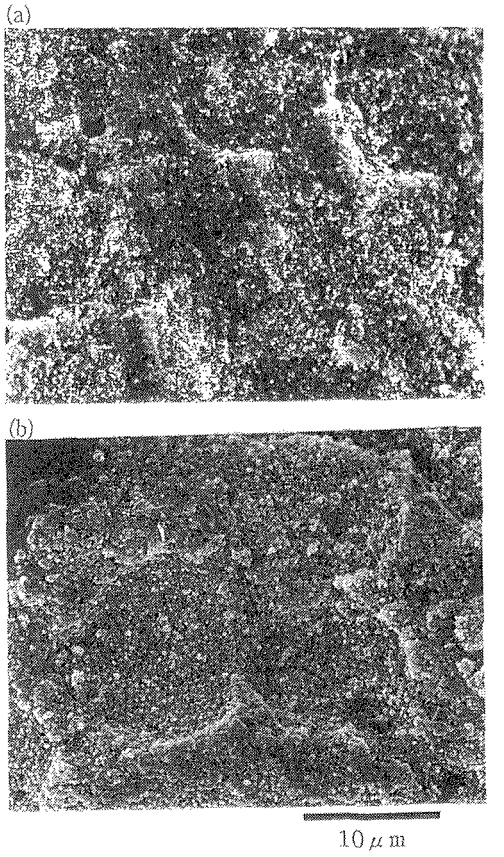

Fig. 2 SEM photographs of porous HA before soaking test (a) and after soaking test with pH 5.0 (b).

3-2 Soaking test for porous β-TCP

No other phases and peaks than β-TCP were revealed by XRD and FT-IR in any specimens. Weight change of porous β-TCP after soaking test with the pH-controlled simulated body fluid is shown in Fig. 1. The weight of porous β-TCP considerably decreased with increasing period in the simulated body fluid with pH 5.2 and 5.0, and slightly decreased in the simulated body fluid with pH 6.0. As these results, it was assumed that the simulated body fluid with pH 5.2 and 5.0 were clearly undersaturation for β-TCP and that β-TCP had higher solubility than HA.

The pK_{IAP} for β-TCP also decreased linearly with an increase of pH value. From the relationship between the pK_{IAP} value and pK_{sp}=29.5 for β-TCP reported previously[15], it was estimated that the simulated body fluid was saturated for β-TCP at about pH 6.0. When pH value was less than 6.0, the solution was obviously undersaturation for β-TCP. From the results of estimation of the K_{IAP} and the K_{sp}, it was assumed that β-TCP was considerably dissolved in the simulated body fluid with pH 5.2 and pH 5.0.

On the surface of porous β-TCP after soaking in the pH-controlled simulated body fluid, a traces of dissolution

was considerably observed with increasing period (Fig. 3). Individual traces on porous β-TCP was deeper than that on porous HA. It was indicated that β-TCP had more solubility than HA. Thus, the result of soaking test corresponded to the result of calculation for β-TCP in the simulated body fluid with pH 5.2 and pH 5.0.

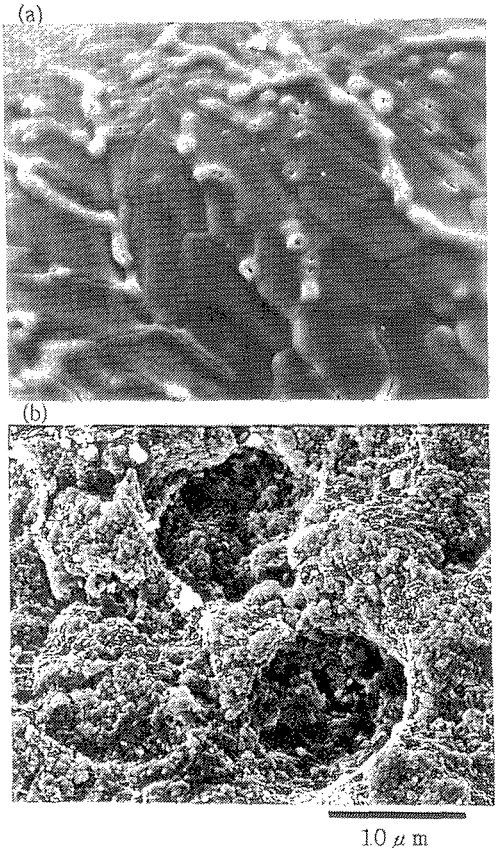

Fig. 3 SEM photographs of porous β-TCP before soaking test (a) and after soaking test with pH 5.0 (b).

4. SUMMARY

Porous HA and porous β-TCP were soaked in the simulated body fluid with pH value from 4.0 to 6.0 in order to investigate the biodegradation mechanism of these materials. According to the estimation of K_{IAP} for these materials, the critical pH value of dissolution for HA was 5.1, and the value for β-TCP was 6.0. The results of soaking test agreed with the K_{IAP} estimation. It was considered that the difference behavior between HA and β-TCP *in vivo* depended on the critical pH value of dissolution.

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