Interlaboratory studies on *in vitro* test methods for estimating *in vivo* resorption of calcium phosphate ceramics

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This study was carried out as Project 5 of Technical Working Area (TWA) 30, Versailles Project on Advanced Materials and Standards (VAMAS). Data obtained using Specimens 3, 4, 7, 8, 9 and 12 (Table 1) measured under conditions A-3, A-4, A-6, B, C, D, E-1, E-2, and F (Table 2) are used in JIS T 0330-3:2012. These data and related materials are reproduced with permission.

Abstract

A potential standard method for measuring the relative dissolution rate to estimate the resorbability of calcium-phosphate-based ceramics is proposed. Tricalcium phosphate (TCP), magnesium-substituted TCP (MgTCP) and zinc-substituted TCP (ZnTCP) were dissolved in a buffer solution free of calcium and phosphate ions at pH 4.0, 5.5 or 7.3 at nine research centers. Relative values of the initial dissolution rate (relative dissolution rates) were in good agreement among the centers. The relative dissolution rate coincided with the [relative volume of resorption pits](#) of ZnTCP *in vitro*. The relative dissolution rate coincided with the relative resorbed volume *in vivo* in the case of comparison between microporous MgTCPs with different Mg contents and similar porosity. However, the relative dissolution rate was in poor agreement with the relative resorbed volume *in vivo* in the case of comparison between microporous TCP and MgTCP due to the superimposition of the Mg-mediated decrease in TCP solubility on the Mg-mediated increase in the amount of resorption. An unambiguous conclusion could not be made as to whether the relative dissolution rate is predictive of the relative resorbed volume *in vivo* in the case of comparison between TCPs with different porosity. The relative dissolution rate may be useful for predicting the relative amount of resorption for calcium-phosphate-based ceramics having different solubility under the condition that the differences in the materials compared have little impact on the resorption process such as the number and activity of resorbing cells.

Keywords

Dissolution; calcium phosphate; resorption; round-robin test

1. Introduction

The evaluation and subsequent optimization of the resorbability of calcium phosphate are crucial in the use of resorbable calcium phosphates. Resorbable calcium phosphates include β -tricalcium phosphate (β -TCP), α -tricalcium phosphate, carbonated hydroxyapatite, low-crystalline hydroxyapatite, and a certain range of biphasic hydroxyapatite and β -TCP. These calcium phosphates are not only osteoconductive but are also gradually resorbed in a long period of time after implantation [1-10]. The rate of resorption needs to be optimized depending on the intended purpose and be balanced with bone tissue regeneration for successful clinical treatments. Moreover, recent findings suggest that the optimized resorption or degradation of calcium phosphates itself can enhance bone tissue regeneration through an immunomodulatory action [11-14].

Possible alternatives to animal testing **for evaluating the resorbability** include cell-based assay, simulation and dissolution tests. Cell-based resorption assay uses a variety of resorbing cells including primary and tumor osteoclasts, differentiated osteoclasts and monocyte/macrophage cell lines [15, 16]. A limitation of cell-based assay is that the assay requires a flat surface for evaluating resorption lacunae or pits. Thus, it is difficult to evaluate the **resorbability of porous bodies and granules**. **Simulation is an alternative to animal testing that is applicable to porous bodies**. Recent simulations have demonstrated an excellent correlation with *in vivo* resorption data for macroporous β -TCP [17, 18]. The simulation is intrinsically based on the paradigm that only osteoclasts and/or macrophages in the vicinity of blood vessels can survive and resorb the surface of the pores. A dissolution test is potentially a chemical alternative to animal testing for the evaluation of resorption because the resorption of calcium

phosphate is substantially a process of cell-mediated dissolution in acidic fluids inside the resorption lacunae underneath osteoclasts or in the phagolysosome of phagocytic cells. The pH values of the acidic fluids have been reported to be 3.9–6.0 [19-23].

Different dissolution tests of calcium phosphates have been carried out under various conditions using either continuous or discontinuous measurement techniques. The continuous measurement techniques include the constant composition method [24-26], the constant pH method [27] and other methods using electrodes [28]. The discontinuous measurement techniques include pharmacopoeia methods such as the BP (British Pharmacopoeia) and USP (United States Pharmacopeia) methods and other intermittent sampling-and-analysis methods [29, 30]. The pH values ranged from ≈ 3 to 7.4 in these dissolution tests. The dissolution tests did not necessarily aim to predict *in vivo* resorption. However, some dissolution tests of calcium phosphates have been found to be predictive of their resorption behavior *in vivo* [31].

On the basis of the background described above, a dissolution test method to predict the resorption of calcium phosphates is proposed as a potential standard method in the present study. The efficacy and limitations of the method were evaluated by round-robin tests as well as comparison with *in vitro* and *in vivo* resorption studies.

2. Materials and methods

2.1. Materials

Specimens of sintered polycrystalline TCP, magnesium-containing TCP (MgTCP) and zinc-containing TCP (ZnTCP) were used (Table 1). TCP25 and TCP35 denote 100% macroporous bodies of TCP with intended relative densities of 25% and 35%, respectively. TCP60, TCP70 and TCP90 denote 100% microporous bodies of TCP with

intended relative densities of 60, 70 and 90%, respectively. 1.0MgTCP, 1.7MgTCP and 3.4MgTCP denote 100% microporous bodies of MgTCP containing 1.0, 1.7 and 3.4 mol% of Mg, respectively, with an intended relative density of 60%. 0.5ZnTCP and 1.0ZnTCP denote 100% microporous bodies of ZnTCP containing 0.5 and 1.0 mol% of Zn, respectively, with an intended relative density of 90%. Scanning electron microscopy (SEM) images of the specimens are shown in the supporting data (Fig. S1). The specimens have block, cylinder and disk shapes with dimensions as listed in Table 1. TCP25 and TCP35 had a block shape and were used in the dissolution study. TCP60, TCP70, 1.0MgTCP, 1.7MgTCP and 3.4MgTCP had a cylinder shape and were used in the dissolution and *in vivo* resorption studies. TCP90, 0.5ZnTCP and 1.0ZnTCP had a disk shape and were used in the dissolution and osteoclastic resorption studies. The cylinder specimens used for the *in vivo* resorption study had 1-mm-thick biphasic (TCP and hydroxyapatite) portions with a Ca/P molar ratio of 1.60 at both ends. The biphasic portion was microscopically non-resorbable in the present animal model (Fig. S2). The biphasic portion was used to define the base line of zero resorption when measuring the resorbed volume in the *in vivo* study. All specimens were prepared by sintering in the temperature range from 1030 to 1100 °C depending on the chemistry and intended relative density. All the specimens except for the macroporous TCP25 and TCP35 were prepared in the research center A. The macroporous specimens were prepared by Covalent Materials Co., Japan, then subjected to quality inspection at research center A.

2.2. Interlaboratory dissolution tests

Interlaboratory dissolution tests were performed at nine research centers (A-I; Table 2). The protocol for the dissolution tests is described in the supporting data. Briefly, the

specimens were dissolved in either 0.05 M potassium hydrogen phthalate buffer solution at pH 4.0, 0.08 M acetic acid-sodium acetate buffer solution at pH 5.5 or 0.05 M Tris-HCl buffer solution at pH 7.3 while stirring the buffer solutions. The dissolution temperature was 25 °C except in the pharmacopoeia method, where it was 37 °C (A-5 in Table 2). The dissolution period was 5, 18, 30, 180 or 300 min, where 30 min was adopted in most cases. The volumes of the solutions were from 35 to 100 mL except in the pharmacopoeia method, where it was 200 mL. The calcium concentration was measured continuously or intermittently. The continuous measurement was made using calcium-ion-selective electrodes. The intermittent measurement was made by collecting 1 mL of solution at intervals of 3 or 5 min except in the pharmacopoeia method, in which 5 mL was collected at intervals of 60 min. The collected solution was analyzed for calcium using atomic absorption spectroscopy (AAS), inductively coupled plasma atomic emission spectrometry (ICP) or inductively coupled plasma mass spectrometry (ICP-MS). Three to ten samples of TCP, MgTCP and ZnTCP were subjected to the dissolution test. The data of calcium concentration at the end of the dissolution period were converted to the dissolved amount (μmol) of TCP, MgTCP or ZnTCP on the assumption that the dissolution proceeded congruently. The dissolved amount divided by the dissolution period gave the dissolution rate ($\mu\text{mol/s}$). The dissolution rate was averaged over every specimen subjected to the dissolution test ($n=3-10$). The average dissolution rate was divided by that of TCP60, TCP25, 1.0MgTCP or TCP90 to obtain the relative dissolution rate. The relative dissolution rates were used to characterize dissolution behaviors and for comparison with the relative amount of resorption *in vitro* and *in vivo*.

2.3. Osteoclastic resorption

Previous data measured at research center A for the volume of osteoclastic resorption pits on the TCP90, 0.5ZnTCP and 1.0ZnTCP disks (Specimens 13-15, respectively) with relative densities of 91-93% was analyzed – the previous paper reported incorrect relative densities (67-83%) for the disks [32]. Briefly, the osteoclasts were isolated from unfractionated bone cells in the bones of 10-day-old Japanese white rabbits. Between 1×10^4 and 3×10^4 mature osteoclasts were obtained from one rabbit. More than 90% of the cells obtained were tartrate-resistant acid phosphatase (TRAP)-positive and multinucleate (three or more nuclei). The osteoclasts were seeded on the TCP and ZnTCP disks previously placed in each well of a 12-well plate and cultured for 2 hours at 37 °C to allow them to attach to the disks. After cultivation for 2 hours, 2 ml of α -MEM with 10% FBS and 5% L-glutamine supplemented with 100 ng/ml of macrophage colony-stimulation factor (M-CSF) (mouse recombinant, Calbiochem) and 50 ng/ml of TRANCE (mouse recombinant RANK ligand, TECHNE Co.) were added to each well. The osteoclasts were further cultured for 24 hours at 37 °C. After cultivation for 24 hours, the osteoclasts were removed from the disks by sonication in a 0.25 M NH_4OH solution. All disks were then dehydrated in a graded series of ethanol concentrations and processed with a critical point dryer. They were subsequently coated with platinum in a cold sputter coater to visualize the resorption pits. The coated disks were examined with a color laser microscope (VK-9500, KEYENCE, Japan) equipped with an image analysis system (VK-9500 analyzer, KEYENCE, Japan). The volumes of 30 randomly selected pits on each of the four disks (120 pits in total) were measured. The pit volumes were averaged over the 120 pits. The average pit volume for 0.5ZnTCP and 1.0ZnTCP was divided by that of the TCP90 to obtain the relative pit volume. The

relative pit volumes were compared with the relative dissolution rates of corresponding ZnTCP disks measured at research center C.

2.4. In vivo resorption

The *in vivo* resorption study was performed at research center A. Cylindrical implants of TCP60, TCP70, 1.0MgTCP, 1.7MgTCP and 3.4MgTCP (Specimens 8-12, respectively) were implanted perpendicular to the long axis of the femoral diaphysis of skeletally mature New Zealand White rabbits (male, 15 months old, 3.0-3.5 kg in weight) for four weeks. The same group of specimens was implanted bilaterally. After sacrifice, all the bone specimens containing the implants were subjected to undecalcified histological processing except for one pair of bone specimens (right and left) randomly selected from each group for decalcified histological processing. For undecalcified histological processing, the bone specimens were immersed in a 70% ethanol solution for 1 week for fixation. The bone specimens were stained with Villanueva Bone (VB) solution, embedded in methyl methacrylate (MMA), cut into two or three sections by cutting parallel to the long axis of the implant and ground to 50- μ m-thick undecalcified sections. The resorption of implants was revealed by the rugged external form of the implants on undecalcified sections observed with a stereoscopic microscope (Fig. 1).

The area of resorption lying between the rugged outline and the original outline was measured by image analysis (Image-Pro PLUS, Ver 6.1). From the area of resorption, the apparent resorbed volume of the implant per unit lateral area of the implant (V) was calculated according to a method described elsewhere [33]. The measurement was performed on bone specimens with no femoral fracture, in-process

implant fracture or deformation. The apparent resorbed volume per cylindrical length of 1 mm (V_I) was calculated using the following equation:

$$V_I = 2\pi rV \text{ (mm}^3\text{)},$$

where r is the radius of the implant. The net amount of resorbed TCP or MgTCP per cylindrical length of 1 mm (A_I) was calculated using

$$A_I = DrDV_I/100M \text{ (}\mu\text{mol)},$$

where Dr , D and M are the relative density of the implant, the density of the TCP or MgTCP and the molecular weight of the TCP or MgTCP, respectively. V_I and A_I were averaged over every specimen ($n=8-10$). The average V_I and A_I were divided by the average V_I and A_I , respectively, of TCP60 or 1.0MgTCP to obtain relative V_I and A_I . The relative V_I and A_I were compared with the relative dissolution rates measured at research centers A-I.

For decalcified histological processing, the bone specimens were fixed in 10% neutral-buffered formalin, decalcified, embedded in paraffin and cut into 5- μm -thick sections with the staining of toluidine blue and TRAP to observe the osteoclasts. The number of TRAP-positive cells existing less than 200 μm from the implant was measured on 6-16 microscopic images within the section. Thus, the average and standard deviation for each group are those for the 6-16 images from one rabbit. All animal experiments were performed under conditions approved by the Institutional Animal Care and Use Committee of National Institute of Advanced Industrial Science and Technology (AIST) and in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH 1996).

2.5. Statistical analysis

All statistical comparisons were conducted using Student's *t*-tests. The standard deviations (σ) of the relative values, $\overline{X_1}/\overline{X_0}$, such as the relative dissolution rate, pit volume, V_I and A_I were calculated using

$$\sigma = \sqrt{\left\{ -\frac{\overline{X_1}}{(\overline{X_0})^2} \right\}^2 \cdot \sigma_0^2 + \left(\frac{1}{\overline{X_0}} \right)^2 \cdot \sigma_1^2}$$

where $\overline{X_0}$ and $\overline{X_1}$ are average values for groups 0 and 1, and σ_0 and σ_1 are the standard deviations for groups 0 and 1, respectively. The significance level was set at $p < 0.05$.

3. Results

The dissolution curves were almost linear during the dissolution periods at pH 4.0, 5.5 and 7.3 (Fig. 2 and Fig. S6). The calculated dissolution rates represented the slopes of the dissolution curves. The dissolution rate of the same specimen under the same pH condition varied by up to 600% depending on the research center and the dissolution conditions such as the solution volume (35-200 mL) and stirring speed (200-450 rpm). However, the relative dissolution rates, i.e., the average dissolution rates of TCP70, TCP25, TCP35, 1.0MgTCP, 1.7MgTCP and 3.4MgTCP relative to that of TCP60, varied by only up to 183% in spite of the differences in solution volumes, stirring speeds and even pH (4.0, 5.5 and 7.3) as shown in Figs. 3-6.

The relative dissolution rates had higher sensitivity to a difference in microporosity

than to a difference in macroporosity. The relative dissolution rates of microporous TCP70 compared with microporous TCP60 were significantly lower than 1.0 except for research center G as shown in Fig. 3. However, the relative dissolution rates of macroporous TCP35 compared with macroporous TCP25 showed no significant difference ($p>0.05$) from 1.0 except for condition H-2 (Fig. 4). Thus, a 10% difference in microporosity has a greater effect on the dissolution rate than the same difference in macroporosity.

The relative pit volume and relative dissolution rate decreased with an increase in the zinc content of TCP (Fig. 7). The relative pit volume for 0.5ZnTCP compared with TCP90 was significantly lower than 1.0. Similarly, the relative pit volume for 1.0ZnTCP was significantly lower than both 1.0 and that for 0.5ZnTCP. The dissolution rates for 0.5ZnTCP and 1.0ZnTCP compared with TCP90 were significantly lower than 1.0. The relative dissolution rate for 1.0ZnTCP was not significantly lower than that for 0.5ZnTCP ($p=0.066$).

The relative pit volumes agreed with the relative dissolution rates without significant differences for both 0.5ZnTCP and 1.0ZnTCP ($p=0.33$ for 0.5 ZnTCP and 0.46 for 1.0 ZnTCP).

The apparent resorbed volume *in vivo*, V_I , for microporous TCP60, TCP70, 1.0MgTCP, 1.7MgTCP and 3.4MgTCP depended on the relative density and magnesium content. Individual V_I values for the same specimen varied by up to 268%. The average value of V_I was from 0.25 to 0.39 mm³ (Fig. 8). There was no significant difference in V_I between TCP60 and TCP70 ($p=0.088$). The TCP60 and TCP70 specimens had

relative densities of 62 and 72%, respectively (Table 1). The MgTCP specimens, which had similar relative densities (65-67%) and different Mg contents, tended to have decreasing V_I with increasing Mg content from 1.0 to 3.4 mol% (1.0MgTCP, 1.7MgTCP and 3.4 MgTCP).

The relative dissolution rate was in good agreement with the relative V_I but in poor agreement with the relative A_I in the case of the microporous TCP specimens (Fig. 3). The relative dissolution rates of microporous TCP70 compared with microporous TCP60 agreed with the relative V_I for TCP70 compared with microporous TCP60 without significant differences ($p=0.54-0.08$) except for condition E-1 ($p=0.03$). However, the relative dissolution rates were significantly different from the relative A_I under five conditions (A-4, C, D, E-1 and A-6 in Fig. 3).

The relative dissolution rate coincided with the relative V_I and A_I among the microporous MgTCP specimens. The relative dissolution rates of 1.7MgTCP and 3.4MgTCP compared with 1.0MgTCP were in the range from 0.88 to 0.95 and from 0.48 to 0.61, respectively (Fig. 5). These relative dissolution rates agreed with the relative V_I and A_I of 1.7MgTCP and 3.4MgTCP compared with 1.0MgTCP without significant differences ($p=0.49-0.11$) (Fig. 5). However, the relative dissolution rates of 1.0MgTCP, 1.7MgTCP and 3.4MgTCP compared with TCP60 were all significantly lower than the corresponding relative V_I and A_I except for condition A-3 for 1.0MgTCP and H-1 for 3.4MgTCP (Fig. 6). More osteoclasts were observed around the 1.0MgTCP, 1.7MgTCP and 3.4MgTCP implants than around TCP60 implants, although the differences were not significant (Fig. 9).

4. Discussion

In the present study, the dissolution tests were designed to test different calcium phosphate phases under a range of undersaturation starting at 1 and ending – for the lowest value – at 0.8 (see supporting data and Table S8) regardless of the specimen. In this range of undersaturation, the dissolution curves were almost linear, which was useful for defining the dissolution rates.

A dissolution rate is a function of both the equilibrium solubility and kinetic factors such as the surface area, particle size, porosity and crystallinity. The resorption of calcium phosphate biomaterials depends on both their equilibrium solubility and kinetic factors [17, 31, 34]. Thus, we focused on measuring the dissolution rate. However, the dissolution rate was unacceptable as a standard index due to large interlaboratory differences. On the other hand, the interlaboratory differences were diminished efficiently by converting the dissolution rate to the relative dissolution rate. The interlaboratory differences in the relative dissolution rates (183% at maximum) were within the scattering in V_I (268% at maximum) obtained from *in vivo* experiments. The maximum value of 183% in interlaboratory difference was obtained in spite of a wide range of differences in solution volumes (35-200 mL), stirring speeds (200-450 rpm) and even pH (4.0, 5.5 and 7.3). Note that a difference of 1.0 in pH gives a difference of 660% in the initial dissolution rate of TCP [27]. Therefore, the relative dissolution rate can be a useful standard index that can be used regardless of a wide range of differences in dissolution conditions.

The relative dissolution rates agreed with the relative pit volumes for ZnTCPs **having different equilibrium solubility**. The zinc incorporated in TCP decreases the equilibrium solubility of TCP [33]. The osteoclastic resorption of calcium phosphate is a process of cell-mediated dissolution in an acidic fluid in the resorption pits [19]. The effect of zinc on decreasing the TCP solubility resulted in the **same rate of** decrease in both the dissolution rate and the volume of osteoclastic resorption pits. Thus, the relative dissolution rates agreed with the relative pit volumes. However, it should be noted that **the relative pit volumes** (Fig. 7) do not take account of **material-mediated** differences in the total number of pits or osteoclasts between TCP90, 0.5ZnTCP and 1.0 ZnTCP. The number of resorbing osteoclasts **slightly** decreases with an increase in the zinc content of TCP *in vitro* [32]. Thus, the relative dissolution rates multiplied by the total number of osteoclasts or resorption pits would be better correlated to **the relative** amount of osteoclastic resorption *in vitro*.

A relative dissolution rate may be useful to predict the amount of resorption of microporous calcium phosphate having different equilibrium solubility when **material-mediated differences in biological process** are negligible. The effect of Mg on decreasing the equilibrium solubility of MgTCP has been substantiated [35, 36]. The relative dissolution rate of MgTCP coincided with the relative V_I and A_I for the MgTCP when the denominator of the relative values belonged to another MgTCP. However, when the denominator belonged to TCP (**TCP60**), the relative dissolution rate of MgTCP significantly differed from the relative V_I and A_I for the MgTCP. The discrepancy when the denominator belongs to TCP arises from differences in the material-mediated biological **process** between TCP and MgTCP, as was **tentatively**

shown by the increased number of osteoclasts around the MgTCP implants compared with TCP implants (Fig. 9). As a result, the relative V_I and A_I for the MgTCP compared with TCP resulted from two different effects of Mg incorporated in the TCP lattice: the effect of Mg on increasing the resorption of TCP and the effect of Mg on decreasing the equilibrium solubility of TCP. The former effect has some link with the dose-dependent effects of Mg on the differentiation and function of osteoclasts and macrophages [37, 38]. Thus, the relative dissolution rate may be useful when material-mediated differences in biological process are negligible. Note that the present study demonstrates that a small amount of Mg incorporated in the TCP lattice increases the resorption of TCP *in vivo*.

The validity of using the relative dissolution rate for predicting the resorption of pure TCP with different porosity remains to be clarified further. In the case of ZnTCP and MgTCP, the specimens had the same porosity and different equilibrium solubility while, in the case of pure TCP, the specimens had the same solubility and different porosity. Regarding the microporosity of TCP, it is reported that an increase in the microporosity of TCP has a greater effect on resorption than an increase in macroporosity [3, 39, 40]. These phenomena are qualitatively similar to the effect of microporosity and macroporosity on the dissolution rates. However, in the previous and present studies, *in vivo* resorption was quantified by measuring the resorbed area or volume histomorphometrically. The measured area or volume such as V_I inherently includes that of micropores that existed in the resorbed portion. Thus, the net amount of resorbed TCP is not V_I but A_I . On the other hand, the difference in A_I was statistically insignificant between microporous TCP60 and TCP70. Thus, it cannot be

ruled out that the agreement between the relative dissolution rate and relative V_I for microporous TCP was an accidental result that arose from the combined effects of the latent micropore volume and some biological factor that maintains a constant calcium metabolism. To address the above issue, the quantification of A_I with higher precision is required. Regarding the macroporous TCP, no *in vivo* study was carried out in the present study. Although the 10% difference in macroporosity gave a value of nearly 1.0 for the relative dissolution rate, it is yet to be clarified whether the 10% difference in macroporosity gives a value of 1.0 for the relative V_I and A_I .

Other limitations of the present study are (i) its uncertain applicability to other calcium phosphates and (ii) that the protocol for the dissolution tests needs to be improved further. The present dissolution test conditions may not be able to predict the *in vivo* resorption of calcium phosphate ceramics different from those tested in the present study. The correlation between the relative dissolution rates and *in vivo* resorption needs to be studied for individual calcium phosphate ceramics. In other words, an assay has to be established for every ceramic to be analyzed. In addition, the protocol of dissolution tests has to be improved to obtain higher precision and better reproducibility of the measured values. Means of improvement include the pretreatment of specimens before immersion, the method of validation, the way the specimen is held in the solution and the way of integration of the effect of particle disintegration [41, 42]. One institute (I) reported the reduced wettability of specimens, probably due to the natural and progressive adsorption of organic hydrophobic moieties in air, which was recovered by heat treatment before immersion.

5. Conclusion

An *in vitro* dissolution test method was proposed to estimate the relative rate of resorption of calcium-phosphate-based ceramics. According to this method, calcium phosphates were dissolved in a neutral or slightly acidic buffer solution free of calcium and phosphate ions. Since the calcium phosphates dissolved linearly over the first 5-180 min, the relative dissolution rates were calculated without difficulty and compared with each other. The results suggest that the measurement of the relative dissolution rate *in vitro* is useful as a standard test procedure to predict relative amounts of resorption for calcium-phosphate-based ceramics having different solubility, provided that material factors, such as a small amount of Mg doping ions, do not affect the biological process, such as the density or activity of resorbing cells. Further study is required to clarify whether the relative dissolution rate is predictive of relative amounts of resorption for calcium-phosphate-based ceramics having the same solubility but different porous characteristics.

Disclosures

There is no potential conflict of interest for this study

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Tables

Table 1 Specimens used in the present study

Footnotes for Table 1;

Parentheses mean the diameters of the non-resorbable biphasic portion at both ends of the cylinder.

§ dis., *in vivo* and ost. indicate dissolution, *in vivo* and osteoclastic resorption studies, respectively.

Table 2 Participating research centers, specimens used and dissolution conditions

Footnotes for Table 2;

The letters indicate the individual research centers while the numbers indicate dissolution conditions in the center.

§ See Table 1.

¶ Ph, Ac and Tr indicate 0.05 M potassium hydrogen phthalate, 0.08 M acetic acid-sodium acetate and 0.05 M Tris-HCl buffers, respectively.

* “Elect.” indicates measurement using a calcium-ion-selective electrode.

Figure captions

Figure 1 Rugged external form of TCP60 (Specimen 8) showing resorption in the implantation period of 4 weeks.

Figure 2 Dissolution curves for microporous TCP60 (Specimen 3) at pH 5.5 measured in different research centers and under different conditions. The plots are presented as mean $\pm$ SD. The number of specimens was 4 or 5.

Figure 3 Relative dissolution rates, relative apparent resorbed volume (V_I) and relative net resorbed amount (A_I) for microporous TCP70 (Specimens 4 and 9) compared with TCP60 (Specimens 3 and 8). The data are presented as mean $\pm$ SD. The number of samples was 3, 4, 5 or 8 in the dissolution tests. The numbers of samples were 10 for the relative V_I and A_I .

Figure 4 Relative dissolution rates for macroporous TCP25 and TCP35 (Specimens 1 and 2) compared with TCP60 (Specimen 3) and TCP25. The data are presented as mean $\pm$ SD. The number of samples was 4, 5 or 8.

Figure 5 Relative dissolution rates, relative apparent resorbed volume (V_I) and relative net resorbed amount (A_I) for microporous 1.7MgTCP (Specimens 6 and 11) and 3.4MgTCP (Specimens 7 and 12) compared with 1.0MgTCP (Specimens 5 and 10). The data are presented as mean $\pm$ SD. The number of samples was 5 in the dissolution tests. The number of samples was 9 for 1.7MgTCP and 10 for 3.4MgTCP in the *in vivo* tests.

Figure 6 Relative dissolution rates, relative apparent resorbed volume (V_I) and relative net resorbed amount (A_I) for microporous 1.0MgTCP (Specimens 5 and 10), 1.7MgTCP (Specimens 6 and 11) and 3.4MgTCP (Specimens 7 and 12) compared with microporous TCP60 (Specimens 3 and 8). The data are presented as mean $\pm$ SD. The number of samples was 4, 5 or 8 in the dissolution tests. The number of samples was 8 for 1.0MgTCP, 9 for 1.7MgTCP and 10 for 3.4MgTCP in the *in vivo* tests.

Figure 7 Relative pit volumes and relative dissolution rates for 0.5ZnTCP (Specimen 14) and 1.0ZnTCP (Specimen 15) compared with TCP90 (Specimen 13). The data are presented as mean $\pm$ SD. The number of resorption pits was 120, which were randomly selected from four disks with 30 pits per disk. The number of disks was 10 in the dissolution tests.

Figure 8 Apparent resorbed volume (V_I) of TCP and MgTCP implants (Specimens 8-12). The data are presented as mean $\pm$ SD. The number of samples was 8 for 1.0MgTCP, 9 for TCP60 and 1.7MgTCP, and 10 for TCP70 and 3.4MgTCP.

Figure 9 Numbers of TRAP-positive cells around TCP and MgTCP implants (A) and histological sections showing TRAP-positive cells around TCP60, 1.7MgTCP and 3.4MgTCP implants (B). “b” indicates bone. The data are presented as mean $\pm$ SD. The mean and SD for each group are those for the 6-16 microscopic images from one rabbit.

Table 1 Specimens used in the present study

Specimen No.	Notation	Mg or Zn content (mol%)	Relative density (%)	Macro- or micro-porous	Shape	Diameter [#] (mm)	Length or thickness (mm)	Study [§]
1	TCP25	0	25.8±0.3	macro	block	5.02±0.03	4.52±0.02	dis.
2	TCP35	0	35.1±0.7	macro	block	4.99±0.02	4.52±0.03	dis.
3	TCP60	0	63±1	micro	cylinder	2.43±0.03	11.3±0.3	dis.
4	TCP70	0	74±1	micro	cylinder	2.44±0.02	11.2±0.2	dis.
5	1.0MgTCP	1.0Mg	68±1	micro	cylinder	2.43±0.01	10.8±0.1	dis.
6	1.7MgTCP	1.7Mg	64±1	micro	cylinder	2.48±0.01	11.1±0.1	dis.
7	3.4MgTCP	3.4Mg	62±1	micro	cylinder	2.45±0.01	11.3±0.3	dis.
8	TCP60	0	62±1	micro	cylinder	2.41±0.03 (2.50±0.03)	11.1±0.3	in vivo
9	TCP70	0	72±1	micro	cylinder	2.45±0.01 (2.52±0.01)	11.2±0.1	in vivo
10	1.0MgTCP	1.0Mg	67±1	micro	cylinder	2.43±0.01 (2.37±0.02)	11.2±0.1	in vivo
11	1.7MgTCP	1.7Mg	65±1	micro	cylinder	2.48±0.01 (2.37±0.01)	11.4±0.1	in vivo
12	3.4MgTCP	3.4Mg	67±1	micro	cylinder	2.45±0.01 (2.34±0.02)	11.2±0.1	in vivo
13	TCP90	0	92±1	micro	disk	13.53±0.02	0.90±0.02	ost. dis.
14	0.5ZnTCP	0.5Zn	93±1	micro	disk	13.45±0.02	0.89±0.02	ost. dis.
15	1.0ZnTCP	1.0Zn	91±1	micro	disk	13.56±0.02	0.91±0.02	ost. dis.

[#] Parentheses mean the diameters of the non-resorbable biphasic portion at both ends of the cylinder.

[§] dis., in vivo and ost. indicate dissolution, *in vivo* and osteoclastic resorption studies, respectively.

Table 2 Participating research centers, specimens used and dissolution conditions

Research center & conditions [#]	Specimen Nos. [§]	Solution			Temp. (°C)	Time (min)	Rotation speed of stirring bar (rpm)	Method [*]	Collection of solution		
		Buff. [¶]	pH	Vol. or weight					Interval (min)	Vol. (mL)	Replenishment (mL)
A-1	3-7	Ph	4.0	35mL	25±2	5	430-450	ICP	5	1	1
A-2	3-7	Ph	4.0	35mL	25±2	30	430-450	ICP	5	1	1
A-3	3-7	Ac	5.5	35mL	25±2	180	430-450	Elect.			
A-4	1-7	Ac	5.5	35mL	25±2	30	430-450	Elect.			
A-5	3, 4	Tr	7.3	200mL	37±1	300	200	ICP	60	5	5
A-6	3, 4, 7	Tr	7.3	35mL	25±2	30	430±15	Elect.			
B	3, 4, 7	Ac	5.5	100mL	25±2	30	430±15	Elect.			
C	3, 4, 7,	Ac	5.5	35mL	25±2	30	430±15	Elect.			
C	13-15	Ac	5.5	35mL	25±2	30	430±15	Elect.			
D	3, 4, 7	Ac	5.5	60mL	25±2	30	430±15	Elect.			
E-1	3, 4, 7	Ac	5.5	60mL	25±2	30	430±15	Elect.			
E-2	3, 4, 7	Tr	7.3	60mL	25±2	30	430±15	Elect.			
F	3, 7	Ac	5.5	80mL	26-32	30	430±15	Elect.			
G	1-4, 7	Ph	4.0	70mL	25±1	30	380	Elect.			
H-1	3, 4, 7	Ph	4.0	60mL	25±2	30	417-420	AAS	5	1	1
H-2	1,2	Ph	4.0	40, 35mL	25±2	30, 18	346-348	AAS	5, 3	1	1
I	1-4, 7	Ac	5.5	100g	25±2	30	300	ICP-MS	5	1	0

[#] The letters indicate the individual research centers while the numbers indicate dissolution conditions in the center.

[§] See Table 1.

[¶] Ph, Ac and Tr indicate 0.05 M potassium hydrogen phthalate, 0.08 M acetic acid-sodium acetate and 0.05 M Tris-HCl buffers, respectively.

^{*} "Elect." indicates measurement using a calcium-ion-selective electrode.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

B

Figure 9

Relative dissolution rate

In vitro resorption

In vivo resorption

Osteoclasts