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In vitro degradation behavior of porous calcium phosphates under diametral compression loading

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Abstract

Four different types of porous calcium phosphates were made on addition of a pore-forming compound (PVA) by sinter processing. Mechanical properties, morphology, and weight change with immersion time in Hanks' physiological solution were evaluated. The in vitro fatigue test was also performed. Experimental results showed the pore microstructure of the as-sintered bodies to be made up of the macropores and micropores. The initial strength of the porous bodies was dependent on the contents of the PVA additive. Porous bodies subjected to cycling fatigue in Hanks' solution remarkably decreased in the strength. With increasing immersion time in solution, the tensile strength and elastic modulus of various porous bodies decreased. The weight loss data confirmed the degradation behavior of the porous bodies in Hank's solution. The biomedical uses of the present porous materials are limited to non-load bearing applications such as bone defect repair.

Keywords: C. Mechanical properties; E. Biomedical application; Calcium phosphate; Degradation

1. Introduction

Tissue engineering is regarded as one of the bioscience for guiding the body to regenerate or repair tissues, when organ and tissue loose or fail [1–3]. The scaffold is the temporary supporting structure of tissue-engineered constructions [1–5]. Aside from suitable surface chemistry, the scaffold architectures provide sufficient space and suitable mechanical properties for tissue regeneration [1,2]. Ideally, the resorption rate of an implant should not exceed the rate of bone formation and the resorption-induced reduction in implant strength should closely match the increase in strength of the healing tissue [2,6]. The use of biodegradable biomaterials as bone scaffolds has attracted a great deal of attention and a variety of fabrication methods have been proposed to produce porous scaffolds with interconnected pore networks [3–5,7].

Porous biodegradable synthetic materials, such as calcium phosphates, poly(lactic acid) (PLA), and poly (glycolic acid) (PGA), are currently tested as implants for the regeneration of damaged and diseased tissues [2–6]. These synthetic biomaterials were chosen on the basis of their biocompability and mechanical properties. Concerning the fabrication of porous ceramic bodies, several techniques, such as the use of polymeric sponge [8] and organic additives [9–11], and molding processes [12], have been used. For example, Prado da Silva et al. [9] used three different organic additives, potato starch, almond crust, and wax spheres, to produce porous structures of a $CaO–P_2O_5$ glass reinforced hydroxyapatite (HA). With poly(vinyl

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butyral) powders, Liu [10] fabricated porous HA granules with controlled porosity, pore size, pore size distribution, and granule size by using a drip-casting process. Flautre et al. [11] used polymethylmethacrylate microbead as the porous agent to make various porosity of HA ceramics.

The available synthetic biodegradable calcium phosphate ceramics for bone tissue regeneration include HA, β-tricalcium phosphate (β-TCP), and calcium polyphosphate [3–4,6,8]. The biodegradation behavior of calcium phosphate materials in both in vitro and animal studies has been reported [6,7,13–14]. Shima et al. [13] implanted porous β-TCP between vertebrae of rabbits and found that these porous implants may fracture and were then reduced to granule. Utilizing solid freeform fabrication to build porous parts of calcium polyphosphate, Porter et al. [14] found a decline in mechanical properties in tris-buffered solution. Pilliar et al. [7] studied the effect of particle size of calcium polyphosphates on in vitro degradation. Their finds indicated that there was a significant strength loss for both the fine and coarse powder samples after soaking in buffered solutions at pH 4 and 7.

Biodegradation of tissue-engineered materials is characterized by changes in the mechanical and physicochemical properties (disintegration and dissolution) of the material after implantation or after immersion in physiological solution. Little information on the variations in mechanical properties of porous calcium phosphates, when immersed in simulated body fluid, has been reported. Additionally, relatively few studies are available about strength durability of porous scaffolds under fatigue. There is a need to use cyclic loading to test the bond durability of the porous bioceramics for evaluation of feasibility in clinical applications. In the present study, porous calcium phosphates were prepared by sintering mixtures of monocalcium phosphate monohydrate (MCPM, $Ca(H_2PO_4)_2{\cdot}H_2O$) and polyvinyl alcohol (PVA). To impart porosity to the ceramic body, the PVA was burned out during firing, leaving free space in the resulting body. The degradation behavior of the porous bodies was characterized by monitoring changes in tensile strength and modulus as well as weight loss. The fatigue test in wet condition was also performed.

2. Materials and methods

Commercially pure MCPM and PVA powders (Showa, Tokyo, Japan) were used. The as-received MCPM powder was directly mixed with PVA in a vacuum mixer (VM-112T, J. Morita, Saitama, Japan) for 5 min to ensure homogeneity. Four different types of the porous bodies were prepared from mixtures of MCPM and PVA powders with different ratios (4:1, 3:1, 2:1, and 3:2 by weight) (Table 1). For simplicity, throughout this study the green body and the sintered body derived from the same mixture are designated with the same code. The mixture was uniaxially pressed in a 12 mm diameter stainless mold at 10 MPa. The green bodies were heated to 900 °C at 10 °C/min and held for 3 h in air, then furnace-cooled to room temperature. The morphology was observed under a scanning electron microscope (SEM, Hitachi S-4200, Japan).

The measurement of the porosity was carried out in two different ways. The first method is to calculate the density of the sintered porous body from measured mass and volume, and then the relative density was determined by the ratio of the measured density to the theoretical density of the materials (2.85 g/cm^3) [14]. The difference between one and relative density is the total porosity. The second measurement was conducted by using a conventional Archimedes immersion technique with ethanol [15]. The average value of six determinations was taken as the porosity of sintered bodies.

The extracellular solution with an ionic composition similar to that of human blood plasma, Hank's balanced salt solution, was used as supporting solution and for the immersion test. The simulated solution consisted of 8.00 g NaCl, 0.35 g $NaHCO_3$, 0.40 g KCl, 0.06 g KH_2PO_4, 0.10 g $MgCl_2{\cdot}6H_2O$, 0.14 g $CaCl_2$, 0.06 g $Na_2HPO_4{\cdot}2H_2O$, 0.06 g $MgSO_4{\cdot}7H_2O$, 1.00 g glucose in 1000 ml distilled H_2O. The solution had an initial pH of 7.4.

For fatigue test, the sintered samples were fatigued under diametral compression test in Hanks' solution at room temperature using Shimadzu servopulser 48000 system (Kyoto, Japan). A fatigue cyclic loading with a stress ratio of $S_{min}/S_{max} = 0$ was imposed at 3 Hz until fracture. Six porous bodies of the same test group were placed in a polystyrene container and Hanks' solution was added into the container to completely cover the samples.

For immersion test, the specimens were immersed in 10 ml of the solution for the predetermined periods of time at 37 ± 0.5 °C. After immersion, the specimens were removed from the vials and placed in a container with fresh Hanks' solution to evaluate the mechanical properties. Their degradation behavior was also monitored through sample weight change. Prior to weighing with a 4-digital balance (AE 240S, Mettler-Toledo AG, Greifensee, Switzerland), the immersed specimens dried at 120 °C for 3 h in an oven. At least seven samples were tested for each measurement. The diametral tensile testing was conducted on an EZ-Test machine (Shimadzu, Kyoto, Japan) at a loading rate of 0.5 mm/min. The diametral tensile strength (T_d) of the sample was calculated from $T_d = 2P/\pi bw$, where P is the peak load (N), b the diameter (mm) and w the thickness (mm) of the sample. The diameter and length of each specimen were measured by using a micrometer. The maximal compression load at failure was obtained from the recorded load–deflection curves. The Young's modulus of the samples was determined from the slope of the initial linear elastic portion of the load–deflection curve. One-way ANOVA statistical analysis was used to evaluate the statistical significance of the tensile strength data. Scheffe multiple comparison testing was used to determine the significance of the deviations in the strength of each sample

Table 1
Composition of various green bodies and porosity and mechanical properties of their respective sintered bodies

Sample code	Composition MCPM:PVA (weight ratio)	Change in diameter (%)	Porosity		Tensile strength ± S.D. (MPa)	Tensile modulus ± S.D. (MPa)
			By geometry	By Archimedes		
C4P1	4:1	3.2 ± 1.1	82.6 ± 0.1	89.8 ± 4.6	0.8 ± 0.1	13.1 ± 1.1
C3P1	3:1	−3.4 ± 0.9	80.1 ± 0.1	83.3 ± 4.1	1.2 ± 0.2	18.0 ± 1.8
C2P1	2:1	−8.4 ± 1.5	76.4 ± 0.1	78.6 ± 3.8	0.7 ± 0.1	15.3 ± 0.9
C3P2	3:2	−7.2 ± 1.0	78.7 ± 0.1	76.0 ± 2.7	0.4 ± 0.1	13.6 ± 0.7

for different immersion times. In all cases, the results were considered statistically different at $p < 0.05$.

3. Results and discussion

3.1. Characterization of as-sintered bodies

3.1.1. Morphology

The morphologies of a series of as-sintered porous bodies are shown in Fig. 1. The SEM micrographs showed the pore structure of calcium phosphate bodies essentially to be as an assembly of macropores and micropores. Macropore sizes as large as hundreds of micrometers were generated and micropores were less than 10 μm. It is obvious that introduction of PVA in the ceramic powder resulted in bodies with macropores and micropores as desired, besides the dimensional change associated with the MCPM itself during the thermal treatment at 900 °C (described later). PVA served as a pore-forming agent because of the evolution of water and carbon dioxide during sinter processing, leading to the development of the highly porous microstructure [16].

3.1.2. Porosity

Table 1 lists the porosity measured by simple weighting and by Archimedes principle. In an earlier report [17], it was found that the optimal sintering temperature (in terms of mechanical properties) for the mixture of MCPM and PVA was in the neighborhood of 900 °C. The final dimensional changes of the bodies ranged from −8.4 to 3.2%. Due to shrinkage effect [18,19], the diameter of all sintered specimens was reduced but C4P1. This change in C4P1 body might be explained by the thermal expansion of MCPM which polymerized into the polyphosphate material. When sintered at 900 °C for 3 h in air, porous bodies of around 80% porosity were achieved. C4P1 sample had the higher porosity possibly due to the double role of PVA both as binder and pore-former.

3.1.3. Mechanical properties

The addition of PVA powder was in the range 20–40 wt.% such that the effect of the organic additive on the mechanical strength can be assessed. The variations in the tensile strength of as-sintered bodies were found to depend on the PVA contents (Table 1). One-way ANOVA analysis of the tensile strength data showed that the variation in strength

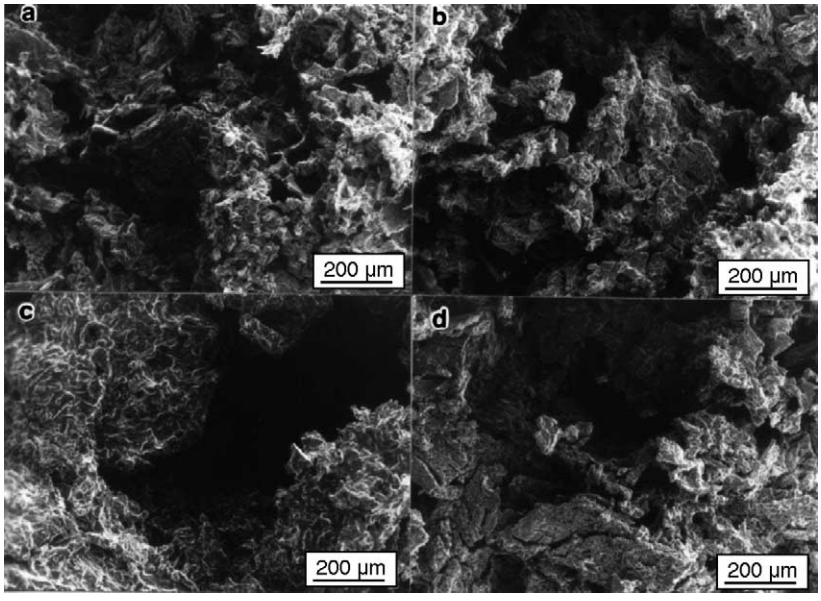

Fig. 1. SEM micrographs of porous calcium phosphate bodies prepared with the weight fractions of PVA particulates of 20% (a), 25% (b), 33% (c), and 40% (d).

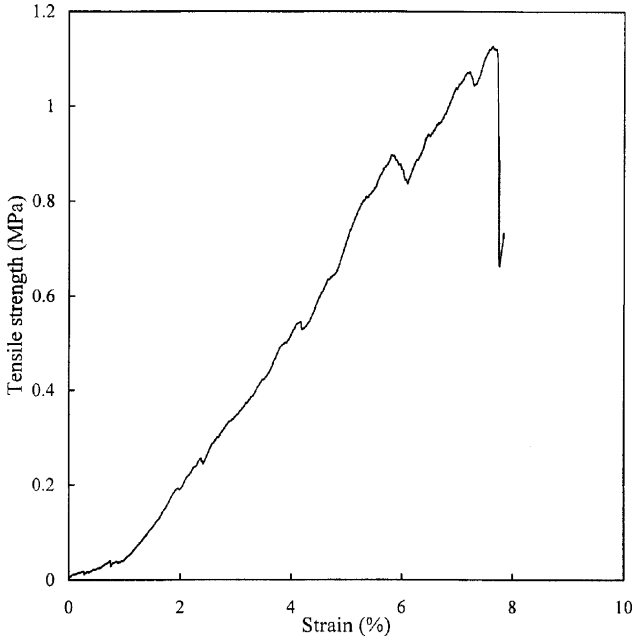

Fig. 2. A typical stress–strain curve for a 3C1P sample.

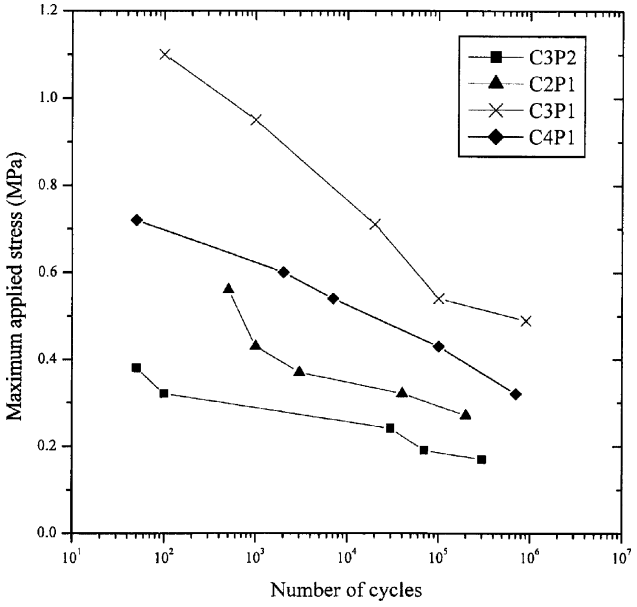

Fig. 3. Maximum stress applied in diametral tensile stress cyclic fatigue versus the number of cycles to failure for different porous bodies in Hanks' solution at room temperature.

between samples was significant ($p < 0.05$). The addition of PVA up to 25 wt.% achieved increased tensile strength. Indeed PVA is a very commonly used binder that can improve the strength, in addition to the role as pore-former [20]. However, higher PVA contents adversely affected the mechanical properties with a reduction of up to 67% of the highest value. The porous bodies consisting of smaller macropores exhibited higher strength in comparison with those with larger macropore [21], according to previous findings [22].

The stress–strain curve showed a response typical for a brittle solid (Fig. 2). The elastic modulus of porous bodies in the range of 12.1–18.0 MPa (Table 1) also varied depending on the amounts of the added PVA, presenting a trend similar to the change in strength.

3.2. Characterization of immersed bodies

3.2.1. In vitro fatigue test

The tissue-engineered porous materials must be evaluated not only by their in vitro strength as described later, but also by resistance to fatigue in solution because of the cyclic nature of in vivo loading [23–24]. However, relatively little information is available in the literature for fatigue behavior in physiological solution.

Results of fatigue experiments are presented as S–N diagrams (the so-called fatigue life diagram) where S is the maximum stress in a cyclic loading, and N is the number of cycles until fracture. Fig. 3 is a plot of the maximum stress applied in diametral compressive cyclic fatigue against the number of cycles to failure at room temperature in Hanks' solution. The graph shows that the stability of the porous bodies was apparently affected by the cyclic loading, with a remarkable decrease in the strength as the number of cycles increased. For example, sample C3P1 fatigued in Hanks' solution for 9×10^5 cycles had a significant degradation down to 40% of the original strength. In addition to the factors of slow or subcritical crack growth, occurring under stress, the decrease of the tensile strength of such brittle ceramic materials was caused by environmental factors [23,25].

The worrisome conclusion may be drawn that porous calcium phosphates as such do have a limited resistance to fatigue failure. However, it provided that the fatigue properties of porous scaffolds should be a noticeable factor. In clinical practice, this means that these porous bodies can only be used as non-load bearing implants.

3.2.2. Weight loss

To further study the immersion-induced dissolution/degradation process, a series of weight change measurements were performed for all immersed samples. Fig. 4 shows that all samples continue to dissolve after immersion in Hanks' solution. The dissolution behavior of immersed calcium phosphate materials in simulated physiological solution has been reported [26,27]. The resulting weight loss indicated that sample C4P1 had the largest weight loss with degradation time, reaching up to 11% after 90 days; however, the weight loss still remained about 6% over 90 days of immersion for all other samples. The decrease in sample weight might be explained by degradation of such porous materials.

3.2.3. Mechanical properties

The variation in the tensile strength with immersion time of the series of porous bodies is presented in Fig. 5. Six immersion regimes of 1, 3, 7, 15, 30 and 90 days were

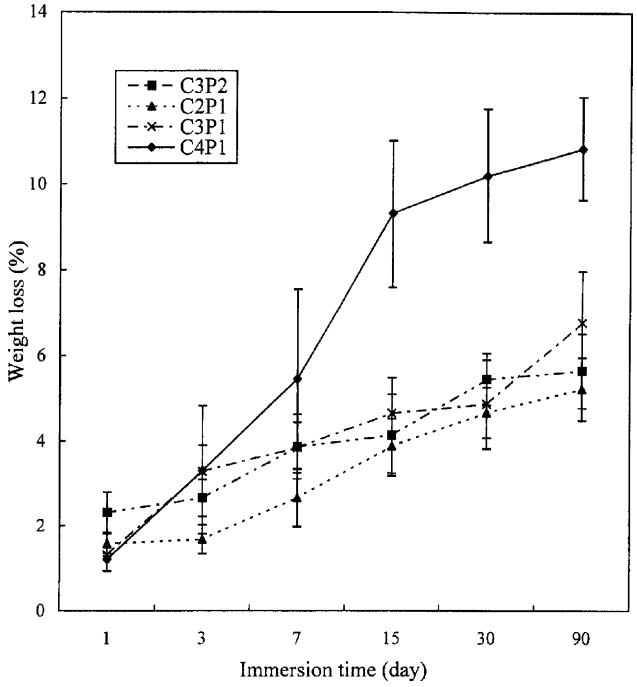

Fig. 4. Weight loss of various porous bodies after immersion in Hanks' solution.

selected for testing the porous bodies. The results revealed that, when immersed in Hanks' solution, the four different types of porous samples gradually lost the strength with increasing immersion time. The strength of sample C3P1 was significantly reduced from the initial strength of 1.2 MPa down to 1.0 MPa after one-day immersion ($p < 0.05$). When immersed for 90 days, its strength decreased to 0.4 MPa. The statistical analysis using Scheffe multiple comparison testing showed that the tensile strength of immersed calcium phosphates mixed with 20 wt.% PVA in green sample (C4P1) significantly declined by about 60% after immersion for 90 days ($p < 0.05$). When the green bodies comprised 33 and 40 wt.% PVA, the resulting porous bodies lost 58% and 64% of the as-sintered strengths after 90-day immersion, respectively. This deterioration in the strength seems unavoidable for biodegradable porous ceramics immersed in physiological solution and has also been observed in other studies [7,14,26]. Porter et al. measured bending strength degradation of 64–100% of the original strength when porous calcium polyphosphates were immersed in a tris-buffered solution. The immersion-induced decline in mechanical strength was due to less stable zones (particle surfaces or interface regions of grains) of porous ceramic body, where the degradation occurred more rapidly [14]. Besides the porous structures of calcium phosphates, the dissolution of the calcium phosphate in the solution and the penetration of water/ions resulting from the solution possibly accounted for the deterioration in mechanical properties [13,28].

The variations of the elastic modulus against immersion time for porous bodies are shown in Fig. 6, as a function of PVA content and immersion time. After immersion in solution over 90 days, there was also a pronounced decrease

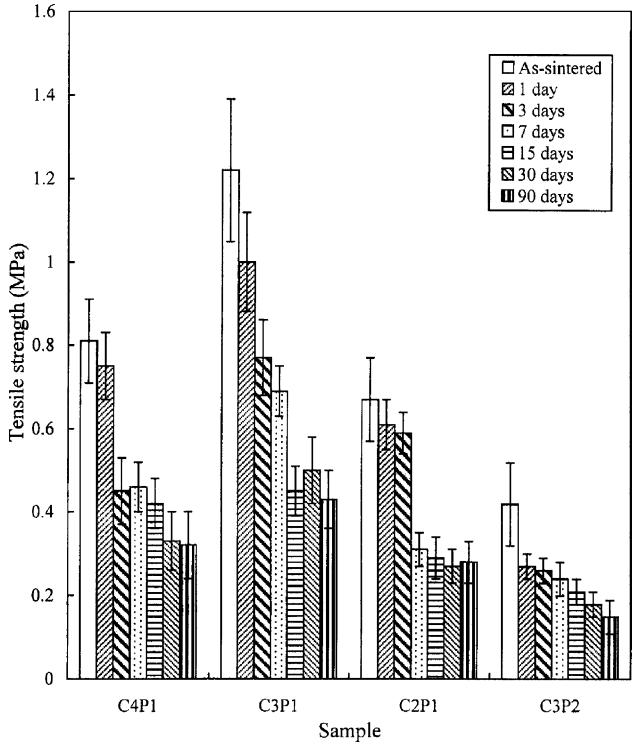

Fig. 5. The variations of tensile strength against immersion time for different porous bodies.

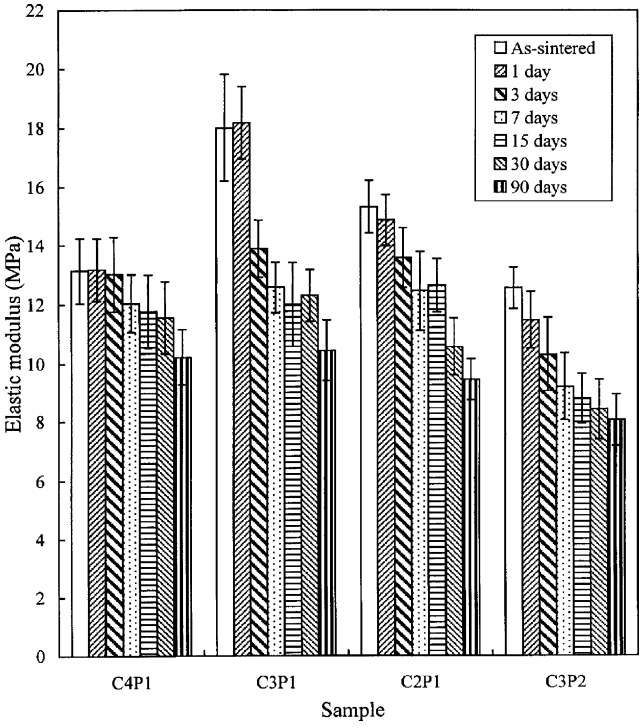

Fig. 6. Tensile modulus of immersed samples as a function of the immersion time.

in the elastic modulus for all porous bodies, similarly to the tensile strength.

4. Conclusions

Results showed the amounts of PVA added as a pore-former and binder to calcium phosphate ceramics to have an important effect on mechanical properties. The fatigue resistance of all porous bodies was found to worstly decline and their texture delaminated when immersed in Hanks' solution. Although possessing a low strength, susceptibility to solution attack, and poor fatigue properties, biomedical uses of present porous materials might be for non-load bearing applications such as bone defect repair.

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