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Preparation and properties of a porous calcium phosphate bone graft substitute

S. M. RABIEE[1,2*], F. MOZTARZADEH[1], H. S. KENARI[3], M. SOLATI-HASHJIN[1]

[1]Department of Medical Engineering, Amirkabir University, Hafis Ave.,
Tehran, Iran, P. O. Box: 15875-4413

[2]Department of Engineering and Technology, Mazandaran University, Babolsar, Iran

[3]Department of Polymer-Chemical Engineering, Sahand University of Technology, Tabriz, Iran

The aim of this study was to obtain a biodegradable porous calcium phosphate implants as synthetic bone graft substitutes. Calcium phosphate used in this study consisted of hydroxyapatite and anhydrous dicalcium phosphate. Four different types of porous calcium phosphates were made on addition of polyurethane by sinter processing. The X-ray diffractometry revealed that β-tricalcium phosphate is a dominating phase. Experimental results showed the pore microstructure of the as-sintered bodies to consist of the macropores and micropores. Surface morphology analysis and porosity evaluations were performed. The variation in the compressive strength, elastic modulus and dissolution behaviour of immersed synthetic bone grafts in simulated physiological solutions was investigated.

Key words: *bioceramics; calcium phosphate; porosity; synthetic bone graft substitute*

1. Introduction

Various phases of calcium phosphates were employed to fabricate porous scaffolds to accommodate bone tissue regeneration in vitro or in vivo. The atomic ratio of Ca/P in calcium phosphates can be varied between 1 and 2 to produce compounds ranging from calcium tetraphosphate ($Ca_4P_2O_9$), hydroxyapatite (HA) $Ca_{10}(PO_4)_6(OH)_2$, tricalcium phosphate (TCP) $Ca_3(PO_4)_2$ to anhydrous dicalcium phosphate (DCP) $CaHPO_4$. The degradability of calcium phosphates generally varies with the Ca/P ratio, the highest being of DCP that usually results in the most extensive bone remodelling around the scaffold [1–5].

Porous biodegradable synthetic materials, such as calcium phosphates, poly(lactic acid) (PLA), and poly(glycolic acid) (PGA), are currently tested as implants for the

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[*] Corresponding author, e-mail: r_rabiee@aut.ac.ir

regeneration of damaged and diseased tissues [6–10]. These synthetic biomaterials were chosen based on their biocompability and mechanical properties. Concerning the fabrication of porous ceramic bodies, several techniques such as the use of polymeric sponge [11] and organic additives [12–14], and molding processes [15], have been used. For example, Prado da Silva et al. [12] 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 butyral) powders, Liu [13] fabricated porous HA granules with controlled porosity, pore size, pore size distribution and granule size by using a drip-casting process. Flautre et al. [14] used polymethylmethacrylate microbead as the porous agent to make of HA ceramics of various porosities. The available synthetic biodegradable calcium phosphate ceramics for bone tissue regeneration includes HA, β-tricalcium phosphate (β-TCP) and calcium polyphosphate [10, 11]. The biodegradation behaviour of calcium phosphate materials in both in vitro and animal studies has been reported [16–18]. Shima et al. [16] 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. [17] found a decline in mechanical properties in tris-buffered solution. Pilliar et al. [18] studied the effect of particle size of calcium polyphosphates on in vitro degradation. Their findings 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.

Previous studies have established that HA in various forms is most widely used but concerns have been raised with regard to the limited degradation properties of this material [19, 20]. Thus, in order to improve their current properties, we have used DCP in combination with HA. In the present study, porous calcium phosphates were prepared by sintering mixtures of HA, DCP and polyurethane (PU). To impart porosity to the ceramic body, PU was burned out during firing, leaving free space in the resulting body.

2. Experimental

Commercially pure hydroxyapatite and anhydrous dicalcium phosphate powders (Merck, Germany) were used. Powder of HA/DCP(HD), with 3:1 weight ratio, was prepared in a planetary ball mil (Retch PMA, Brinkman, USA) for 30 min to ensure homogeneity. The grain was observed by scanning electron microscopy (SEM: Stereoscan 360-Leica Cambridge). The specific surface area was determined by 15-point BET measurement (Micromeritics Gemini 2360). The particles are composed of primary particles with sizes smaller than 10 µm. Specific surface area of the powder was 57 m^2/g. The as-received HD powder was directly mixed with PU in a vacuum mixer (VM-112T, J. Morita, Saitama, Japan) for 15 min to ensure homogeneity. The PU powders have different size fractions, i.e. 100 µm and 400 µm. Four different types of the porous bodies were prepared from mixtures of HD and PU powders with different ratios (4:1, 3:1, 2:1 and 1:1 by weight) (Table 1). The mixture was uniaxially pressed in a cylindrical stainless mold (6×12 mm^2) at 5 MPa. The green bodies were heated up to 1200 °C in a schedule which minimized disruption during pyrolysis and allowed the bioceramic to achieve high density. This heating schedule consisted of the heating rate of 0.5 °C/min up to 800 °C, 1 h held at this temperature and rapid heating at 5 °C/min from 800 to 1200 °C, 3h held at 1200 °C and then cooling in the furnace. The structure of the sintered material was analyzed by X-ray diffraction method using a Siemens D5000 diffractometer, operating at K_α radiation.

Table 1: Composition of various samples and their properties

Sample code	Composition HD:PU [wt. ratio]	Change in diameter [%]	Porosity		Compressive strength ±S.D. [MPa]	Compressive modulus ±S.D. [MPa]
			By geometry	By Archimedes		
HD4	4:1	2.9±1.1	72±0.1	79.8±4.6	8.0±1.1	131±11
HD3	3:1	–3.1±0.9	70.1±0.1	73.3±4.1	12.1±1.2	180±18
HD2	2:1	8.1±1.5	66.4±0.1	68.6±3.8	7.4±1.4	153±9
HD1	1:1	–6.9±1.0	68.7±0.1	66.0±2.7	4±0.6	136±7

The measurement of the porosity was carried out in two different ways. The first method consisted in calculation 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. The difference between the unity and the relative density is the total porosity. The other measurement was conducted by using a conventional Archimedes immersion technique with ethanol [21]. The average value of six determinations was taken as the porosity of sintered bodies. The extracellular solution with the 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 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 $MgCl_2.6H_2O$, 0.14 g $CaCl_2$, 0.06 g $Na_2HPO_4.2H_2O$, 0.06 g $MgSO_4.7H_2O$, 1.00 g glucose in 1000 ml of distilled H_2O. The solution had the initial pH equal to 7.4.

For the immersion test, the specimens were immersed in 10 ml of the solution for the predetermined periods of time at 37 ºC. 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 behaviour was also monitored through the sample weight change. Prior to weighing with a 4-digital balance (AE 240S, Mettler-Toledo AG, Greifensee, Switzerland), the immersed specimens were dried at 100 ºC for 3 h in an oven. At least seven samples were tested for each measurement. All mechanical testing was performed using a Zwick/Roell 2005 with a crosshead speed of 0.01 mm/s. The compressive strength (Cs) of the sample was calculated as $Cs = F/A$, where F is the maximum compressive load (N), A the average cross-sectional area (mm^2) of the sample. The diameter and length of each specimen were measured by using a micrometer. The largest compression load at failure was obtained from the recorded load–deflection curves. Young's moduli of the samples were 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 compressive strength data. Tukey HSD multiple comparison testing was used to determine the significance of the deviations in the strength of each sample for different immersion times. In all cases, the results were considered statistically different at $p < 0.05$.

3. Results and discussion

Porous bioceramics of HA/DCP generally seem to biodegrade much more rapidly than do bioceramics made of HA, because dicalcium phosphate resorbed much more rapidly than hydroxyapatite implants of a similar structure [1, 22]. The bioresorbability of calcium phosphate materials appears to be dependent on their chemical/crystal composition, their structure as "materials", and on the environment at the implantation site [4, 5, 22]. Jarcho proposed the existence of two different biologic resorption pathways: one involving solution-mediated processes (implant solubility in physiologic solutions) and the second involving cell-mediated processes (phagocytosis) [4]. Contrary to expectations, the material more closely resembling the body own hard tissue component, the hydroxyapatite, was found to dissolve much more slowly than many of calcium phosphates not naturally occurring in bone if similar ceramic structures and similar degrees of purity were used [4, 5, 23]. It can be stated that implants of crystalline HA have a lesser tendency to bioresorb because of their chemistry and their small surface area. Table 1 lists the porosities measured by simple weighting and by Archimedes principle. The final dimensional changes of the bodies ranged from –8.1 to 2.9%. Due to shrinkage effect [27, 28], the diameters of all sintered specimens were reduced, except for HD4. This change in HD4 body might be explained by the thermal expansion of HD which polymerized into the polyphosphate material. When sintered at 1200 ºC in air, porous bodies of ca. 70% porosity were achieved.

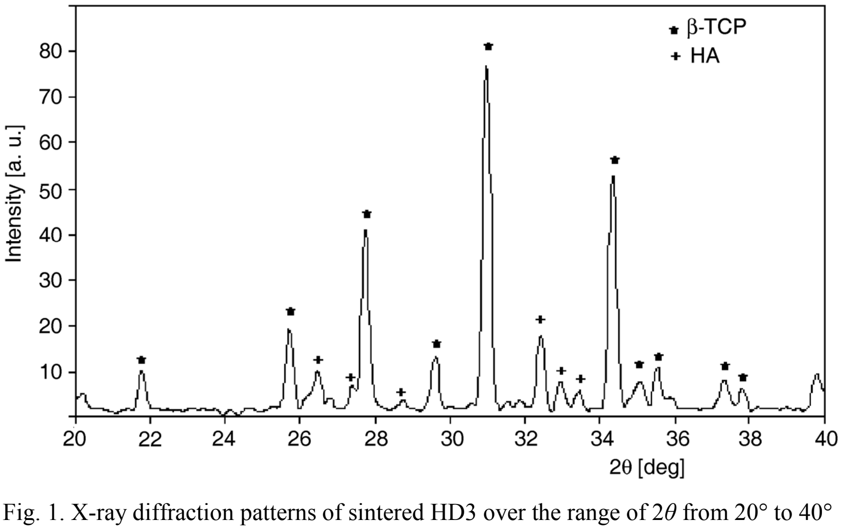

Fig. 1. X-ray diffraction patterns of sintered HD3 over the range of 2θ from 20° to 40°

XRD patterns of sintered samples showed biphasic mixtures of β-TCP and HA (Fig. 1). No obvious difference could be detected in the XRD patterns of mixtures of HD and PU (HD1, HD2, HD3 and HD4). The conversion of $CaHPO_4$ into $Ca_2P_2O_7$ began at 700 ºC and above that temperature, $Ca_2P_2O_7$ and HA transformed into $Ca_3(PO_4)_2$ according to Eqs. (1) and (2) [24, 25, 32]. A little part of HA phase did not change into other phases during the sintering.

$$2CaHPO_4 \rightarrow Ca_2P_2O_7 + H_2O \tag{1}$$

$$Ca_2P_2O_7 + Ca_{10}(PO_4)_6(OH)_2 \rightarrow 4Ca_3(PO_4)_2 + H_2O \tag{2}$$

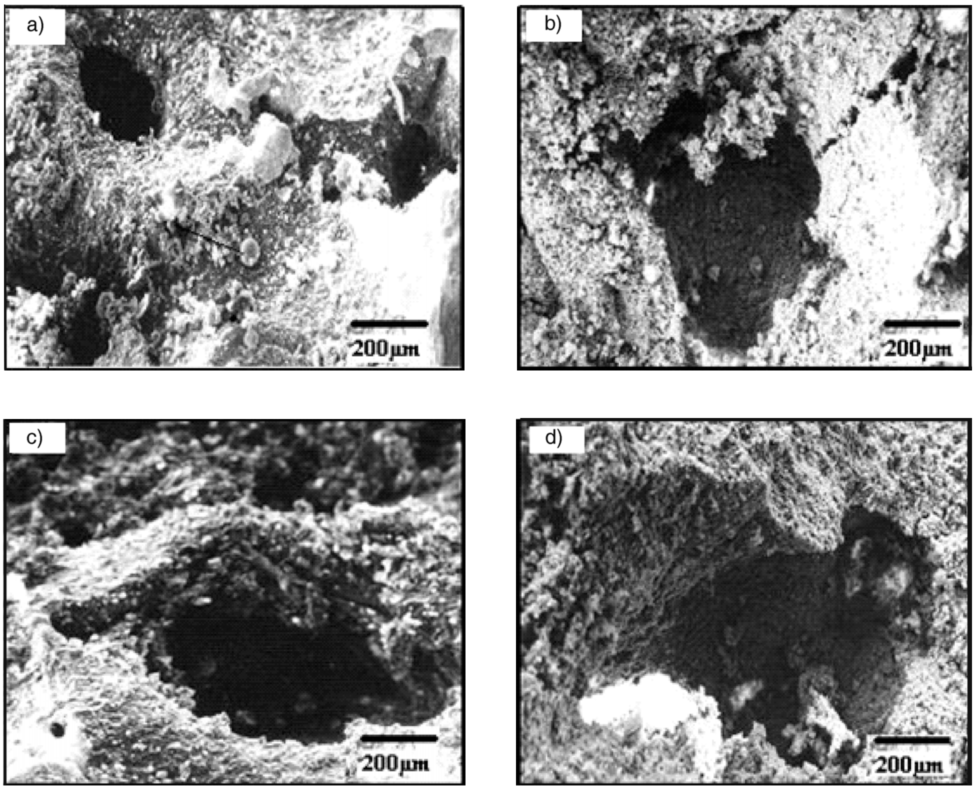

Fig. 2. SEM micrographs of sintered calcium phosphate
with various ratios of PU: a) HD4, b) HD3, c) HD2, d) HD1

The morphologies of a series of as-sintered porous bodies are shown in Fig. 2. 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 20 µm. It is obvious that introduction of PU in the HD powder resulted in bodies with macropores and micropores. PU 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 [26].

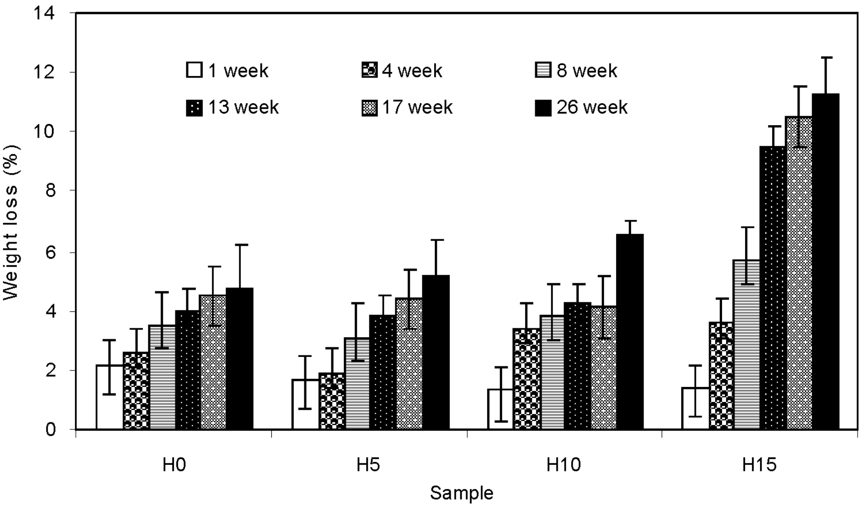

Fig. 3. Weight losses of porous bodies after immersion in Hanks' solution

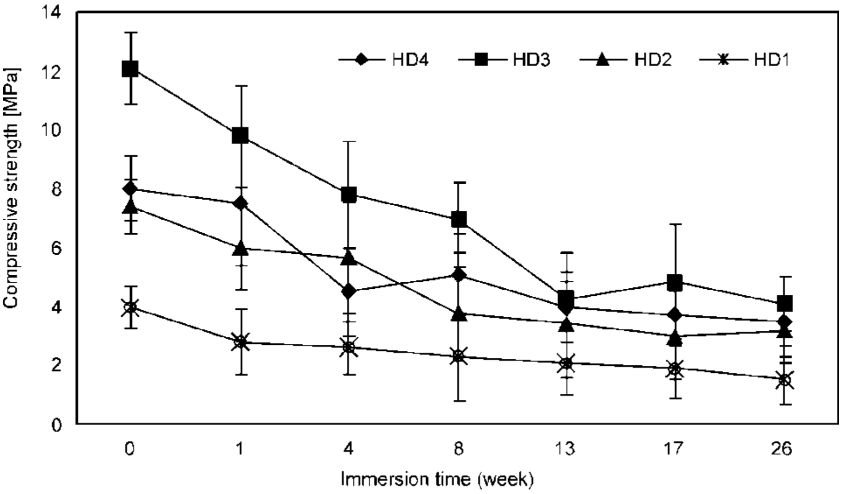

Fig. 4. compressive strength of immersed samples as a function of the immersion time

To further study the immersion-induced dissolution/degradation process, a series of weight change measurements were performed for all immersed samples. Figure 3 shows that all samples continue to dissolve after immersion in Hanks' solution. The dissolution behaviour of immersed calcium phosphate materials in simulated physiological solution has been reported [29, 30]. The resulting weight loss indicated that sample HD4 had the largest weight loss with degradation time, reaching up to 11% after 26 weeks; however, the weight loss still remained about 6% over 26 weeks of immersion for all other samples. The decrease in sample weight might be explained by degradation of such porous materials.

The variation in the compressive strength with immersion time of the series of porous bodies is presented in Fig. 4. Six immersion regimes of 1, 4, 8, 13, 17 and 26 weeks were selected for testing the porous bodies. The results revealed that, when immersed in Hanks' solution, four different types of porous samples gradually lost the strength with increasing immersion time. The strength of the sample HD3 was significantly reduced from the initial strength of 12.1 MPa down to 9.8 MPa after one-week immersion ($p < 0.05$). When immersed for 26 weeks, its strength decreased to 4.1 MPa. The statistical analysis using Tukey HSD multiple comparison testing showed that the compressive strength of immersed calcium phosphates mixed with 20 wt. % PU in green sample (HD4) significantly declined by about 50% after immersion for 26 weeks ($p < 0.05$). When the green bodies comprised 33 and 50 wt.% PU, the resulting porous bodies lost 48% and 54% of the as-sintered strengths after 26-week 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 [17, 18, 29]. 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 due to less stable zones (particle surfaces or interface regions of grains) of porous ceramic body, where the degradation occurred more rapidly [17]. 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 [16, 31].

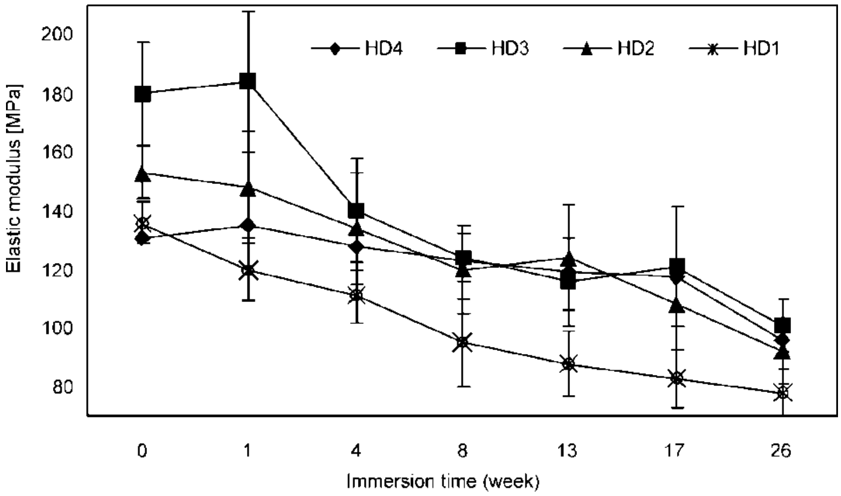

Fig. 5. The variations of the elastic modulus against immersion time for porous samples

The variations of the elastic modulus against immersion time for porous bodies are shown in Fig. 5 as a function of PU content and immersion time. After immersion in solution over 26 weeks, there was also a pronounced decrease in the elastic modulus for all porous bodies, similarly to the compressive strength.

4. Conclusions

The porous HA/DCP bioceramic with various amounts of polyurethane powder as a pore-former can be easily fabricated. The XRD pattern of sintered porous samples showed β-TCP and amount of HA phases. Results showed the compressive strength and elastic modulus of the porous bodies was dependent on the contents of the PU additive. The weight loss data confirmed the degradation behaviour of the porous bodies in Hank's solution. The results revealed that, when immersed in Hanks' solution, the different types of porous samples gradually lost the strength and weight with increasing immersion time.

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