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Preparation of Porous $Ca_{10}(PO_4)_6(OH)_2$ and β-$Ca_3(PO_4)_2$ Bioceramics

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Submicrometer-sized, pure calcium hydroxyapatite (HA, $(Ca_{10}(PO_4)_6(OH)_2)$) and β-tricalcium phosphate (β-TCP, $Ca_3(PO_4)_2$) bioceramic powders, that have been synthesized via chemical precipitation techniques, were used in the preparation of aqueous slurries that contained methyl cellulose to manufacture porous (70%–95% porosity) HA or β-TCP ceramics. The pore sizes in HA bioceramics of this study were 200–400 μm, whereas those of β-TCP bioceramics were 100–300 μm. The pore morphology and total porosity of the HA and β-TCP samples were investigated via scanning electron microscopy, water absorption, and computerized tomography.

I. Background

HUMAN cortical (i.e., dense) bone consists of cylindrical channels (i.e., osteons) that are held together by a framework of hard tissue, mainly natural hydroxyapatite. Cylindrical fibers of collagen (the major organic component of bone) fill the pores (190–230 μm in size) of these bones. The inorganic matrix of cortical bone consists of a porous structure with an interconnected porosity of ~65%.[1] For bone ingrowth to occur readily into a porous ceramic bone-substitute material, which mimics the natural bone structure, the typical pore size must be >100 μm.[2] Trabecular (i.e., spongy) bone, on the other hand, differs from cortical bone in that the former is more open-spaced than the latter and has noncylindrical collagen-filled pores. The pore sizes in trabecular bone are larger, in the range of 500–600 μm.[1]

Slosarczyk[3] prepared porous (35% porosity) calcium hydroxyapatite ceramics by impregnating a polyurethane-based sponge (or foam, which was used by other researchers[4–7]) with a basic (pH ≥9) slurry that consisted of a suspension of fine $Ca(OH)_2$ precipitates in the presence of dissolved H_3PO_4. Fabbri *et al.*,[8] on the other hand, used commercial calcium hydroxyapatite (HA, $(Ca_{10}(PO_4)_6(OH)_2)$) powders to prepare pH-regulated ceramic slurries to impregnate cellulose-based foam bodies that had different porosities (in the range of 70%–83%).

Other methods also have been used to manufacture porous bioceramics. Among these methods is the blending of appropriate amounts of calcium phosphate powders with fine organic powders, such as polvinyl butyral (PVB),[9–11] starch, polystyrene, or polymethyl metacrylate,[12] followed by controlled organic burnout, to achieve a porous structure that is created by the gas bubbles that form.

Ryshkewitch[13] was the first to prepare porous ceramics, using a viscous slip of an oxide powder (either Al_2O_3 or ZrO_2, in an aqueous solution that contained ~0.2% polyvinyl alcohol (PVA)) that was treated with a solution of H_2O_2. Porous (5%–60% porosity) Al_2O_3 or ZrO_2 bodies were prepared successfully after slow firing at a peak temperature of 1850°C.[13] Klein and coworkers[14,15] used the same technique as Ryshkewitch[13] did to manufacture porous HA bioceramics.

The use of "coral" (made by marine invertebrates, which extract calcium and carbonate from the sea reservoir to naturally assemble the limestone matrix in which they live) to produce porous HA bioceramics also has been examined.[16] Calcium carbonate coral skeletons were reacted with diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) via a hydrothermal exchange reaction between carbonate and phosphate salts and converted to HA. Those researchers claimed that the *in situ* porous structure (~65% porosity) of corals was preserved.[16]

Successful experiments on animals (usually rats, rabbits, and dogs) led to clinical applications of porous calcium phosphate bioceramics in humans over the last two decades. These experiments included the grafting of periodontal defects, post-traumatic long bone defects, and augmentation of the alveolar ridge and the maxillofacial skeleton.[17–22]

This study[23,24] is a systematic attempt to prepare highly porous HA and β-tricalcium phosphate (TCP) bioceramics. A novel, methyl cellulose gel-casting technique, using aqueous suspensions of chemically synthesized,[25,26] phase-pure HA and β-TCP powders, was applied.

II. Experimental Procedure

(1) Sample Preparation

Pure HA and β-TCP powders were prepared via a wet-chemical synthesis technique, based on the precipitation of HA or β-TCP precursors from aqueous solutions (of appropriate amounts and concentration) of calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$) and $(NH_4)_2HPO_4$. The details of the powder synthesis and characterization procedures also have been described elsewhere previously.[25,26]

(A) Porous HA Preparation: First, 0.152 mol of $Ca(NO_3)_2{\cdot}4H_2O$ (99% pure; Lot No. A815220, Riedel-de-Haen, Seelze, Germany) and 0.090 mol of $(NH_4)_2HPO_4$ (99% pure; Lot No. A839106, Merck, Darmstadt, Germany) were completely dissolved in 1450 mL of deionized water. Then, 340 mL of 25 vol% NH_4OH solution was immediately added to the opaque solution. The reaction beaker was heated at a temperature of 65°C for 90 min under vigorous stirring. The beaker was later sealed, and the solution was heated to its boiling point.[25] After 2 h of boiling, the solution was cooled to room temperature and the precipitates were allowed to settle overnight. HA precursors were recovered from their mother liquors via vacuum filtration, using a Büchner funnel. The resulting calcium phosphate creams were transferred to an empty beaker, and methyl cellulose powder (the viscosity of a 2% aqueous solution at 25°C was 4000 cP; Lot No. 73H0365, Sigma Chemical Co., St. Louis, MO) was added (in the range of 8–13 g/L). Then, 400–800 mL of deionized water was added; this mixture was subjected to ultrasonication, using an ultrasonic disruptor (Model XLS-2015, Misonix, Inc., Farmingdale, NY) for ~45 min.[23,24] The final mixtures were dried into ceramic cakes in 24 h at a temperature of 90°C in a stagnant-air oven.

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Table I. Thermal Treatment of Porous HA or β-TCP Parts

Treatment temperature (°C)	Treatment time (min)	
	HA	β-TCP
25–250	400	400
250–250	20	20
250–1250	300	450
1250–1250	300	300
1250–25	400	600

(B) Porous β-TCP Preparation: First, 0.075 mol of $Ca(NO_3)_2 \cdot 4H_2O$ was dissolved in 190 mL of deionized water and heated to a temperature of ~40°C. Subsequently, 0.054 mol of $(NH_4)_2HPO_4$ were dissolved in 350 mL of deionized water in a separate beaker, and half of this solution was added simultaneously to the $Ca(NO_3)_2 \cdot 4H_2O$. The resulting opaque solution was stirred for 5 min, and the remaining half of the $(NH_4)_2HPO_4$ solution was added to the reaction beaker immediately. Nine milliliters of a 1:1 (by volume) concentrated (26 vol%) NH_4OH–H_2O mixture also was added, to stabilize the pH at a value of 5, after 5 min of stirring at 40°C. Then, the solution was stirred at 60°C for 65 min. Later, the solution was cooled to room temperature during stirring. The white precipitates finally were aged and allowed to settle for 18 h at room temperature in the reaction beaker.[25]

Two grams of sodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) and 2 g of trisodium phosphate ($Na_3PO_4 \cdot 12H_2O$) (each 99% pure, Horasan Kimya, Ankara, Turkey) were weighed and placed in a glass beaker (capacity of 100 mL). Ninety milliliters of deionized water was added into this beaker, and this mixture was stirred at room temperature until a clear solution was obtained. Then, the prepared "citrate–phosphate solution" was stored at a temperature of 3° ± 1°C.

β-TCP precursors were recovered from their mother liquors via vacuum filtration, using a Büchner funnel. First, wet TCP precursor creams (twice the amount described earlier) were placed into an empty beaker. Then, methyl cellulose powder was added (4–6 g/L) into this cream, together with 375 mL of deionized water. This mixture was subjected to ultrasonication for ~35 min. Finally, a 40 mL aliquot of the above-mentioned citrate–phosphate solution was added to the beaker and stirred vigorously for 10 min or ultrasonicated for a few more minutes.[23,24] The ceramic-slurry mixtures of β-TCP precursor and methyl cellulose finally were dried in a stagnant-air oven at a temperature of 90°C for 24 h.

(C) Sintering: The dried HA (or β-TCP) parts were sintered in an electrically heated box furnace in an air atmosphere. The heating and cooling schedules that were used to sinter the HA or β-TCP parts are given in Table I.

(2) Sample Characterization

The pore morphology and pore-size distribution of the HA and β-TCP bioceramic samples were investigated using scanning electron microscopy (SEM) (Model JSM-6400, JEOL, Tokyo, Japan). The samples first were sputter-coated with a layer of gold–palladium alloy (150 Å thick). Lineal analysis of the SEM micrographs was used to measure the pore sizes.[27]

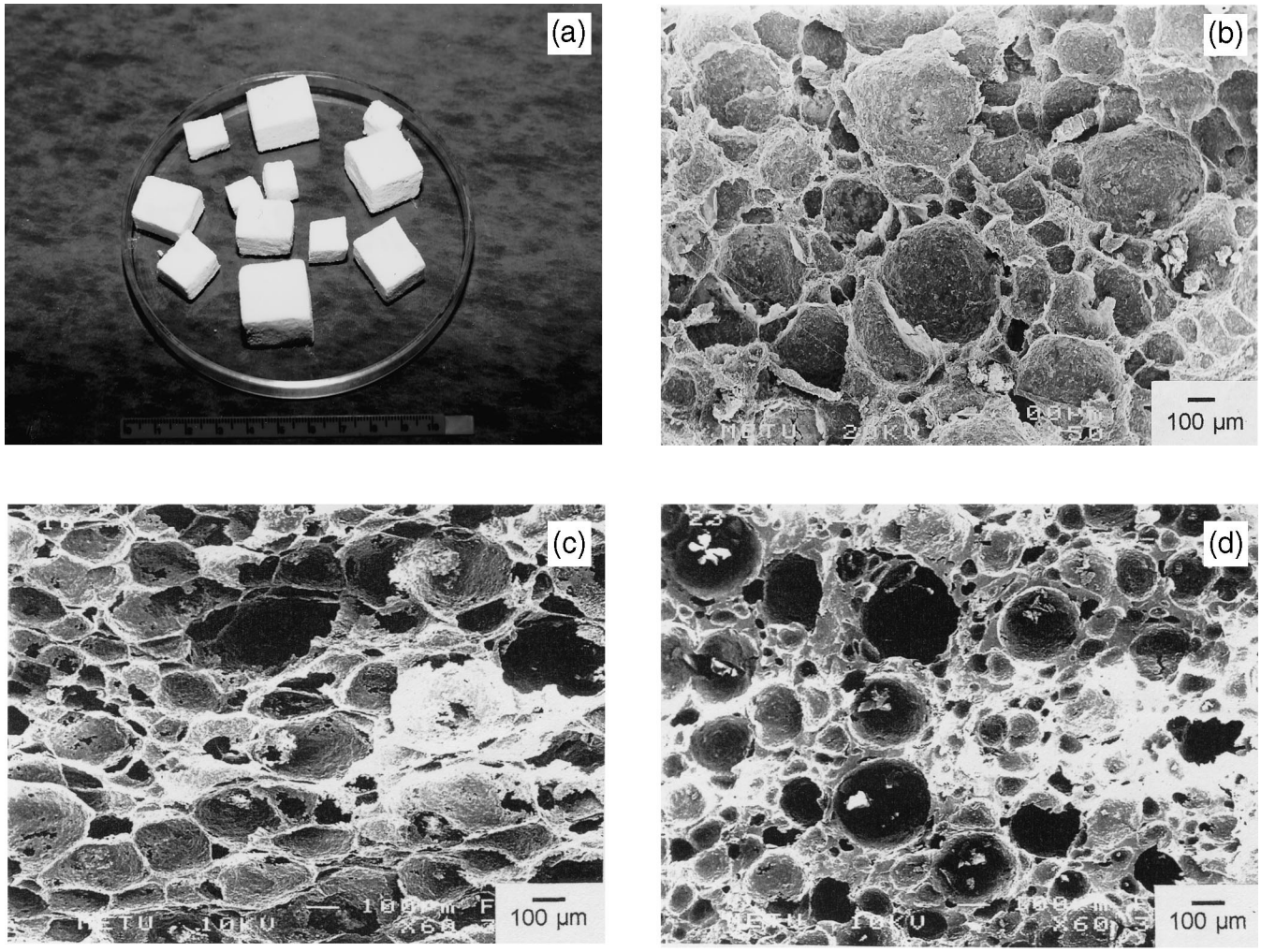

Fig. 1. (a) Photograph of green HA or TCP parts. SEM micrographs of green HA samples and sintered HA samples (90% porosity) are shown in Figs. 1(b) and (c), and Fig. 1(d) shows an SEM micrograph of a sintered β-TCP sample (75% porosity).

The density and porosity were measured using the Archimedes principle. Further details of the methods used to measure the percentage of water absorption and apparent porosity values have been described elsewhere.[28]

The phase purity of the porous HA or β-TCP blocks was checked using powder X-ray diffractometry (XRD) (Model D-Max/B, Rigaku Co., Tokyo, Japan) with a step size of 0.05 and a count time of 5 s; the XRD operating conditions were 40 kV and 20 mA, using a graphite filter and monochromated Cu$K\alpha_1$ radiation. These blocks were confirmed to be single-phase HA or β-TCP. Computerized tomography (CT) runs were performed with a third-generation scanner (Model Tomoscan TX60, Philips Research Laboratories, Eindhoven, The Netherlands) to determine the total porosity.

The fracture strengths were determined using three-point bending tests. These strengths S were calculated using the general flexure stress formula:

$$S = \frac{3PL}{2bd^2} \tag{1}$$

where P is the fracture load (in kilograms), L the span length, b the width of the sample, and d the thickness of the sample. (L, b, and d are all given in millimeters.) Samples used in these tests had typical dimensions of 30 mm × 15 mm × 5 mm.

III. Results and Discussion

HA and β-TCP powders that were synthesized in this study (after calcination) consisted of spherical, submicrometer-sized particles (for HA, the average particle size was 0.65 μm; for TCP, the average particle size was 0.8 μm). These powders had typical surface areas, as measured by the Brunauer–Emmett–Teller (BET) method, in the range of 45–50 m^2/g.[26]

Dried (at 90°C) and porous cakes of HA (or β-TCP) had almost the same shapes of the beakers in which they were produced and dried; then, they were cut into any desired prismatic shapes, using a surgeon's blade, as shown in Fig. 1(a). The outer skins of the 90°C-dried green HA or TCP chunks were much denser than their bulk; for this reason, the outer rims or skins of such cakes were removed and not used in any further experiments. The green (before sintering) HA or TCP samples, such as those shown in Fig. 1(a), were highly porous; they possessed a typical microstructure, as depicted in the SEM micrograph of Fig. 1(b). The unique cellular structure, which was caused by the gelation of methyl cellulose in HA or TCP suspensions, caused the formation of macropores during the drying stage, with a rather broad pore-size distribution around a mean value of a few hundred micrometers.

The SEM micrograph in Fig. 1(c) shows the typical pore morphology of the sintered HA samples (Ca/P = 1.674, as determined via inductively coupled plasma (ICP) analysis); a porosity of 90% ± 1% was observed. The technique of water absorption may be regarded as yielding porosity figures that are ~5% higher than the actual porosity (as confirmed by CT runs that were performed on the same samples), which, most probably, should be caused by the excess water that is still retained at the sample surfaces while the "wet weights" were measured on an analytical balance. Such samples were produced from HA suspensions that had a methyl cellulose content in the range of 6–8 g/L; then, these samples were sliced in random directions before sintering. These specimens consisted of spherical or ellipsoidal "cells" in their matrixes. The typical cell diameter in all the porous HA samples, regardless of the sample-specific percentage-porosity values to which they have been ascribed, had a value in the range of 200–400 μm (according to lineal analysis[27]).

The larger cells (or macropores), as shown in Fig. 1(c), were either always surrounded by micropores or interconnected with each other, which allows them to have high water-absorption figures (in the vicinity of 250%). The ability to absorb water also would render them highly biocompatible (in terms of the soft tissue reactions) when they are exposed with human body fluids in *in vivo* applications. The sintered HA samples, whose microstructures are shown in Figs. 1(c), had a flexure strength of 3.5 ± 0.15 MPa. Note that the flexural strengths of the trabecular bones of the human body were 2–10 MPa.[1]

The SEM micrograph shown in Fig. 1(d) displays the typical pore morphology of the sintered β-TCP samples (Ca/P = 1.502, as determined via ICP analysis); the sample had a porosity of 75% ± 1%. Such samples were produced from TCP suspensions that had a methyl cellulose content in the range of 3.9–5 g/L. The typical "spherical" cell diameter in the β-TCP samples, regardless of the sample-specific percentage-porosity values that they possessed, was in the range of 100–300 μm. The sintered TCP samples, whose microstructures were exemplified in Fig. 1(d), had a average flexure strength of 5.0 ± 0.15 MPa.

Such extremely porous HA or β-TCP samples may be useful as biocompatible implants, especially in the treatment of areas where tumoral formations have crowded certain portions of the trabecular parts of bones. These highly porous samples also may serve as long-term, slowly disintegrating drug-delivery agents in the calcium-phosphate-based hard tissues of the human body.

Throughout this work, the concentration of methyl cellulose (in units of g/L) in the prepared aqueous bioceramic (HA or TCP) slurries has been studied over a range of 3.9–7.9 g/L, as a function of the percentage porosity achieved in the sintered calcium phosphate (either β-TCP or HA) parts. The relationship between the concentration of methyl cellulose and percentage porosity is shown in Fig. 2. The apparent correlation between the polymer concentration and the achieved level of porosity clearly indicates that it was possible to pretailor the desired amounts of porosity over a range of 70%–92% (±1%) for the calcium phosphate bioceramics that were prepared from the submicrometer-sized, chemically uniform powders of this study. The scatter that is observed in the experimental porosity values at higher polymer concentrations (i.e., 7.5 and 7.9 g/L) might have resulted from the heterogeneities that resulted from the dispersion habits of the polymeric species in an aqueous ceramic slurry beyond a certain concentration, which then may cause an unpredictable increase in the percentage of closed pores following sintering. Finding the exact reasons of such scatter in the porosity values of calcium phosphate bioceramics certainly would warrant further research in this field.

IV. Conclusions

Macroporous, sintered calcium hydroxyapatite (HA) bioceramics were produced, using a novel aqueous combination of methyl

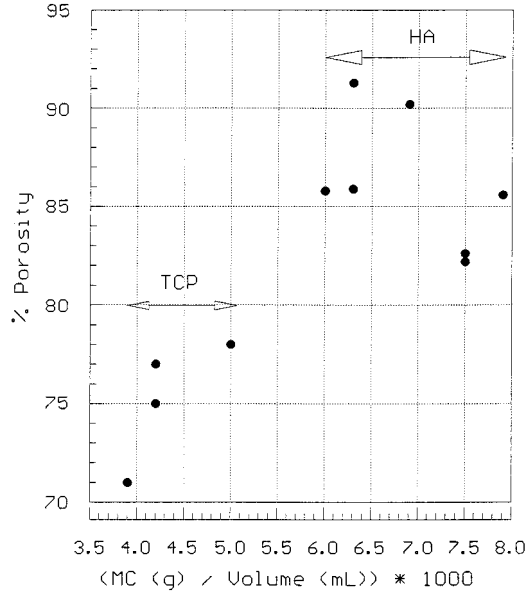

Fig. 2. Concentration of methyl cellulose in the bioceramic slurries, versus the percentage porosity in the sintered HA or β-TCP parts.

cellulose and synthetic submicrometer-sized HA powders, with final porosity values in the range of 80%–92% (±1%). The pore sizes of the sintered HA bioceramics varied over a range of 200–400 μm.

Macroporous, sintered β-TCP bioceramics also were produced, using the same novel technique, with final porosity values in the range of 71%–78% (±1%). The pore sizes of the β-TCP parts varied over a range of 100–300 μm.

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