

## MILLIMETER-SIZED GRANULES OF BRUSHITE AND OCTACALCIUM PHOSPHATE FROM MARBLE GRANULES

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### ABSTRACT

Brushite (DCPD, dicalcium phosphate dihydrate,  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ) and octacalcium phosphate (OCP,  $\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$ ) granules with diameters in the millimeter range were prepared by using marble (calcium carbonate) granules as the starting material. The method of this study simply comprised of soaking the marble granules in aqueous solutions containing phosphate and/or calcium ions at temperatures between 20° and 37°C. This process did not cause any size change between the initial marble and final brushite or octacalcium phosphate granules. Such DCPD and OCP granules of higher *in vitro* solubility than hydroxyapatite could be useful in maxillofacial, dental and orthopedic void/bone defect filling and grafting applications. Samples were characterized by X-ray diffraction, inductively-coupled plasma atomic emission spectroscopy, and scanning electron microscopy.

### INTRODUCTION

It is easy to synthesize powders of brushite (DCPD, dicalcium phosphate dihydrate,  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ) [1-5] and octacalcium phosphate (OCP,  $\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$ ) [6-10]. The  $\log K_{sp}$  (solubility product) values for DCPD, OCP and hydroxyapatite (HA,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) are known to be -6.6, -96.6 and -117.1, respectively [11]. Therefore, one may expect solely on the basis of the above solubility values that both DCPD and OCP shall exhibit *in vivo* percentages of biodegradation and bone replacement higher than that of hydroxyapatite.

DCPD is an acidic calcium phosphate and when implanted in the form of a cement paste containing some  $\beta$ -TCP ( $\beta$ - $\text{Ca}_3(\text{PO}_4)_2$ ) granules, the material was initially surrounded by macrophages and fibrous tissue due to the initial pH drop, but still showed a higher biodegradation rate than that of a control hydroxyapatite cement over the long run [12, 13]. Unfortunately, *in vitro* and *in vivo* data on pure DCPD powders are lacking to the best of our knowledge. On the other hand, to the advantage of OCP, when it was implanted as a dry-sieved powder (sieving apparently causing a certain degree of aggregation of the particulates) such fibrous tissue encapsulation was not observed, and OCP biodegraded well [14].

The difficulty arises if one wants to produce granules or bodies of DCPD or OCP, instead of powders, which should have at least the strength to withstand the handling by the surgeon during implantation. DCPD or OCP cannot be sintered, in stark contrast to HA or  $\beta$ -TCP bioceramics, mainly because at temperatures above 60°C they start to transform into  $\text{CaHPO}_4$  (DCPA, monetite, dicalcium phosphate anhydrous) and HA, respectively. In the literature, there were only scarce attempts to prepare granules of monetite [15-17]. The starting materials in these studies were either brushite cement paste containing  $\beta$ -TCP [15, 16] or pure  $\beta$ -TCP [17]. The so-called brushite cement pastes are prepared by reacting orthophosphoric or sulphuric acid (cement setting solution) with  $\beta$ -TCP (69.74 wt%),  $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$  (30 wt%), and  $\text{Na}_2\text{P}_2\text{O}_7$  (<0.26 wt%), therefore, such pastes contained a significant amount of unreacted  $\beta$ -TCP in their cores [16, 18].

The down-to-earth approach taken in this study was to use granules of natural marble (calcite,  $\text{CaCO}_3$ ) as the starting material and transforming them firstly into DCPD and secondly to OCP by soaking in specially prepared aqueous solutions in glass media bottles (without stirring) at temperatures between 20° (room temperature, RT) and 37°C. The sizes of the obtained DCPD and OCP granules closely imitated the initial sizes of the marble granules used.

EXPERIMENTAL PROCEDURE

Marble granules were purchased from Merck KGaA, Darmstadt, Germany, (Cat. No: 105986). The granules were 0.9 to 2 mm in size. Marble granules were used as-received, without any further purification or chemical treatment, only after dry sieving manually by using a sieve with 0.9 mm openings to remove very small amounts of marble dust that could be present.

For the preparation of DCPD granules, ten grams of  $NH_4H_2PO_4$  (Cat. No: 101126, Merck) was dissolved in 50 mL of doubly distilled water. The pH of the obtained solution was  $4.1 \pm 0.1$  at RT. The solutions were prepared in 100 mL-capacity glass media bottles. In some experiments, that 10 g of  $NH_4H_2PO_4$  was replaced with 13.557 g of  $NaH_2PO_4 \cdot 2H_2O$  (Cat. No: 106342, Merck) or 11.826 g of  $KH_2PO_4$  (Cat. No: 104877, Merck). Two grams of marble granules were then placed into the phosphate solution. The granules were kept in sealed glass bottles at RT for about 20 h without stirring. Granules were separated from the solution by using a small sieve with 0.9 mm openings, followed by washing with 1.5 L of distilled water and overnight drying at 37°C.

An alternative phosphate solution was also prepared, for transforming marble granules into DCPD, by slowly adding 120 mL of concentrated (85%)  $H_3PO_4$  (Cat. No: 100573, Merck) to 730 mL of doubly distilled water, followed by the drop-wise addition of 137 mL of concentrated (28-30%)  $NH_4OH$  (Cat. No: 105423, Merck); the resultant solution again had a pH value equal to  $4.1 \pm 0.1$  at RT. One mL of this solution had  $1.889 \times 10^{-3}$  mole P. In using this alternative solution to form the DCPD granules, 3 g of marble granules were placed into 69 mL of the above solution and were kept unstirred for 20 h at RT.

In synthesizing the OCP granules, DCPD granules as produced above were used as the starting material. The OCP synthesis solution was prepared by adding the following chemicals at RT, one by one, to 1420 mL of double distilled water under vigorous stirring; 12.448 g of NaCl (Cat. No: 106404, Merck), 0.559 g KCl (Cat. No: 104933, Merck), 0.426 g  $Na_2HPO_4$  (Cat. No: 106586, Merck) and 0.735 g  $CaCl_2 \cdot 2H_2O$  (Cat. No: 102382, Merck). 8.5 g of Tris (Cat. No: 108382,  $(HOCH_2)_3CNH_2$ , Merck) was then added to this slightly turbid solution, followed by titrating the solution to pH  $7.4 \pm 0.2$  by adding 60 to 67 mL of 1 mol/L HCl solution (Cat. No: 109057, Merck). The details of preparing the above solution are given in Table 1.

Table 1 Preparation of the solution used in converting DCPD granules into OCP granules

Reagent	Order	Amount (g)
H <sub>2</sub> O	1	1420
NaCl	2	12.448
KCl	3	0.559
Na <sub>2</sub> HPO <sub>4</sub>	4	0.426
CaCl <sub>2</sub> ·2H <sub>2</sub> O	5	0.735
Tris	6	8.500
1 M HCl	7	60 to 67 mL (to adjust the pH at 7.4 at RT)

To produce OCP granules, 500 mL of this transparent solution (with a Ca/P molar ratio of 1.667) was placed into a 500 mL-capacity glass media bottle and 2.2 g of DCPD granules were added into the bottle. The bottle was kept undisturbed at 37°C in a microprocessor-controlled oven for one week, however the solution in the bottle was fully replenished at the end of the first 3 days. This solution (of Table 1) was inspired from the one described by Wen *et al.* [19]. Granules were finally separated from

the solution by using a small sieve with 0.9 mm openings, followed by washing with 1.5 L of distilled water and overnight drying at 37°C.

All granules were characterized by using a scanning electron microscope (SEM; Model AG, Karlsruhe, Germany) after either gently mixing with a small amount of Play-Doh clay (Play-Doh®) or after grinding them into a fine powder. The SEM was operated with a Cu tube at 40 kV and 40 mA and a step size of 0.02° and a preset time of 5 s.

Scanning electron microscopy (SEM; Model AG, Karlsruhe, Germany) was used to study the morphology of the granules, after sputter-coating with a thin layer of gold to impart electrical conductivity to the specimen surface.

Chemical analyses of granules were performed using inductively coupled plasma emission spectroscopy (ICP-AES; Model 51E, PerkinElmer, Waltham, MA) solution analyses, roughly 100 mg portions of granules (Cat. No: 101799, Merck) solution.

RESULTS AND DISCUSSION

The chemical analyses of the initial marble granules were found to consist of 55.5 wt% CaO, 40.5 wt%  $CO_2$ , and 4.0 wt%  $Fe_2O_3$ . The values above were the average of three analyses. X-ray diffraction (XRD) analyses of the granules revealed a single-phase calcite of high crystallinity, confirmed by the XRD pattern (JCPDS file (PDF) of 5-0586 of International Centre for Diffraction Data) of the pristine marble granules, observed at  $29.4^\circ$   $2\theta$  with a scan rate of 0.5°/min and 15,000 counts-per-second. Figure 1 depicted the morphology of the starting marble granules, with the inset showing a digital camera image of the granules on a scale to facilitate the direct evaluation of granule size.

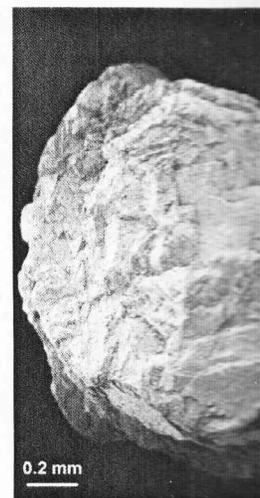


Figure 1 SEM micrograph of the starting marble granules. The inset shows a digital camera image of the granules on a scale to facilitate the direct evaluation of granule size.

the solution by using a small sieve with 0.9 mm openings, followed by washing with 1.5 L of distilled water and overnight drying at 37°C.

All granules were characterized by using an X-ray diffractometer (XRD; Advance D8, Bruker AG, Karlsruhe, Germany) after either gently mounting the granules in the sample holder on modeling clay (Play-Doh®) or after grinding them into a powder by using an agate mortar and pestle. XRD was operated with a Cu tube at 40 kV and 40 mA equipped with a monochromator. Samples were scanned with a step size of 0.02° and a preset time of 5 s.

Scanning electron microscopy (SEM; EVO 40, Zeiss, Dresden, Germany) was used to evaluate the morphology of the granules, after sputter-coating the granules with a 25 nm-thick gold layer to impart electrical conductivity to the specimen surfaces.

Chemical analyses of granules were performed by using inductively-coupled plasma atomic emission spectroscopy (ICP-AES; Model 61E, Thermo Electron, Madison, WI). For the ICP-AES analyses, roughly 100 mg portions of granules were dissolved in 7.5 mL of concentrated (69%) HNO<sub>3</sub> (Cat. No: 101799, Merck) solution.

#### RESULTS AND DISCUSSION

The chemical analyses of the initial marble granules were performed by using ICP-AES and the granules were found to consist of 55.5 wt% CaO, 2100 ppm MgO, 960 ppm SiO<sub>2</sub>, 430 ppm Al<sub>2</sub>O<sub>3</sub>, and <200 ppm Fe<sub>2</sub>O<sub>3</sub>. The values above were the average of three measurements. No other impurities were detected. X-ray diffraction (XRD) analyses of the marble granules showed that they were consisting of single-phase calcite of high crystallinity, conforming very well with the standard powder diffraction file (PDF) of 5-0586 of International Centre for Diffraction Data (ICDD). The (104) reflection of the pristine marble granules, observed at 29.4° 2θ, was having an X-ray diffraction intensity higher than 15,000 counts-per-second. Figure 1 depicted the SEM morphology of the initial, white marble granules, with the inset showing a digital camera macrograph of the same; incorporating a millimetric scale to facilitate the direct evaluation of granule sizes.

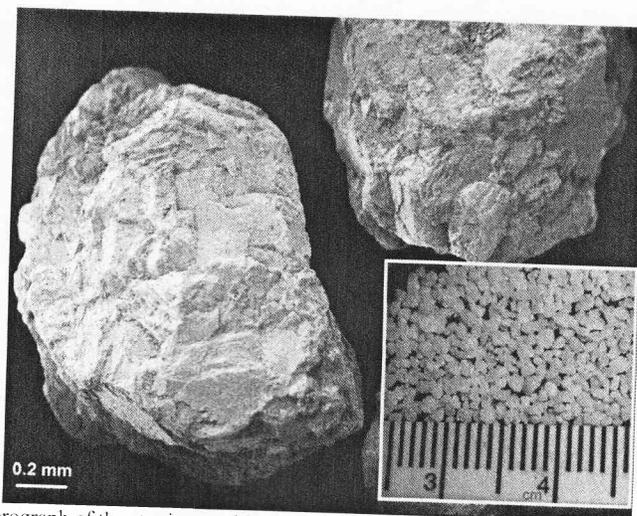


Figure 1 SEM micrograph of the starting marble granules (inset: optical macrograph of the same)

Upon soaking the marble granules in the pure ammonium dihydrogen phosphate solution for 20 h at RT, the granules were covered with characteristic DCPD crystals (Figures 2a and 2b). The SEM morphology did not exhibit any noticeable change when the marble granules were similarly soaked in the alternative  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ ,  $\text{KH}_2\text{PO}_4$  or  $\text{H}_3\text{PO}_4\text{-NH}_4\text{OH}$  solutions (as described in Chapter 2) and all four liquid media were able to transform the marble granules into DCPD. The solution pH increased to the range of 5.1 to 5.3 at the end of 20 h of ageing. The XRD pattern of the DCPD granules was given in Figure 3, all the peaks belonging to the DCPD phase (peak positions matching perfectly well with those given in ICDD PDF 09-0077). DCPD granules obtained by using  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ ,  $\text{KH}_2\text{PO}_4$  and  $\text{H}_3\text{PO}_4\text{-NH}_4\text{OH}$  solutions also had the same XRD data (not shown). The XRD data was collected from the DCPD granules without grinding them into a powder (after mounting the granules directly in the sample holder). This was the reason why no  $\text{CaCO}_3$  peak was observed in this figure. The high intensity (020) reflection located at  $11.68^\circ 2\theta$  was characteristic of DCPD. One distinctive feature of these DCPD granules was that they were shining especially under the direct sun light due to the brushite crystals shown in Figures 2a and 2b. It was actually impossible to obtain any calcium phosphate phase other than DCPD when immersing, at RT, such a reactive calcium carbonate/marble material in a pure phosphate solution having a pH value of 4 [2, 8].

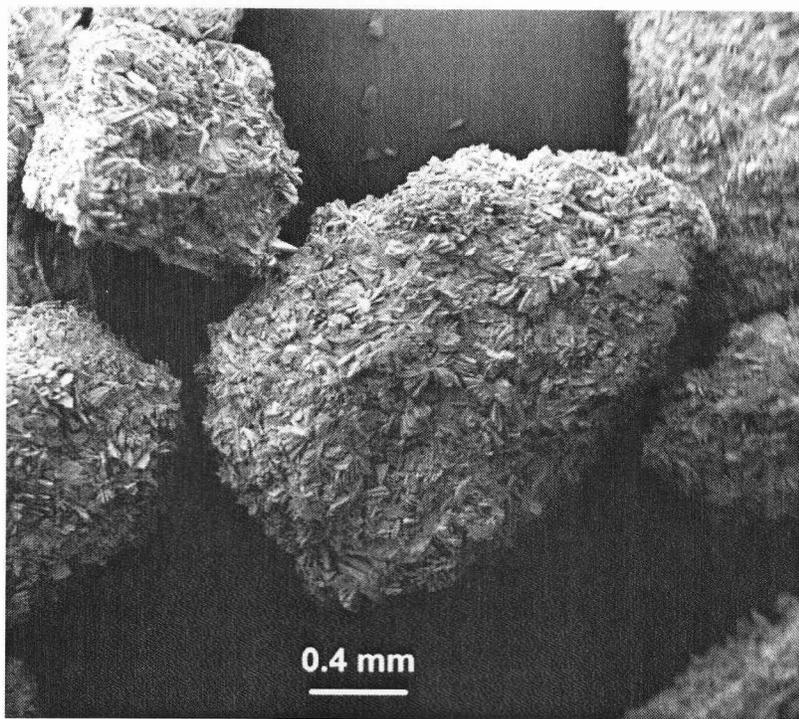


Figure 2a SEM micrograph of DCPD granules (low magnification)

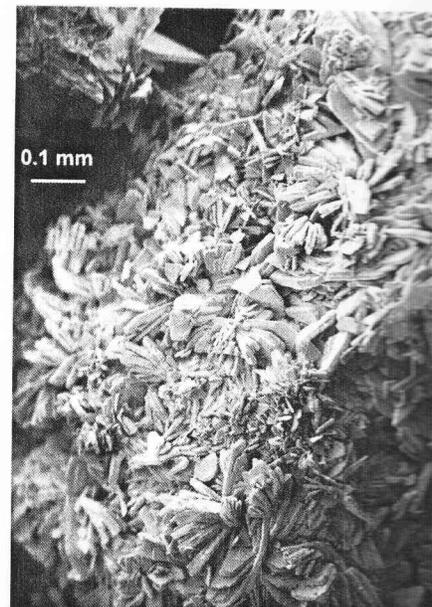


Figure 2b SEM micrograph of DCPD granules (high magnification)

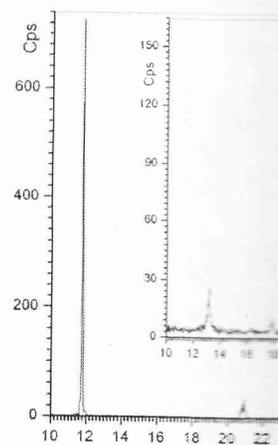


Figure 3 XRD chart of DCPD granules prepared by soaking marble granules in the  $\text{NH}_4\text{H}_2\text{PO}_4$  solution with no stirring (inset: XRD of granules in the  $\text{NH}_4\text{H}_2\text{PO}_4$  solution)

phosphate solution for 20 h (Fig. 2a and 2b). The SEM images were similarly soaked in the solution (described in Chapter 2) and the solution pH increased. The DCPD granules were obtained by matching perfectly well with the  $\text{NH}_4\text{H}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ ,  $\text{KH}_2\text{PO}_4$  solution. XRD data was collected from the granules directly in the solution, as shown in this figure. The high contrast is the distinctive feature of the granules under bright sun light due to the reflection to obtain any calcium carbonate/marble



Figure 2b SEM micrograph of DCPD granules (high magnification)

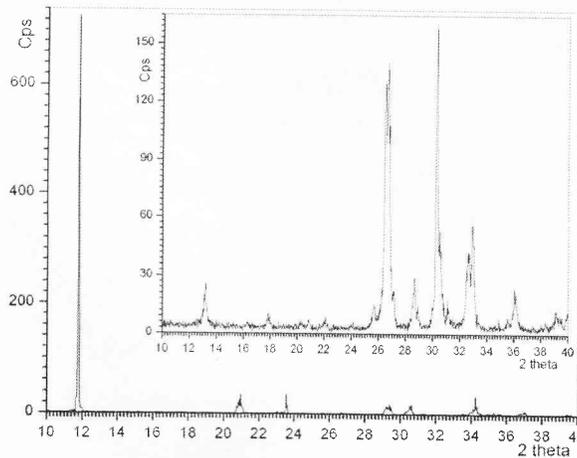


Figure 3 XRD chart of DCPD granules obtained by using the  $\text{NH}_4\text{H}_2\text{PO}_4$  solution at RT, no stirring (inset: XRD of DCPA powders obtained after stirring the marble granules in the  $\text{NH}_4\text{H}_2\text{PO}_4$  solution for 20 h at  $65^\circ\text{C}$ ).

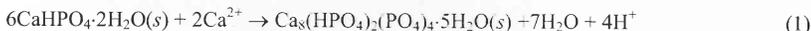
ICP-AES analyses of the DCPD granules showed that the Na and K amounts were less than 100 ppm, even when we used  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  or  $\text{KH}_2\text{PO}_4$  solutions. If one added, for instance, precipitated chalk (i.e., calcite) powder into the same solution and stirred it vigorously at RT for 20 h, one would have obtained brushite powder. Similarly, when we added the same amount of marble granules into the same volume of the ammonium phosphate solution in a sealed glass bottle and stirred the solution at  $65^\circ\text{C}$  (at 400 rpm) constantly for 20 h, we obtained only a powder (no granules left) consisting of single-phase monetite ( $\text{CaHPO}_4$ ), as shown in the XRD data of inset of Figure 3, where peak positions and intensities matched with those of ICDD PDF 09-0080. Increased temperature and vigorous stirring both forced (i) the DCPD to transform into DCPA and (ii) the marble granules to crumble into a powder.

It would be very difficult, if not impossible, to obtain OCP (octacalcium phosphate) directly from marble in an unstirred hydrothermal ageing solution of pH 7.4. At that pH, apatitic CaP is the phase to form. This was why we preferred DCPD granules as the starting material in producing the OCP granules. On the other hand, it would have been very easy to convert the DCPD granules into apatitic CaP granules by soaking them in a  $\text{Ca}^{2+}$ -containing solution with pH on the basic side (e.g., 8.5 to 10), but our intention here was not to produce low solubility HA. The solution developed [19] for the conversion of DCPD to OCP had a Ca/P molar ratio of 1.667 and the mildly acidic DCPD granules (with Ca/P ratios roughly equal to 1, depending on the amount of  $\text{CaCO}_3$  remaining still unreacted in the cores of those granules) would help to slowly lower both the nominal Ca/P ratio in the ageing bottles to around 1.33 (i.e., to that of OCP) and the solution pH to around 6.5 to 6.8 (stability range of OCP) [9]. The DCPD-to-OCP transformation was a surface reaction.

Figures 4a and 4b depicted both the XRD data and the SEM micrographs of the OCP granules obtained by using the DCPD granules prepared in  $\text{NH}_4\text{H}_2\text{PO}_4$  solutions. OCP granules produced by using the DCPD granules obtained in the other solutions of this study also showed identical XRD and SEM data. The peak positions and intensities of the XRD pattern of the OCP (without grinding the granules into a powder, Fig. 4a) granules were in good match with that of ICDD PDF 26-1056. The intermingling nanocrystals seen in Figure 4b depicted the characteristic habit of OCP crystals [10].

The OCP granule synthesis process described here is a benign one since it starts with a solution of neutral pH and proceeds at the human body temperature. The solution pH values were measured to be in the range of 6.5 to 6.8 at the end of ageing periods.

The overall conversion of brushite to OCP can be visualized by the below reactions. Reactions help to explain the effect of  $\text{Ca}^{2+}$  and  $\text{HPO}_4^{2-}$  ions present in the ageing solutions, as well as the slight pH decreases observed.



$\text{Mg}^{2+}$  ions are known [20] to significantly inhibit the nucleation and growth of cryptocrystalline apatitic calcium phosphate in aqueous media, and for this reason the presence of magnesium in the original marble granules were deemed to be simply advantageous for the synthesis of OCP. We did not assess the influence of other elements, such as Si, Al and Fe, present in smaller amounts in the initial marble granules.

Upon grinding (with an agate mortar/pestle) both the so-called DCPD and OCP granules of this study into a fine powder and then collecting their XRD data, the small amounts of  $\text{CaCO}_3$  remaining in the cores of the granules became visible, as shown in Figure 5a. This was quite an expected observation, if there were no calcite remaining in the cores, the granules would have turned into powder by the end of the hydrothermal ageing process.

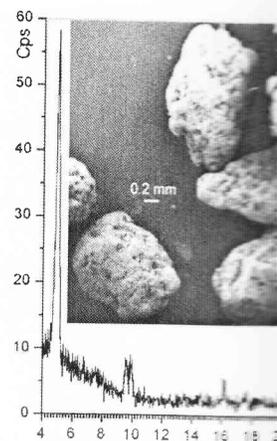


Figure 4a XRD and

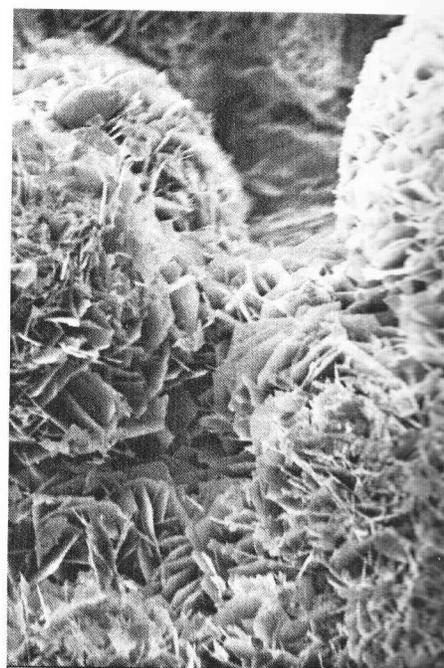


Figure 4b High magnification SEM photomicro

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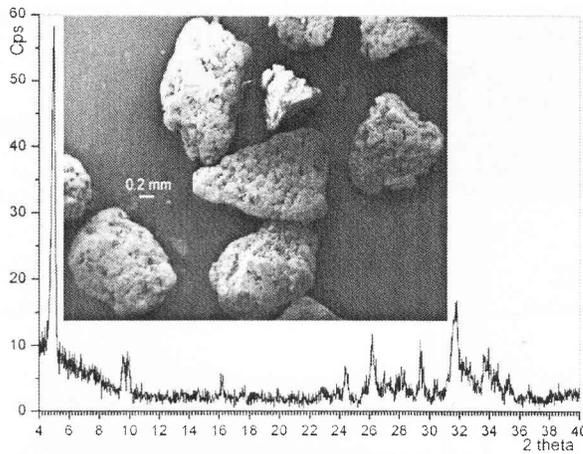


Figure 4a XRD and SEM data of OCP granules

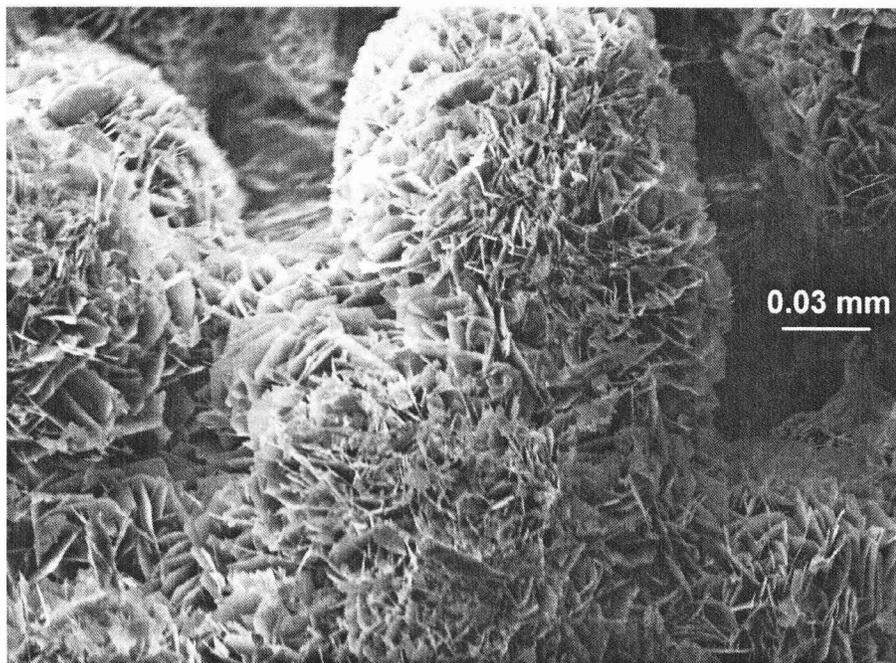


Figure 4b High magnification SEM photomicrograph of OCP granules showing surface nanotexture

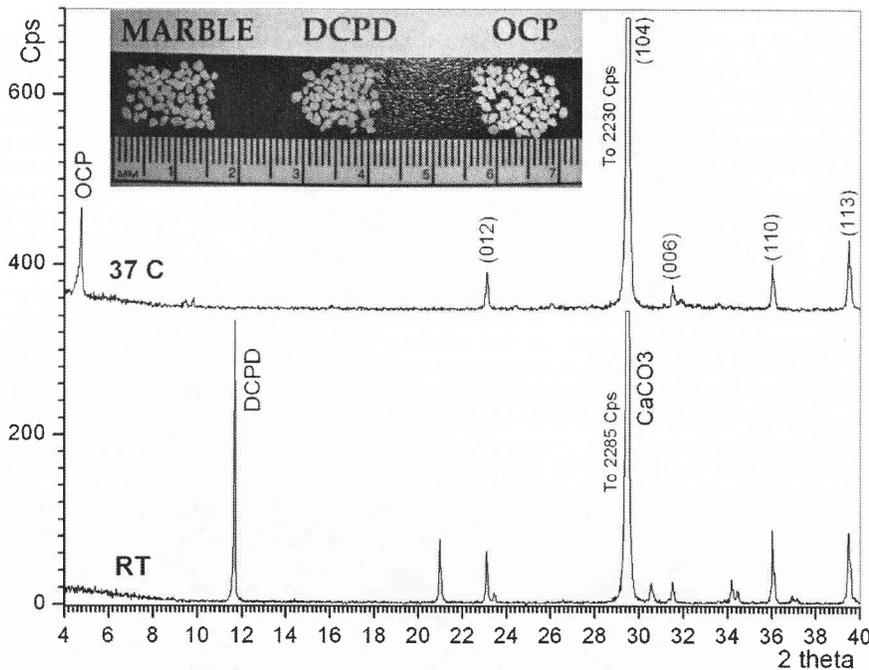


Figure 5a XRD charts of DCPD (bottom) and OCP (top) granules (after grinding into a fine powder); reflections of calcite are indicated by their crystallographic planes (104) and observed intensities (inset shows the size similarity between the granules)

The strongest (104) reflections of calcite located at  $29.4^\circ 2\theta$  in both DCPD and OCP samples (Fig. 5a) were still much lower than the 15,000 Cps intensity of the initial marble granules. The percentage decrease in the XRD intensity of the (104) reflection of calcite (from the initial marble granules (15,000 Cps) to the final DCPD or OCP granules (2300 Cps)) corresponded to about 85%. Therefore, the process reported here was starting at the surfaces of the marble granules, proceeding inwards and then stopping at one point where the formed DCPD or OCP layers were partially obstructing the reach of the solution to the remnant calcitic marble cores at RT and  $37^\circ\text{C}$ .

What would happen if only the temperature and ageing time was increased, without stirring, in preparing the DCPD granules? The bottom trace of Figure 5b showed the XRD pattern of granules heated at  $50^\circ\text{C}$  for 96 h without stirring, whereas the top trace showed the granules heated at  $70^\circ\text{C}$  for 96 h again without stirring. In both cases, the end products were intact granules, not powders; in contrast to the stirred sample of Figure 3. It must be noted that while the intensity of the (104) reflection of the calcite phase was decreasing from 2285 Cps (RT, 20 h) to 1942 Cps ( $50^\circ\text{C}$ , 96 h), upon increasing the solution ageing temperature to  $70^\circ\text{C}$ , DCPD phase completely decomposed to DCPA with a further decrease in the intensity of the calcite peak (Fig. 5b, top trace).

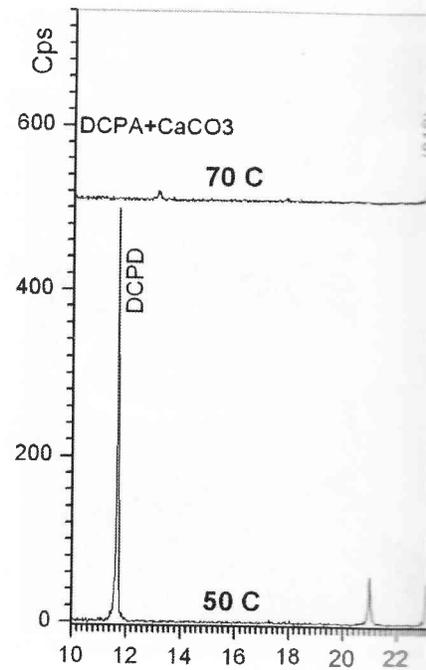


Figure 5b XRD charts of marble granules heated in the  $\text{NH}_4\text{H}_2\text{PO}_4$  solution without stirring (granules were ground into powder)

DCPD is a low-temperature phase. The remnant calcite in the cores of the granules was dissolved in the aqueous ageing solution (in going from RT to  $70^\circ\text{C}$ ), and the DCPD phase did not penetrate more inwards through the DCPD layer, leaving the cores of marble at the granule cores. Increasing the ageing temperature to  $70^\circ\text{C}$ , the DCPD phase completely decomposed to DCPA.

We further pursued this high-temperature ageing of marble granules to 140 mL of the ammonium phosphate solutions of  $\text{H}_3\text{PO}_4$  and  $\text{NH}_4\text{OH}$ , see Chapter 2. The ageing was done in a Teflon instrument (Instrument Company, Moline, IL) with a Teflon inner vessel. The instrument was heated in a microprocessor-controlled oven. The granules were aged there for 24 h without any risk of evaporating the solution, as there was a pressure gauge.

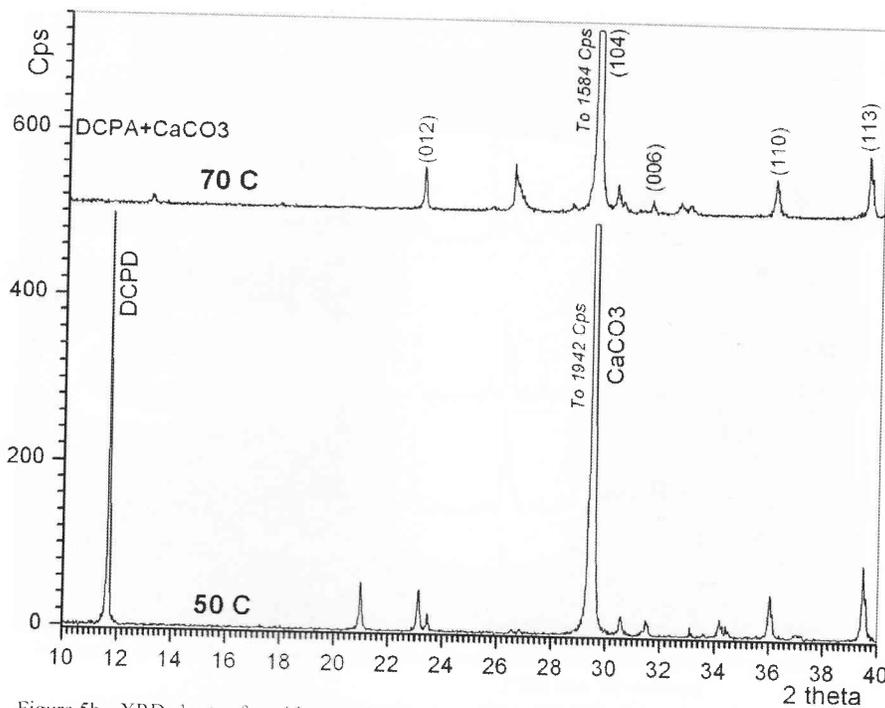


Figure 5b XRD charts of marble granules heated at 50°C for 96 h (bottom) and 70°C for 96 h (top) in the  $\text{NH}_4\text{H}_2\text{PO}_4$  solution without stirring (after grinding the granules into a fine powder)

DCPD is a low-temperature phase. The reason behind the decrease in the XRD intensity of the remnant calcite in the cores of the granules was most probably the reduced surface tension of the aqueous ageing solution (in going from RT to 50°C and 70°C), and thus the solution was able to penetrate more inwards through the DCPD layer and reaching a bit more of the non-reacted, shielded marble at the granule cores. Increasing the ageing time also helped this transformation.

We further pursued this high-temperature transformation of marble granules by adding 2.35 g of marble granules to 140 mL of the ammonium phosphate solution (prepared by mixing concentrated solutions of  $\text{H}_3\text{PO}_4$  and  $\text{NH}_4\text{OH}$ , see Chapter 2) in a non-stirred pressure vessel (Model 4760, Parr Instrument Company, Moline, IL) with a Teflon<sup>®</sup> cup inside, as shown in Fig. 6. The pressure vessel was heated in a microprocessor-controlled oven. By this way, we were able to reach 200°C (and stay there for 24 h) without any risk of evaporating the solution. This pressure vessel was not equipped with a pressure gauge.



Figure 6 Pressure vessel assembly used in testing the feasibility of forming granules between 90° and 200°C

The XRD traces of marble granule samples heated at temperatures between 90°C (96 h) and 200°C (24 h) are given in Figure 7. None of these samples disintegrated into a powder, they were all recovered as strong granules. Starting from somewhere between 90° and 110°C, a new phase was appearing:  $\beta$ -TCP. Heating runs at 150° and 200°C, on the other hand, also resulted in another phase: HA. Therefore, as shown in Figure 7, the samples heated in the pressure vessel at 110°, 150° and 200°C crystallized  $\beta$ -TCP besides DCPA, and the samples heated at 150° and 200°C were consisting of four phases: DCPA,  $\beta$ -TCP, HA, and calcite. The appearance of HA and  $\beta$ -TCP phases should be at the expense of calcite and DCPA. Crystallization of  $\beta$ -TCP at a temperature as low as 110°C was noteworthy [21]. The decrease observed in the amount of calcite (Fig. 7), with an increase in the temperature of the pressure vessel from 150° to 200°C, implied that a further increase in temperature up above 200°C could have totally consumed the calcite phase. Nevertheless, to prepare granules comprising the phases of HA and  $\beta$ -TCP was not one of our initial purposes, and for this reason we opt not to deliberate much on these multi-phase high temperature-high pressure samples.

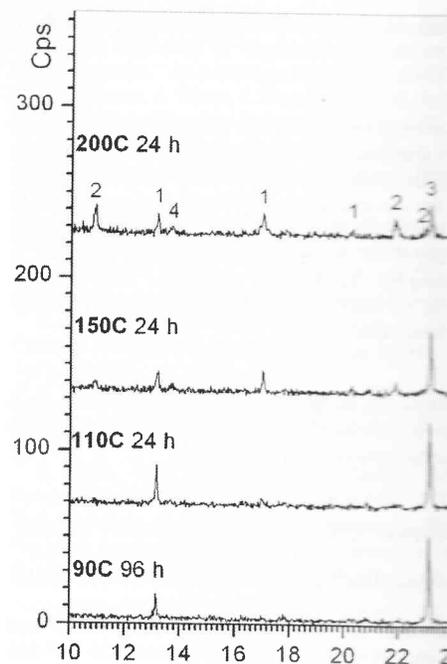


Figure 7 XRD traces of marble granules heated at different temperatures and times. The solution prepared by adding  $\text{NH}_4\text{OH}$  to the solution. Phases = 1: DCPA, 2: HA, 3: calcite, 4:  $\beta$ -TCP.

The DCPD and OCP granules of this study were found to be more degradable since their cores were not made out of the previous granule synthesis studies [16-18], but of DCPD. Calcite is more soluble than  $\beta$ -TCP by about an order of magnitude. The *in vitro* cell culture study of Monchau *et al.* [22] showed that the degradation of DCPD granules was faster than that of  $\beta$ -TCP with one another by using both human and rat osteoblasts.

The preparation of biphasic DCPD-OCP granules was as easy as weighing and mixing the DCPD (log  $K_{sp}$ ) with another. Such biphasic granule mixtures may have a wide range of solubility/resorbability level, between the solubility of the two biomaterials.

It is obvious that the weakest part of the granules was the compressive strength measurements on the marble granules. It was impossible to crush the DCPD and OCP granules.

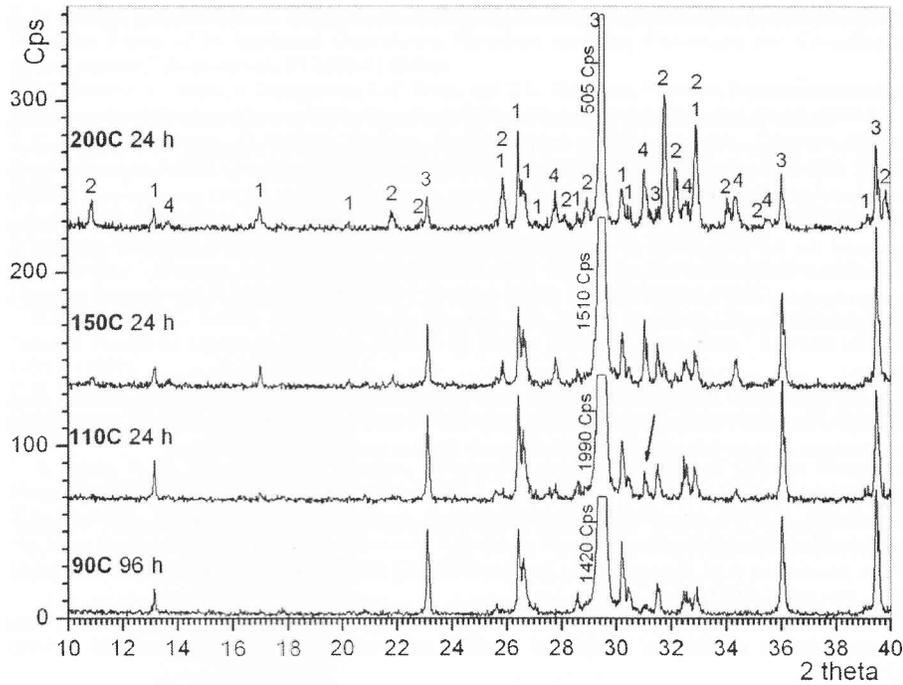


Figure 7 XRD traces of marble granules heated in a pressure vessel between 90° and 200°C, in a solution prepared by adding NH<sub>4</sub>OH into H<sub>3</sub>PO<sub>4</sub>. Phases = 1: DCPA, 2: HA, 3: calcite, 4: β-TCP.

The DCPD and OCP granules of this study may be expected to show a high *in vivo* degradability since their cores were not made out of β-TCP ( $\log K_{sp} = -81.7$  [11]) as was the case with the previous granule synthesis studies [16-18], but were out of calcite, CaCO<sub>3</sub> ( $\log K_{sp} = -8.55$  [11]). Calcite is more soluble than β-TCP by about an order of magnitude, and this fact was also proved by the *in vitro* cell culture study of Monchau *et al.* [22], which directly compared HA, β-TCP and CaCO<sub>3</sub> with one another by using both human and rat osteoclasts.

The preparation of biphasic DCPD-OCP granules (in any desired weight percentages) would be as easy as weighing and mixing the DCPD ( $\log K_{sp} = -6.6$ ) and OCP ( $\log K_{sp} = -72.5$ ) granules with one another. Such biphasic granule mixtures may thus provide a tool of fine-tuning the desired *in vivo* solubility/resorbability level, between the solubilities of the end members DCPD and OCP, of these biomaterials.

It is obvious that the weakest part of this *proof-of-the-concept* study was the absence of compressive strength measurements on the marble, DCPD and OCP granules (although it was still impossible to crush the DCPD and OCP granules into a powder by using the fingertips), as well as the

*in vitro* and *in vivo* characterization, however, our follow-up study is already focusing right on those aspects.

#### CONCLUSIONS

Marble (calcite,  $\text{CaCO}_3$ ) granules over the size range of 0.9 to 2 mm were used as the starting material to prepare brushite (DCPD,  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ) granules, without changing the starting granule sizes. DCPD granule synthesis consisted of soaking the marble granules at room temperature in an acidic (of pH 4) phosphate solution, without stirring, for less than a day. DCPD granules were found to contain small amounts of residual marble in their cores. DCPD granules were then used as the starting material for the preparation of octacalcium phosphate (OCP,  $\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$ ) granules. A non-stirred Tris-buffered solution (of pH 7.4) containing  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$  and  $\text{HPO}_4^{2-}$  ions was used in synthesizing OCP granules at  $37^\circ\text{C}$  in one week. DCPD and OCP granules were found to contain a small amount of residual marble ( $\text{CaCO}_3$ , calcite) in their cores.

#### Notes

Certain commercial instruments or materials are identified in this paper to foster understanding. Such identification does not imply recommendation or endorsement by the author, nor does it imply that the instruments or materials identified are necessarily the best available for the purpose.

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# Advances in Bioceramics and Porous Ceramics V

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## Sets forth new advances and new applications resulting from bioceramics and porous ceramics research

This issue of the *Ceramic Engineering and Science Proceedings* is one of nine issues that has been published based on content presented in January 2012, during the 36th International Conference on Advanced Ceramics and Composites (ICACC) in Daytona Beach, Florida. It features papers from two popular symposia held during the ICACC meeting:

- **Next-Generation Bioceramics** explores new research into ceramic materials designed to support and enhance the treatment of dental and medical disorders. Presentations from researchers in academia, government, and industry address the processing, characterization, modeling, and use of bioceramic materials for dental and medical applications. Specific topics covered at the symposium include bioinspired and biomimetic ceramic materials; nanoscale bioceramic materials; in vitro and in vivo evaluation of bioceramic materials; and bioceramic materials for drug delivery.
- **Porous Ceramics: Novel Developments and Applications** examines some of the latest advances and innovations in processing methods and synthesis, structure and properties, modeling and novel characterization tools, mechanical behavior, micro- and mesoporous ceramics, and ceramic membranes. Moreover, participants discuss new and emerging applications of porous ceramics in environmental sciences, energy, defense, biomedicine, and aeronautics.

Charts, tables, and illustrations throughout this issue help readers better understand the latest advances in bioceramics and porous ceramics research and the many applications of this research.

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