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Transformation of Brushite ($CaHPO_4{\cdot}2H_2O$) to Whitlockite ($Ca_9Mg(HPO_4)(PO_4)_6$) or Other CaPs in Physiologically Relevant Solutions

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Brushite (dicalcium phosphate dihydrate, DCPD, $CaHPO_4{\cdot}2H_2O$) and whitlockite [WH, $Ca_9Mg(HPO_4)(PO_4)_6$] are usually found in the mammalian metabolism in the form of diverse pathological calcifications, dental calculi, urinary tract stones, salivary gland deposits, cardiovascular or pulmonary calcified deposits, and even as prostate or cartilage calcifications. The hydrothermal transformation of synthetic brushite crystals into single-phase whitlockite, octacalcium phosphate, or apatitic calcium phosphate was observed over the time period of 1 to 21 d and at 37°C, 70°C, and 115°C in nonstirred physiologically relevant solutions developed for this work. The strong influence of the physiologically relevant ions such as Mg^{2+} and HCO_3^- on hydrothermal transformations is exposed. The formation of the nanoglobules and nanofibrils of X-ray amorphous calcium phosphate or Mg-doped calcium phosphate on the surfaces of brushite crystals are observed for the first time in biomimetic solutions containing 10 mM Mg^{2+} and/or 27 mM HCO_3^-. The experimental conditions leading to the formation of such nanofibrils on brushite crystal surfaces are also found to stop the further transformation of brushite into any other calcium phosphate (CaP) phases even at high solution temperatures. Samples were characterized by scanning electron microscopy and powder X-ray diffraction.

I. Introduction

BRUSHITE (DCPD, dicalcium phosphate dihydrate, $CaHPO_4{\cdot}2H_2O$), named after the mineralogist George Jarvis Brush (1831–1912), is the predominant mildly acidic phase of the $Ca(OH)_2$–H_3PO_4–H_2O system to precipitate between pH 2 and 6.5,[1–3] when Ca^{2+} and HPO_4^{2-} ions are simultaneously present in an aqueous solution of the stated pH range. When Ca-chloride (or Ca-nitrate or Ca-acetate) solutions are mixed with those of basic Na-(or K- or ammonium-) phosphate at room temperature (RT, 22°C ± 1°C), monoclinic (ICDD PDF 9-0077) brushite crystals instantly form. The crystal structure of brushite was ascertained by Curry and Jones[4] *via* neutron diffraction.

Brushite synthesis, if performed in the way outlined above, does not require any strict pH control in stark contrast to stoichiometric Ca-hydroxyapatite [HA, $Ca_{10}(PO_4)_6(OH)_2$ or $3Ca_3(PO_4)_2{\cdot}Ca(OH)_2$] synthesis since the latter necessitates the solution pH to be maintained above 12 at all times. For instance, if the pH during stoichiometric Ca-hydroxyapatite synthesis falls below 12, then Ca- and OH-deficient, apatite-like (apatitic) calcium phosphates would precipitate. Precipitated brushite crystals were recently shown to go through a maturation process[5] in their mother liquors. The maturation of brushite revealed itself as follows. The very first crystals formed in the synthesis solutions (e.g., 1 h residence time in solutions) exhibited the crystal structure and characteristic X-ray diffraction pattern of ICDD PDF 9-0077, but these crystals had a Ca/P molar ratio of only 0.8 in opposition to the stoichiometric ratio of 1.0.[5] Crystals soaked in the same synthesis solution for 24 h reached a Ca/P molar ratio of 0.97.[5] On the other hand, the *in vivo* and *in vitro* maturation of apatitic calcium phosphate (Ap-CaP) was studied by Cazalbou et al.[6]

Brushite is found in the human metabolism in the form of pathological calcifications; such as, dental calculi (or tartar),[7] urinary stones [8], and in chondrocalcinosis (together with $Ca_2P_2O_7{\cdot}2H_2O$ (CPPD) crystals [9]). Brushite has a high solubility (pK_{SP} of 6.59 at 25°C) in comparison to stoichiometric hydroxyapatite (pK_{SP} of 116.8 at 25°C).[10] The solubility of brushite is also significantly higher than that of octacalcium phosphate, OCP, $Ca_8(HPO_4)_2(PO_4)_4{\cdot}5H_2O$ (with a pK_{SP} of 96.6 at 25°C).[10] Tang et al.[11] experimentally measured the dissolution rate of brushite in water at RT as 4.26×10^{-4} mol/m^2/min. High solubility of brushite, in comparison to hydroxyapatite, led to the development of injectable orthopedic or dental paste formulations based on brushite[12–14] with β-TCP (β-tricalcium phosphate, β-$Ca_3(PO_4)_2$) and MCPM [monocalcium phosphate monohydrate, $Ca(H_2PO_4)_2{\cdot}H_2O$] as the starting materials. Apelt et al.[15] reported in a comparative *in vivo* study that such nonapatitic cements, with microstructures comprised of coarse β-TCP granules embedded in a brushite matrix, were rapidly biodegraded by macrophage activity and showed faster new bone formation compared to commercially available hydroxyapatite cements.

Brushite, as a mildly acidic CaP, is known to be not stable in aqueous solutions of neutral pH and rapidly transforms to apatitic calcium phosphate (Ap-CaP) in basic media containing dissolved NaOH, KOH, or NH_4OH.[16] Similarly, brushite slowly transforms into carbonated Ap-CaP when soaked in Tris [*tris(hydroxymethyl)aminomethane*, $(HOCH_2)_3CNH_2$]-buffered SBF (synthetic body fluid) solutions [17] and into OCP when immersed in Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, $C_8H_{18}N_2O_4S$]-buffered DMEM solution (Dulbecco's Modified Eagle Medium) at the human body temperature of 37°C and at the blood pH of 7.4 for about 48 h.[18] SBF and DMEM solutions contain Mg^{2+} ions at the concentrations of 1.5[17] and 0.814 mM,[18] respectively. High solubility brushite, therefore, has the unique ability of acting (*in vitro*) like a precursor to calcium phosphates of lower solubility, such as HA or OCP, especially in solutions mimicking the inorganic ion concentrations of blood plasma. Brushite can be deposited on titanium[19,20] or Mg-alloy[21] substrates at ambient temperatures in aqueous solutions. Mechanically stable millimeter-sized brushite granules developed for use in orthopedic and oral surgery were also shown to be suitable for high throughput production.[22] Therefore, the close monitoring of the hydrothermal transformations of brushite, into other benign CaP phases, in physiologically relevant abiotic solutions (containing Mg^{2+}, Na^+, Cl^-, HCO_3^-, HPO_4^{2-} and SO_4^{2-} ions at concentrations equal to

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those of the human blood) would be useful to future development work on brushite cements, brushite coatings, and brushite granules.

Rowles[23] reported that brushite powders soaked in distilled water containing only 1 mM Mg^{2+}, at 37°C for 24 d, led to the formation of whitlockite [WH, $Ca_9Mg(HPO_4)(PO_4)_6$ or $3Ca_3(PO_4)_2{\cdot}MgHPO_4$] at the expense of brushite. The natural mineral whitlockite is named after Herbert Percy Whitlock (1868-1948) and the trigonal (rhombic symmetry) crystal structure (ICDD PDF 04-09-3397) of whitlockite is similar to that of β-TCP (ICDD PDF 55-0898). The powder X-ray diffraction spectra of WH and β-TCP look somewhat similar to one another. Nevertheless, WH and β-TCP are not isostructural due to the difference between their space groups, as shown in Table I. This structural similarity caused some researchers to inadvertently name Mg- and HPO_4-free β-TCP [i.e., β-$Ca_3(PO_4)_2$] as whitlockite.[24–30] Although the crystallographic unit cell of WH was recently compared to that of β-TCP by Jang et al.,[31] a more comprehensive analysis of the crystal structure of Mg-containing WH single crystals was provided earlier by Gopal et al.[32] The crystal structure of Mg-free β-TCP, on the other hand, has been described by Dickens et al.[33]

Whitlockite [$Ca_9Mg(HPO_4)(PO_4)_6$] was reported to be present in human tooth enamel,[34] in the knees of arthritic patients [35] and in the calcifications of the articular cartilage,[36] in the human supragingival calculus[7] and dental plaque,[37] as pathological calcification spots on aorta,[38, 39] in the pulmonary calcified deposits of tuberculosis patients,[40] in human prostate calculi,[41] in urinary tract stones,[42, 43] and even in the stones of the parotid (salivary) glands.[44] Although brushite and whitlockite both are phases of diverse pathological calcifications in the human metabolism, the literature on the *in vitro* inorganic transformation of synthetic brushite powders into whitlockite in Mg- and HCO_3-containing saline solutions remained scarce, and this provided the impetus for this study.

This study is designed to examine, for the first time, the hydrothermal transformation of synthetic brushite (the starting phase) to whitlockite in solutions, free of any organics, such as Tris or Hepes, mimicking the inorganic ion concentrations of physiological fluids. Throughout this study, the temperature of hydrothermal reaction solutions were varied between 37°C and 115°C, whereas the same solutions contained Mg^{2+} (from 1.5 to 10 mM) and Na^+, K^+, Cl^-, SO_4^{2-}, HCO_3^-, or HPO_4^{2-} ions in physiologically relevant concentrations. The simple inorganic solutions and experimental conditions reported here might be of interest to readers who wish to further study

1. The transformation of brushite into whitlockite (WH), or
2. The transformation of brushite into OCP, or
3. The transformation of brushite into apatitic CaP (Ap-CaP), or
4. The transformation of brushite into whitlockite-apatitic CaP or whitlockite-OCP biphasic mixtures, or
5. The inhibition of the transformation of brushite to monetite (DCPA, $CaHPO_4$) or to any other calcium phosphates in aqueous or alcoholic solutions heated above 37°C.

II. Experimental Procedure

The synthesis of brushite powders consisted of preparing two solutions, as described elsewhere.[18, 45] Solution A was prepared as follows: 1.650 g of KH_2PO_4 (≥99.9%, Cat. No: 1.04873; Merck KGaA, Darmstadt, Germany) was dissolved in 1400 mL of deionized water, in a 2L-capacity glass beaker, followed by the addition of 6.026 g of Na_2HPO_4 (≥99.9%, Cat. No: 1.06586; Merck), which resulted in a clear solution of pH (Orion Star 215 pH-meter; Thermo Scientific, Dreieich, Germany) 7.5 at RT. The pH meter was calibrated by using solutions having standard pH values of 4.01 and 7 (Solutions PHA-4-CAL-PH and PHA-7-CAL-PH; Omega Engineering Ltd., Manchester, UK).

Solution B (of pH 6.4) was prepared by dissolving 8.028 g of $CaCl_2{\cdot}2H_2O$ in (≥99.9%, Cat. No: 1.02382; Merck) 400 mL of deionized water. Solution B was then rapidly added to solution A under stirring and the crystals formed were aged in solution for 24 h at RT, while stirring at 500 rpm (final solution pH 5.3). Solids recovered by filtration (and washing) from their mother liquors were dried at 35°C (24 h) to obtain approximately 7 g of flat plate-shaped,[18] optically transparent brushite crystals. Only these crystals were used in the hydrothermal transformation experiments.

The hydrothermal reaction solutions were prepared, as detailed in Table II of the next chapter, by dissolving appropriate amounts of NaCl (>99%, Cat. No: 106400, Merck), $MgCl_2{\cdot}6H_2O$ (>99%, Cat. No: 105833, Merck), KCl (>99%, Cat. No: PX-1405, Merck), $NaHCO_3$ (>99%, Cat. No: SX-0320, Merck), Na_2SO_4 (>99%, Cat. No: SX-0761, Merck), or $NaH_2PO_4{\cdot}H_2O$ (>99%, Cat. No: 106349, Merck) in preboiled (an essential step to eliminate dissolved bicarbonate ions in water) deionized water. All of the hydrothermal transformation solutions were optically transparent and were free of any turbidity prior to the reaction(s).

Brushite crystals (0.35 g) were placed in 100 mL aliquots of the specific hydrothermal reaction solutions, and the solutions were contained in 100 mL-capacity sterile glass media bottles having plastic screw caps with O-rings. The brushite suspensions were first ultrasonicated for 1 min by dipping the sealed glass media bottles in an ordinary ultrasonic cleaning bath. The bottles were then placed in a microprocessor-controlled oven for durations (and temperatures) specified in Table II. The bottle contents were not stirred or agitated during the course of hydrothermal transformation reactions. Biological mineralization processes do never employ stir bars, stir rods, stir plates, etc. The solids were finally separated from the solutions by filtration (and washing with deionized water), followed by drying at 35°C (24 h). All experiments were repeated three times and the XRD and SEM analyses were also repeated on such samples of reproduction.

The particle/crystal morphology analyses of the samples were performed by scanning electron microscopy (SEM; Neon 40-EsB, Zeiss, Jena, Germany). The samples for the SEM analyses were sputter coated with a 5 nm-thick layer of Au–Pd alloy prior to imaging at 5 kV. The samples for energy-dispersive X-ray spectroscopy (EDXS) analyses were not sputter coated. Samples for X-ray diffraction (XRD; D8 Advance, Bruker, Karlsruhe, Germany) runs were first ground in an agate mortar with an agate pestle. All XRD scans (λ = 0.15406 nm) were performed at 40 kV and 40 mA

Table I. Structural Similarity Between β-TCP and Whitlockite (WH)

Compound	Crystal structure	Lattice parameters	Space group	ICDD-PDF†
β-$Ca_3(PO_4)_2$ (β-*TCP*)	Trigonal	a = 1.0418 nm c = 3.7346 nm	*R*-3*c* (167)	00-55-0898
$Ca_9Mg(HPO_4)(PO_4)_6$ (WH)	Trigonal	a = 1.035 nm c = 3.7085 nm	*R*3*c* (161)	04-09-3397

†ICDD-PDF = International Centre for Diffraction Data–Powder Diffraction File.

Table II. Experimental Conditions and XRD Data of Products

Experiment	Brushite (g)	Salt concentration in 100 mL aqueous solution (mM)						Temp. (°C)	Time (d)	Final pH†	Phase(s) by XRD
		$MgCl_2$	NaCl	KCl	$NaHCO_3$	Na_2SO_4	NaH_2PO_4				
1	0.35	—	—	—	—	—	—	70	1	6.3	DCPA
2	0.35	—	—	—	27	—	—	70	1	6.4	OCP
3	0.35	1.5	—	—	—	—	—	70	1	4.5	WH
4	0.35	1.5	—	—	—	—	—	37	2	7.4	DCPD
5	0.35	1.5	—	—	—	—	—	37	7	5.9	DCPD + OCP
6	0.35	1.5	—	—	—	—	—	37	21	5.2	WH
7	0.35	1.5	—	—	—	—	—	115	1	5.3	WH
8	0.35	1.5	—	—	—	—	1	37	21	5.5	WH + OCP
9	0.35	1.5	95	5	27	0.5	1	37	21	7.0	Ap-CaP
10	0.35	1.5	95	5	27	0.5	1	70	1	7.0	WH + Ap-CaP
11	0.35	1.5	95	5	—	—	—	37	21	5.4	WH + Ap-CaP
12	0.35	10	—	—	—	—	—	70	1	6.4	DCPD
13	0.35	10	95	5	27	0.5	1	70	1	6.8	DCPD
14	0.35	1.5 mM $MgCl_2 \cdot 6H_2O$ in pure ethanol						70	1	N/A	DCPD

†All pH values reported here showed the maximum variability of ±0.1.

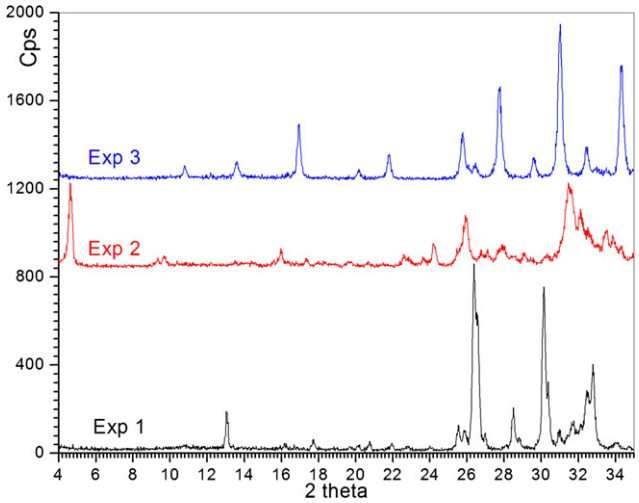

Fig. 1. XRD traces of the samples of experiments 1 through 3 of Table II.

with a step size of 0.02° and a 5 s preset time while using a rotating specimen holder.

III. Results and Discussion

The select experiments and their qualitative XRD phase analysis results are shown in Table II together with the solution preparation details for each experiment. The presentation and the discussion of the experimental findings are, therefore, directly based on Table II.

(1) Brushite to Monetite

Brushite ($CaHPO_4 \cdot 2H_2O$) decomposes in dry air into monetite (DCPA; dicalcium phosphate anhydrous, $CaHPO_4$) when heated to temperatures at or above 45°C[17] and also starts its decomposition to DCPA when it is heated in deionized or distilled water at or above 40°C.[46] Experiment 1 showed that 0.35 g of brushite crystals of this study (having the characteristic crystal morphology and XRD pattern reported previously[5,17]) completely transformed into DCPA upon heating in 100 mL of deionized water at 70°C for 1 d. Water is incorporated into the crystal structure of brushite and the water layers of brushite appear as bilayers parallel to the (020) faces of crystals.[4, 47] Water molecules inside the crystal structure are linked to the HPO_4^{2-} groups by bulk hydrogen bonds, but these hydrogen bonds are broken when the bilayers of water molecules were interrupted by the crystal surface termination, and the presence of such broken hydrogen bonds explains the instability of brushite crystals to heating (either dry or wet) at temperatures even below 50°C.

(2) Brushite to OCP

Experiment 2 provides, perhaps, the simplest and the most economical method of OCP synthesis performed at 70°C. The aqueous solution of Exp. 2, with an initial pH of 7.8 ± 0.1, only contains 27 mM HCO_3^- (i.e., the bicarbonate ion concentration of the human blood plasma) and the procedure of Exp. 2 does not involve any stirring and pH control during synthesis in contrast to more tedious yet popular OCP synthesis techniques.[48, 49] The XRD data of experiments 1 and 2 are given in Fig. 1. Although the final solution pH measured in Exps. 1 and 2, at the end of 24 h at 70°C, were close to one another (Table II), the solution pH alone does not strongly dictate which CaP phase will form. The solution of Exp. 2 matched the HCO_3^- concentration (27 mM) of blood plasma, while its Na^+ concentration was only 19% of that of blood.

The positive effect of CO_3^{2-} ion in enhancing the formation of OCP was previously reported by Iijima et al.,[50] although their synthesis experiments did not utilize DCPD as the starting material. The carbonate content, if any, of the powder samples of Exp. 2 were not measured.

(3) Brushite to Whitlockite

In order to form whitlockite (WH), out of brushite crystals, the aging solution must have Mg^{2+} ions. The Mg^{2+} concentration of 1.5 mM was selected, since it is the [Mg^{2+}] of the human blood plasma.[51] Experiment 3 resulted in the formation of single-phase whitlockite crystals at a final solution pH of 4.5 at the end of 24 h at 70°C. Figure 1 shows the XRD trace of the Exp. 3 sample and all peaks present in that trace do match with those listed in the ICDD PDF 04-09-3397 of WH. Figure 2(a) shows the morphology of the starting brushite flat plate-shaped crystals, whose XRD data were previously reported elsewhere.[45] Figures 2(b) and (c) depict the particle morphology of hexahedral WH crystals (of about 200 nm in the largest dimension) formed in Exp. 3, from the brushite crystals of Fig. 2(a), at two different magnifications. The flat plates (looking like brushite crystals) seen in the right half of Fig. 2(b) did not produce any brushite peaks in the XRD data of Exp. 3 (Fig. 1). Stulajterova and Med-

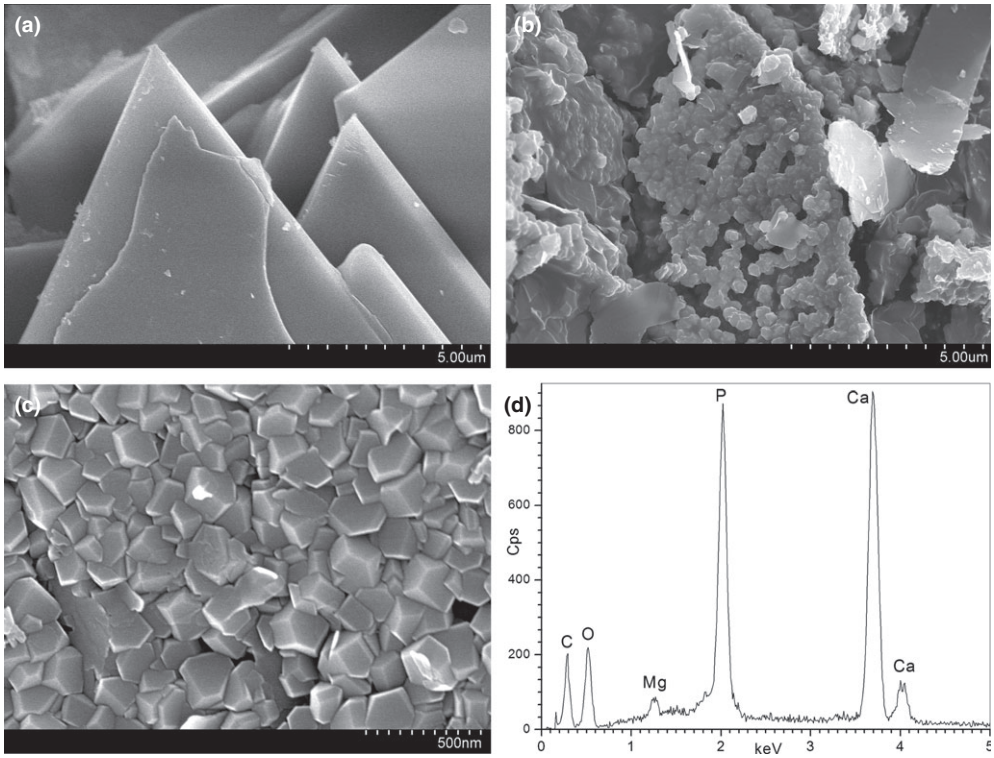

Fig. 2. (*a*) flat plate morphology of starting brushite crystals, (*b*) and (*c*) hexahedral whitlockite crystals formed in experiment 3, (*d*) EDXS spectrum of the whitlockite crystals of experiment 3.

vecky[52] reported that brushite hydrolyzed for long times in Ca^{2+} ion-containing aqueous solutions at elevated temperatures (as we did; however, in our case the source of aqueous Ca^{2+} was the dissolving brushite platelets themselves) may cause the drastic loss of brushite reflections from the XRD data of such samples while the particles still maintaining their plate-like shapes, in strong confirmation of our observations in this study. The morphology of the synthetic, hexahedral whitlockite crystals of this study [Fig. 3(c)] resemble those found and reported in the human tooth enamel,[34] human dental calculus,[43, 53] calculi of human prostate,[41] and human articular cartilage.[36] The mineralized sections of mature bones do not contain whitlockite in opposition to what is claimed by Jang et al.[31, 54] The characteristic EDXS data for the Exp. 3 sample is given in Fig. 2(d) and the semi-quantitative EDXS analysis indicated the presence of 2.2 ± 0.1 wt% Mg. The theoretical Mg wt% of WH is 2.31.

Would it be possible to synthesize WH, from brushite, at 37°C in less than 1 d in a nonstirred solution? The answer was not affirmative. Increasing the aging time at 37°C, in the 1.5 mM Mg^{2+} solution of Exp. 4, to 2 d did not cause the formation of any CaP phase other than DCPD. However, one week of aging at 37°C in the 1.5 mM Mg^{2+} solution of Exp. 5 resulted in the formation of the secondary phase of OCP. In confirmation of the early yet inspirational contribution of Rowles,[23] brushite crystals aged in a 1.5 mM Mg^{2+} solution at 37°C for 21 d (Exp. 6) resulted in the complete disappearance of brushite in favor of the formation of single-phase WH. The message received from experiments 3 through 6 was: WH formation, taking place at the expense of brushite crystals, proceeds in 1.5 mM Mg^{2+} solutions *via* a dissolution–reprecipitation process, and the kinetics of which is enhanced by an increase in the aging temperature from the human body temperature of 37°C to 70°C. Increasing the temperature of aging to 115°C (Exp. 7 of Table II) did again result in the formation of single-phase WH.

Calcium phosphate dissolution has a unique character; calcium and orthophosphate ions do not leach out, from any CaP phase, at exactly the same rate, and this is why one needs to consult two different solubility diagrams to compare the solubilities of CaPs with one another, one diagram with the axes of log [Ca] and pH, and the other with log [P] and pH. Two such diagrams have previously been provided by Chow and Takagi[55] and they indicate higher rates for Ca release in comparison to that of phosphate ions. The recently reported Ca-deficiency in brushite crystals,[5] and the associated phenomenon of the maturation of brushite during its synthesis, actually helps the above discussion. When DCPA (formed upon the initial conversion of DCPD into DCPA) undergoes its dissolution in aqueous solutions, the solution became progressively enriched in [Ca^{2+}] and this by itself explains the transition from DCPD/DCPA to OCP to WH (in the presence of Mg^{2+}), which represents a continuous increase in the Ca/P molar ratio of the reprecipitating phases.

(4) Brushite to Whitlockite + OCP Biphasics

Experiment 6, as mentioned previously, is a slow but sure way of transforming brushite (DCPD) into single-phase whitlockite (WH) at the human body temperature of 37°C in 21 d in a simple aqueous solution only containing 1.5 mM Mg^{2+}. In accord with the above discussion, if brushite dissolution continuously increases the Ca^{2+} concentration of the solution, one way to slowdown or to counter this [Ca^{2+}] enrichment would be to add 1 mM phosphate ions to the same solution and repeat the experiment with the new solution at 37°C for 21 d. The human blood plasma contains 1 mM phosphate ions. This physiologically relevant solution initially containing 1.5 mM Mg^{2+} and 1 mM phosphate ions was tested in experiment 8 and it resulted in the formation of WH + OCP biphasics *in lieu* of single-phase WH. Since the formulations of Exp. 6 and 8 were tested at the same temperature for the same aging time, they thus provide a direct comparison for the influence of 1 mM phosphate ions (in the solution of Exp. 8) on the phase constitution of solids recovered. The XRD traces of the samples of experiments 6 and 8 are given in Fig. 3(a). The characteristic morphology of a

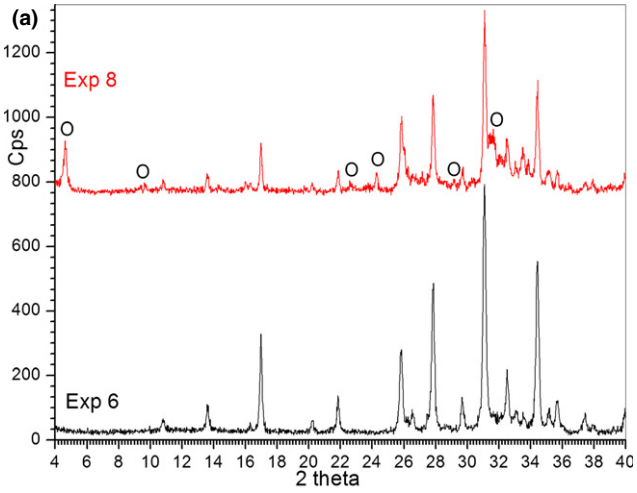

Fig. 3. (*a*) XRD traces of the samples of experiments 6 and 8; O indicates OCP peaks in the trace of experiment 8; experiments 6 trace belongs to single-phase WH, (*b*) SEM photomicrograph of the sample of experiment 8; the flake-like tiny crystals are of OCP, where the background hexahedral crystals are of WH.

WH + OCP biphasic sample (e.g., Exp. 8) is shown in the SEM photomicrograph of Fig. 3(b), where the occasional OCP flake-like crystals seemed to contaminate the hexahedral WH crystals. It should be emphasized that WH is not a CaP phase, but it is rather a CaMgP (calcium–magnesium phosphate) phase with a Ca + Mg/P molar ratio of 1.4286.

(5) Brushite to Apatitic CaP

The biomimetic synthesis of phase-pure Ap-CaP is not so difficult when using the route of hydrothermal transformation of brushite at the human body temperature of 37°C. The simple solution used in Exp. 9 has 124 mM Na^+, 103 mM Cl^-, 1.5 mM Mg^{2+}, 5 mM K^+, 1 mM PO_4, and 0.5 mM SO_4^{2-}. This extremely stable (with respect to any colloidal particle formation or decomposition) solution is named as saline ionic essentials medium (SIEM).[56] The above-mentioned ions are all present in the human blood plasma at the stated concentrations. The only slight mismatch in the composition of the solution of Exp. 9 is seen in its Na^+ concentration, where the Na^+ concentration of blood plasma is equal to 142 mM. A quantity of 350 mg of brushite was soaked in 100 mL of SIEM at 37°C for 21 d to synthesize single-phase, poor crystallinity Ap-CaP (Fig. 4) at the final pH of 7. This is a nonstirred synthesis process, which required no pH adjustments or control whatsoever.

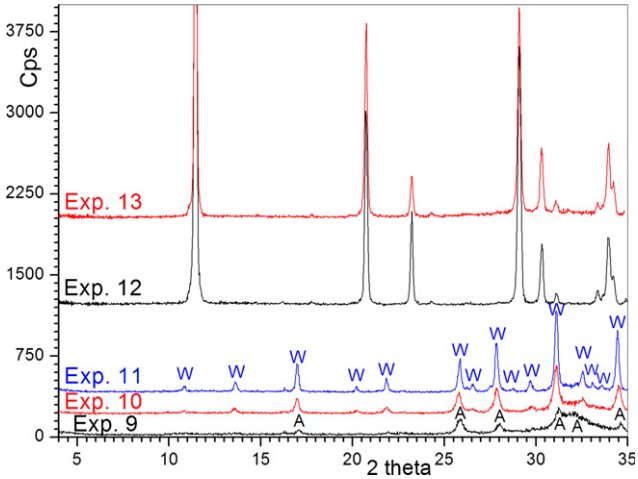

Fig. 4. XRD traces of the samples of experiments 9 through 13 (A: apatitic CaP, W: whitlockite).

(6) Brushite to Whitlockite + Apatitic CaP Biphasics

Experiment 10 helps to describe the synthesis of WH + Ap-CaP biphasic samples (Fig. 4) by only changing the hydrothermal transformation temperature from 37°C to 70°C, in combination with a drastic decrease in reaction time. Note that the solution composition and the brushite amount in both Exps. 9 and 10 were identical. Experiment 10 shows that it is possible to synthesize WH crystals at 70°C in a physiologically relevant, biomimetic solution having a pH of 7. In comparison to Exp. 10, experiment 11 (performed at the biomimetic temperature of 37°C for 21 d) uses a solution free of bicarbonate, sulfate and phosphate ions to facilitate a drop in pH to around 5, and the obtained samples were comprised of high crystallinity WH, only contaminated with small amounts of Ap-CaP (Fig. 4). The hump (Fig. 4, Exp. 11) at around 32° 2θ is indicative of the presence of Ap-CaP.

(7) Inhibition of Brushite Transformation

A water-based solution (100 mL) containing only 10 mM Mg^{2+} was found to completely inhibit the transformation of brushite crystals (0.35 g) into any other CaP phase, which can be detected by powder XRD, such as DCPA, OCP, WH, or Ap-CaP, even upon heating the said crystals in that solution at 70°C for 1 d (Exp. 12). The SIEM solution [of Section III(5)] with a [Mg^{2+}] raised to 10 mM was also found to inhibit the transformation of brushite into any such CaP phases within the detection ability of powder XRD (Exp. 13; 70°C, 1 d). The XRD traces of experiments 12 and 13 are shown in Fig. 4, indicating that only the crystalline brushite phase was present in both samples.

Experiments 3 and 12 are directly comparable with one another. While experiment 3, which resulted in WH formation, employed a solution with a [Mg^{2+}] of 1.5 mM, the same concentration was increased to 10 mM in Exp. 12; as the remaining experimental parameters were unchanged. Similarly, the experiments 9 and 13 are comparable with one another. In Exp. 9, the [Mg^{2+}] of the SIEM solution was 1.5 mM and the resultant sample was comprised of phase-pure Ap-CaP. Increasing the [Mg^{2+}] in Exp. 13 to 10 mM inhibited the Ap-CaP formation.

Although the strong influence of Mg^{2+} ions present in the aqueous synthesis media exert to stop the phase transformation of brushite into any other CaPs, such as OCP or Ap-CaP, was known,[57] electron microscopical evidence on this inhibition was not abundant in the literature. The SEM analysis performed on the samples of Exps. 12 and 13 was powerful enough to disclose what was happening on the surfaces

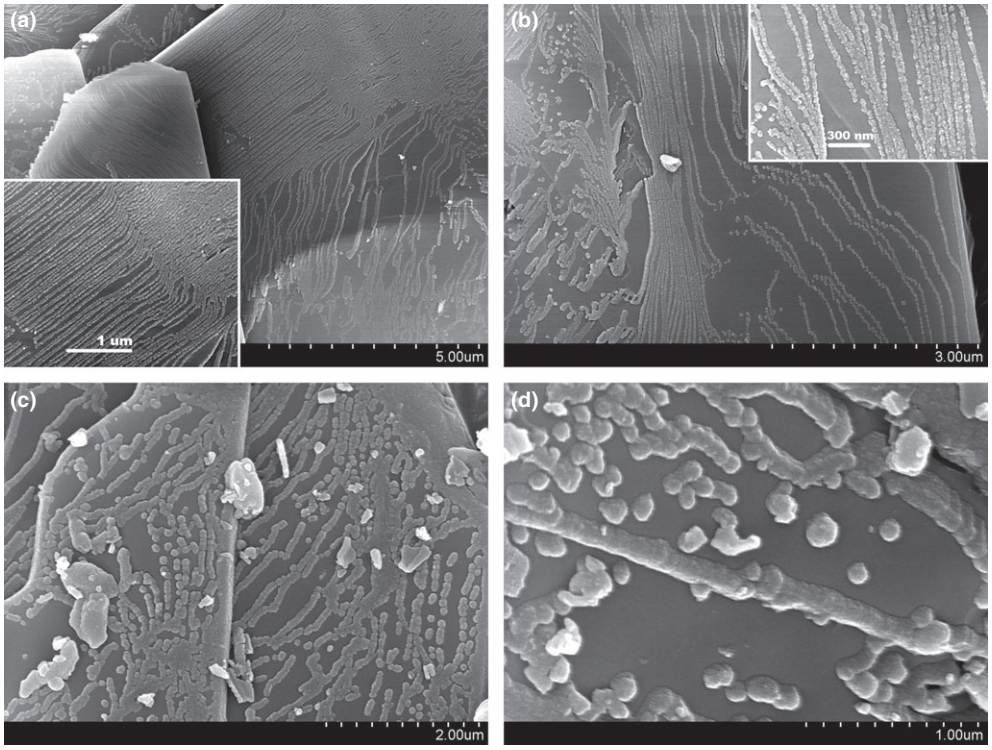

Fig. 5. SEM photomicrographs of the samples of experiment 12, (*a*) and (*b*); experiment 13, (*c*) and (*d*).

of brushite crystals. The photomicrographs of Fig. 5, for the samples of both Exps. 12 and 13, show the formation of nanofibrils of X-ray amorphous calcium phosphate or Mg-doped calcium phosphate (which do not show up in the powder XRD runs) on the surfaces of large brushite crystals. The surface nanofibrils actually start forming as a monolayer of individual yet much smaller globules [Figs. 5(b) and (f)] on the flat brushite surfaces, eventually coalescing with one another to form the nanofibrils. With the progress in surface coverage of brushite crystals by such nanoglobules/nanofibrils of X-ray-amorphous calcium phosphate or Mg-doped calcium phosphate, the inherent solubility of brushite crystals diminish and they would no longer be able to transform into OCP, WH, or Ap-CaP. X-rays still see the underlying brushite crystals, by readily penetrating through those nano-thick layers (Fig. 5) of X-ray amorphous fibrils, as single-phase brushite of high crystallinity (as shown in the XRD data of Fig. 4). The role of Mg in biomineralization (*in vitro*) is discussed, in detail, elsewhere.[56] Experiment 14, on the other hand, shows the presence of such strong inhibition of brushite transformation to persist at 70°C (1 d) in pure ethanol solutions having even a much lower [Mg^{2+}] of 1.5 mM.

In brief, the solutions and hydrothermal techniques reported in this study can be adopted to transform brushite-coated metallic implant materials into OCP, whitlockite, or apatitic calcium phosphate. The same can also be used to hydrothermally transform brushite granules, brushite cement bodies, or 3D-printed brushite bioceramic scaffolds into whitlockite, OCP, or apatitic calcium phosphate.

IV. Conclusions

The hydrothermal transformation of brushite (DCPD) to whitlockite (WH), OCP, apatitic calcium phosphate (Ap-CaP), whitlockite + octacalcium phosphate (WH + OCP) biphasics, and whitlockite + apatitic calcium phosphate (WH + Ap-CaP) biphasic samples were separately studied in unique solutions developed in this study. The solutions, free of any organics, such as Tris or Hepes, were partially or completely mimicking the inorganic ion concentrations of physiological fluids.

1. DCPD crystals transformed to single-phase OCP, in less than a day, when statically heated at 70°C in a simple aqueous solution only containing 27 mM HCO_3^-.
2. DCPD crystals transformed to single-phase WH, in less than a day, when statically heated at 70°C in an aqueous solution only containing 1.5 mM Mg^{2+}. DCPD crystals also transformed to WH in less than 3 weeks when heated at 37°C in the same solution of 1.5 mM Mg^{2+}.
3. DCPD transformation at 70°C, to any other CaP, was completely inhibited when the aqueous hydrothermal solutions contained 10 mM Mg^{2+}. DCPD crystals heated at 70°C in 1.5 mM Mg^{2+}-containing ethanol solutions did not transform to any other CaP as well.
4. DCPD crystals transformed to single-phase Ap-CaP, in less than 3 weeks, when statically heated at 37°C in a completely inorganic solution mimicking the Na^+, Mg^{2+}, K^+, Cl^-, HCO_3^-, SO_4^{2-}, and phosphate ion concentrations of the human blood plasma.

Disclaimer

Certain commercial instruments or materials are identified in this article solely 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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