Heating of Calcium Phosphate Crystals: Morphological Consequences and Biological Implications

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Abstract

Sintering hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) affects the chemical composition, the crystallinity, and the morphological features as demonstrated by means of X-ray diffraction (XRD), infrared spectroscopy (IR), and scanning electron microscopy (SEM). When heated to 1230°C, 16.7% of HA had decomposed to β-TCP. SEM investigations showed homogeneous, sharp angular polyhedric blocks of 30 to 50 μm with rare surface pores. On heating at 1230°C, β-TCP had entirely transformed to α-TCP. During sintering, the size of the powder grains increased and progressive bridging between the grains was observed. At 1230°C, a network within round-shaped polyhedric blocks of 50 to 90 μm was formed. In both, HA and β-TCP, surfaces were smooth. The chemical composition and the crystallinity of calcium phosphate ceramics determine their dissolution behavior and osteogenic properties. Nevertheless, their temperature dependent morphological features, such as, particle shape and size, surface texture, and porosity, as demonstrated in the present study, also influence the resorption rates, tissue responses, and wound healing duration. This should be emphasized more by clinicians in choosing an appropriate material for bone substitution.

Key Words: Calcium phosphate crystals, sintering, size and shape modifications, surface texture, chemical composition, crystallinity, biological implications, dental preprosthetic surgery, periodontal surgery, bone defect filling.

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Introduction

Synthetic calcium phosphates are applied in orthopaedic, maxillofacial, otorhinoplastic, and in dental preprosthetic and periodontal surgery in order to fill bone defects and to enhance bone repair (Nery and Lynch, 1978; de Groot, 1983; LeGeros, 1988; Passuti and Daculsi, 1989; Daculsi et al., 1990, 1992; Hamel, 1992). Before use, many are sintered at high temperatures. Besides the modification of the chemical composition and crystallinity of these materials, sintering also causes modifications of the size, shape and surface texture of the powder grains. These modifications are temperature dependent. They affect the surfaces in contact with tissue fluids and bone cells, and are thought to influence the biological reactions of these materials and their solubility properties. The morphological modifications have not been taken in account sufficiently by clinicians in dental prosthetics, periodontology, and osteoarticular surgery in choosing an appropriate material.

The aim of this study was to point out the modifications of the chemical composition and the crystallinity of hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) during calcination, to demonstrate the size, shape and surface texture modifications which affect the powder grains and to discuss possible biological consequences of those modifications.

Materials and Methods

Sample preparation

Carbonated HA and β-TCP powders prepared by Dr. R.Z. LeGeros (University of New York) were used. Both HA and β-TCP were passed through a 100 μm sieve. In free atmospherical conditions, the samples were heated from room temperature to respectively 900°C and 1230°C with a Vectra furnace. Each heating period was for one hour followed by maintaining the specified temperature for 90 minutes. Cooling was performed for one hour. Then, all heated and unheated powder samples underwent X-ray diffraction (XRD), infrared (IR) and scanning electron microscope (SEM) examinations.

Table 1. FWHM and D-value variations at different temperatures.

	Temp. °C	FWHM (002)	FWHM (310)	D (Å) (002)	D (Å) (310)
HA	20	0.1436	0.1409	614.7	626.5
HA	900	0.1449	0.1575	609.2	560.4
HA	1230	0.1113	0.1246	793.1	708.4

Table 2. FWHM and crystallinity index (CI) value variations at different temperatures.

	Temp. (°C)	FWHM	D (Å)
β-TCP	20	0.1457	0.620
β-TCP	900	0.1470	0.625

X-ray diffraction

The powders were examined using an INEL XRG 3000 CPS diffractometer at 40 kV and 30 mA connected to a CATO Multianalyser. The samples were exposed for about 30 minutes to a Cu-radiation ($\lambda = 1.5406$ Å). The 2θ reflections, relative peak intensities, and full width at half maximum (FWHM) values were calculated using a computer program developed at the "Institut des Matériaux", Nantes, France. Peak broadening is inversely related to both crystal size and internal lattice perfection (Vetter *et al.*, 1991). So, using Scherrer's equation (Posner *et al.*, 1963; Bonar *et al.*, 1983; Chantraine *et al.*, 1988; Vetter *et al.*, 1991)

$$D = \lambda/(\beta \cos\theta) \qquad [1]$$

D (the mean crystallite size in Å) values could be determined; these are not absolute values and used only for comparison (Chantraine *et al.*, 1988). λ is the X-ray wavelength (Å) employed; β the FWHM measured; and θ the reflectance angle. As β and θ were measured in degrees, not in radians, these values were divided by 57.3, the radian-degree conversion factor, before applying the above equation for D (Vetter *et al.*, 1991).

Infrared spectroscopy

From unheated and heated HA and β-TCP samples, pellets of 300 mg KBr/1 mg HA and 300 mg KBr/1 mg β-TCP were prepared and examined by means of a Nicolet 20 SXC FTIR spectrometer (32 scans) with 4 cm^{-1} resolution. On HA scans, CO_3 peaks were identified. On β-TCP scans, the crystallinity indices were calculated (Rey *et al.*, 1991).

Scanning electron microscopy

All powder samples were carbon coated and examined at 7 kV in a JEOL JSM 6300 scanning electron microscope to assess the size, shape, and surface texture modifications of the powder grains.

Results

Hydroxyapatite (HA)

The FWHM-values at hkl reflections 002 and 310 had increased from room temperature to 900°C and had strongly decreased at 1230°C. Consequently, the D-value variations, which do not give the crystal sizes directly but represent crystal size variations (Vetter *et al.*, 1991), indicated crystal size decrease at 900°C and high crystal growth at 1230°C (Table 1).

Infrared scans showed CO_3 peaks at 670 and 878 cm^{-1}. Furthermore, when heated to 1230°C, a β-TCP phase appeared at 944 cm^{-1}. X-ray diffraction investigations confirmed that HA had partly decomposed to β-TCP. The degree of decomposition to β-TCP, estimated by the relative peak heights of the strongest apatite (211) and β-TCP (0210) hkl reflections (Apfelbaum *et al.*, 1990), was 16.7%.

β-Tricalcium phosphate (β-TCP)

The FWHM-values calculated at the hkl reflection 2010 and the crystallinity indices (CI) measured on IR-scans exhibited little differences from 20 to 900°C which do not seem to be significant (Table 2).

At 1230°C, X-ray diffraction investigations showed that β-TCP had transformed to α-TCP.

Scanning electron microscopy (SEM) observations

Scanning electron microscopy investigations of HA, for room temperature and 900°C samples, showed loosely packed clusters of round-shaped powder grains of about 0.2 μm in diameter. Spaces between clusters were about 1 to 3 μm. All surfaces appeared rough, bridging between powder grains was not observed. When sintered at 1230°C (Figs. 1 and 2), the particles and clusters had coalesced to form sharp angular polyhedric blocks of about 30 to 50 μm, without or with rare surface pores of 0.2 to 2 μm. The boundaries of the original particles were either visible or had disappeared. The surfaces seemed to be smooth.

At room temperature, β-TCP showed loose bridging between round-shaped particles of about 2 μm in diameter. At 900°C, bridging between powder grains had progressed. The diameter of the bridged particles had increased to 4 to 6 μm. The grain boundaries could be seen. The width of the free spaces was about 1 to 4 μm. When sintered at 1230°C (Figs. 3 and 4), general bridging was observed forming a true network within polyhedric blocks of about 50 to 90 μm without any sharp angles. The spaces between the powder grains were about 1 to 6 μm. The boundaries between bridged powder grains persisted; all surfaces seemed to be smooth (Fig. 5).

Discussion

With regard to HA, the variability of the chemical and crystal structures of the used hydroxyapatites makes
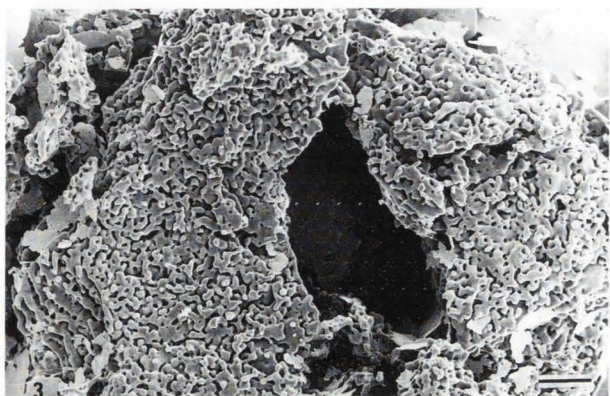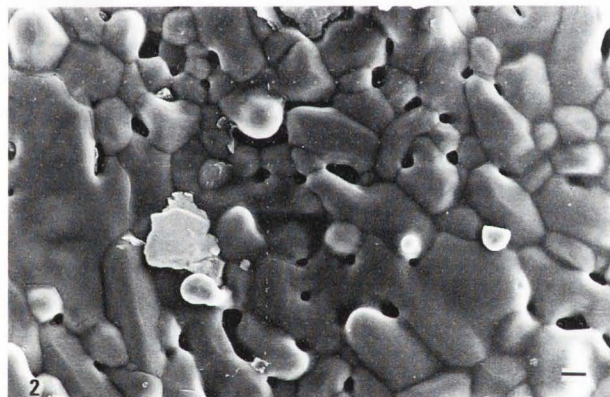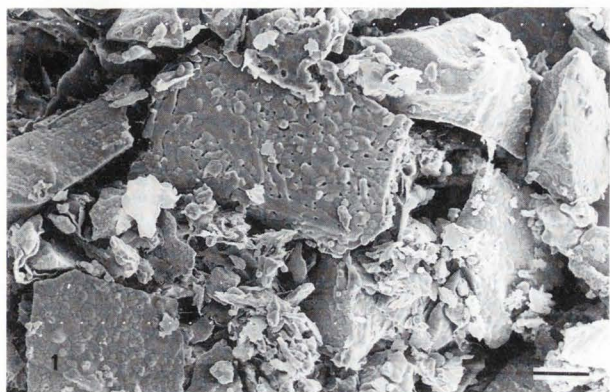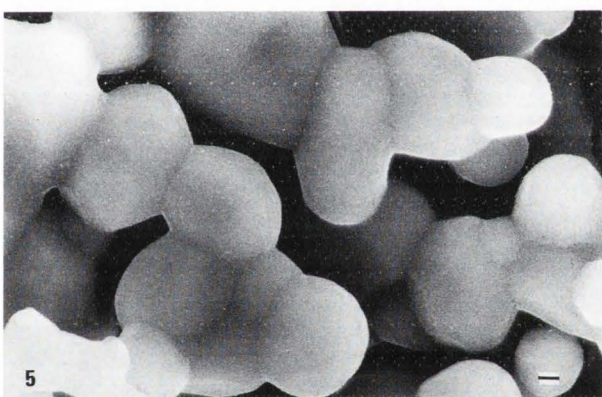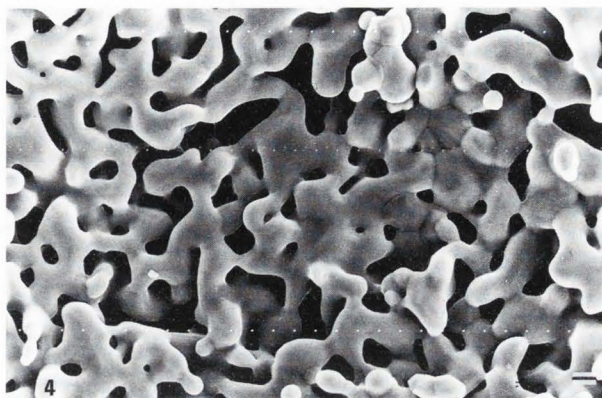
Figure 4. Detail of α-TCP formed by bridging of β-TCP powder grains sintered at 1230°C, showing a network structure. Bar = 2 μm.

Figure 5. Smooth surface texture and grain boundaries of coalesced α-TCP grains formed from β-TCP powder grains sintered at 1230°C. Bar = 200 nm.

Figure 1. Sharp angulated polyhedric blocks of coalesced HA powder grains sintered at 1230°C. Bar = 10 μm.

Figure 2. Surface of coalesced HA powder grains sintered at 1230°C showing some pores of 0.2 to 2 μm. Bar = 2 μm.

Figure 3. At 1230°C sintered β-TCP powder grains forming polyhedric blocks of α-TCP showing bridging phenomenon. Bar = 10 μm.

the comparison between different laboratory studies difficult (de Groot, 1983). Moreover, HA shows variable chemical stoichiometry and crystal structure according to the conditions used in its synthesis (Sakae, 1988). Beside these factors, the calcination of HA and β-TCP powders also affects the stoichiometry of the powder crystals, and modifies their solid phase constituents. On one hand, HA partially decomposes to β-TCP at about 1000°C, while on the other hand, rhombohedric β-TCP transforms to monoclinic α-TCP between 1120 and 1180°C (Fowler and Kuroda, 1986; Binder-Royer, 1990). During cooling, Binder-Royer (1990) observed a limited transformation of α-TCP to β-TCP, a finding not reproduced by our study. Therefore, both synthesis and manufacturing conditions are important, because the employed methods and the sintering temperatures affect the chemical composition and the crystallinity of HA and β-TCP powders and consequently their biological behavior (Daculsi and LeGeros, 1989; Ducheyne *et al.*, 1993).

With regard to the crystal size modifications (D value variations) found in our study, they are in accordance with findings in the literature (Young and Brown, 1982; Sakae *et al.*, 1989). These facts are well known. But the morphological modifications of calcium phosphate powder grains during sintering, as shown in our SEM study, seem not to have been taken sufficiently into account by clinicians. Indeed, the size and shape of these particles, their surface texture, and their porosity, are assumed to be important factors for dissolution behavior, and hence, for the rate of biodegradation (Klein *et al.*, 1983; LeGeros, 1988; LeGeros *et al.*, 1988; van Blitterswijk and Grote, 1989). Therefore, sintered HA blocks were estimated not to be appropriate for bone defect filling because of their lack of porosities (Dehen and Niederdellmann, 1989). Studies on dry HA powders (Thomann *et al.*, 1990) and laser irradiated HA (Meurman et al., 1992) showed that their surfaces provided significant amounts of calcium and phosphate ions adsorbed onto the apatite surface. The presence of these ions slows the dissolution rate (Ducheyne *et al.*, 1993) because the interface is charged (Meurman *et al.*, 1992). Furthermore, the solubility rates of calcium phosphates in decreasing order are: α-TCP, β-TCP, HA (Fowler and Kuroda, 1986; LeGeros *et al.*, 1988; Meurman *et al.*, 1992; Ducheyne *et al.*, 1993).

Plasma spraying of calcium phosphates revealed that β-TCP was more readily resorbable than HA which, *in vivo*, underwent minor surface dissolution (Ellies *et al.*, 1992). β-TCP is an extremely unstable form of calcium phosphate (Okazaki and Sato, 1990) and its dissolution rate is at least 10 to 20 times higher than HA (Klein *et al.*, 1983). HA ceramics possessed a higher osteogenic potential than β-TCP (van Blitterswijk and Grote, 1989), but β-TCP seemed biodegradable in contrast to HA which showed no detectable resorption after 9 months implantation (Klein *et al.*, 1983).

All implanted biomaterials encounter inflammation caused by the wound inflicted during healing (van Blitterswijk and Grote, 1989). The presence of foreign bodies leads to the so-called foreign-body reaction presenting macrophages and multinucleated cells which exhibit signs of phagocytosis, while the exudate cells show proliferative activity 4 weeks after implantation (van Blitterswijk and Grote, 1989). However, in the case of HA implants, there is little proliferative activity of exudate cells. As related by van Blitterswijk and Grote (1989), the implant shape and the surface structure have a direct influence on the biological performance of biomaterials. Size is an important parameter: above a certain size, whole particles cannot be phagocytosed. Surface texture affects tissue responses, making them more severe for rough than for smooth surfaces. Furthermore, round particles induce a less unfavorable reaction than angular material, and particles seem to cause a more severe inflammatory response than porous bodies. Similarly, round particles lead to faster resorption of inflammation than sharp ones. To combine the advantages of both HA and β-TCP, so-called biphasic calcium phosphates (BCP) of various β-TCP/HA ratios have also been used for bone defect filling, but systemic studies in animal models where HA/β-TCP ratios were varied and sequential time points studied, are lacking. BCP resulted in rapid bone replacement after implantation into periodontal defects in monkey (Weng *et al.*, 1991) and in man (Nery *et al.*, 1990). BCP containing 80% β-TCP and 20% HA (80/20 BCP) formed dense bone much more than 50/50 BCP (Weng *et al.*, 1991; Hamel, 1992). Six months after implantation, most of the implanted granules had disappeared in the defects (Weng *et al.*, 1991). An *in vivo* study of root socket filling after tooth extraction in beagle dogs by means of 85/15 BCP (Hamel, 1992), showed active resorption and advanced dissolution of the powder grains and bone apposition, 7 months after implantation. A complementary radiographic study of human root sockets filled by 40/60 BCP exhibited densified root sockets, 10 months later, which was interpreted as new-bone formation (Hamel, 1992). In addition, the alveolar process importance in BCP filled sites was about twice that in control sites (Hamel, 1992).

Conclusions

Calcium phosphate ceramics such as HA, β-TCP, or BCP of various β-TCP/HA ratios are frequently used in bone filling. In the present study, the SEM investigations were of particular interest. On one hand we found homogeneous angular blocks of HA/β-TCP presenting a high volume/surface ratio at 1230°C, while on the other hand, during sintering, progressive bridging between powder grains of β-TCP followed by the formation of round shaped α-TCP blocks with a low volume/surface ratio, which were composed of a network of round shaped structures. In both sintered HA and β-TCP, surfaces were smooth. Besides the chemical composition and the crystallinity of the employed materials, the temperature dependent morphological features, such as, granule size, surface texture, and porosity, which determine the dissolution behavior, tissue responses and wound healing duration should also be considered by clinicians in odontology and in osteoarticular surgery to choose an appropriate material. It seems to be of great interest to study the morphological modifications of BCP powders during sintering to determine the optimal sintering temperatures. As systemic studies in animal models where HA/TCP ratios were varied and sequential time points studied seem not to have been made, such studies are needed to assess the behavior of synthesized calcium phosphates in bone sites.

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Discussion with Reviewers

C. Rey: Please describe the starting materials more precisely. Is HA stoichiometric? How was the stoichiometry measured?
Authors: β-TCP was stoichiometric with a Ca/P ratio of 1.5. HA was prepared by precipitation. It was carbonated and non-stoichiometric.

C. Rey: The transformation of α-TCP into β-TCP depends essentially on the cooling rate of the sample. Two different experiments cannot be compared and the discrepancy observed might not be due to "synthesis or manufacturing conditions" but simply to different cooling rates.
Authors: In our study, we did not observe transformation of α-TCP to β-TCP during cooling, but we mentioned this transformation because it was observed by Daculsi and LeGeros (1989) and by Ducheyne et al. (1993).