Biphasic calcium phosphate bioceramics: preparation, properties and applications

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Biphasic calcium phosphate (BCP) bioceramics belong to a group of bone substitute biomaterials that consist of an intimate mixture of hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$, and beta-tricalcium phosphate (β-TCP), $Ca_3(PO_4)_2$, of varying HA/β-TCP ratios. BCP is obtained when a synthetic or biologic calcium-deficient apatite is sintered at temperatures at and above 700 °C. Calcium deficiency depends on the method of preparation (precipitation, hydrolysis or mechanical mixture) including reaction pH and temperature. The HA/β-TCP ratio is determined by the calcium deficiency of the unsintered apatite (the higher the deficiency, the lower the ratio) and the sintering temperature.

Properties of BCP bioceramics relating to their medical applications include: macroporosity, microporosity, compressive strength, bioreactivity (associated with formation of carbonate hydroxyapatite on ceramic surfaces *in vitro* and *in vivo*), dissolution, and osteoconductivity. Due to the preferential dissolution of the β-TCP component, the bioreactivity is inversely proportional to the HA/β-TCP ratio. Hence, the bioreactivity of BCP bioceramics can be controled by manipulating the composition (HA/β-TCP ratio) and/or the crystallinity of the BCP.

Currently, BCP bioceramics is recommended for use as an alternative or additive to autogeneous bone for orthopedic and dental applications. It is available in the form of particulates, blocks, customized designs for specific applications and as an injectible biomaterial in a polymer carrier. BCP ceramic can be used also as grit-blasting abrasive for grit-blasting to modify implant substrate surfaces. Exploratory studies demonstrate the potential uses of BCP ceramic as scaffold for tissue engineering, drug delivery system and carrier of growth factors.

1. Introduction

Albee, in 1920, reported the first successful application of a calcium phosphate reagent (described as ''triple calcium phosphate'') for the repair of bone defect in human [1]. More than 50 years later, clinical use of a ''tricalcium phosphate'' preparation in surgically created periodontal defects in animals was reported by Nery *et al*. [2] and use of dense hydroxyapatite (HA) as immediate tooth root replacements was reported by Dennnissen [3]. In the early 1980s, synthetic HA and β-tricalcium phosphate (β-TCP), became commercially available as bone substitute materials for dental and medical applications largely through the efforts of Jarcho *et al*. [4–8].

The term biphasic calcium phosphate (BCP) was first used by Nery *et al*. [9, 10] to describe the bioceramic that consisted of a mixture of HA and β-TCP, based on the X-ray diffraction analysis which showed that the ''tricalcium phosphate'' preparation material used in their early publication [2] was actually a mixture of 20% HA and 80% β-TCP [8]. The first studies on BCP with varying HA/β-TCP reported by LeGeros *et al*. [11–14] demonstrated that the bioactivity of these ceramics may be controled by manipulating the HA/β-TCP ratios. Subsequently, focussed studies on BCP by Daculsi and co-workers [15–19] led to the significant increase in manufacture and use of commercial BCP bioceramics (Table I) as bone substitute materials for dental and orthopedic applications [16, 19–27].

This paper briefly reviews the preparation, properties and present and future applications of BCP ceramics.

2. Preparation of BCP bioceramics

2.1. Calcium deficient apatites

Biphasic calcium phosphate or mixtures of HA and β-TCP are obtained when calcium-deficient biologic (e.g. enamel, dentin and bone mineral) or synthetic apatites are sintered at or above 700 °C [28, 29] according to the following reaction:

$$\underset{\text{CDA}}{Ca_{10-x}M_x(PO_4)_{6-y}(HPO_4)_y(OH)_2} \rightarrow \underset{\text{HA}}{Ca_{10}(PO_4)_6(OH)_2} + \underset{\beta\text{-TCP}}{Ca_3(PO_4)_2}$$

TABLE I BCP ceramics: commercial products

Product	Manufacturer	HA/β-TCP ratio
Ceratite	NGK Spark Plug, Japan	20/80
MBCP, Triosite, HATRIC	Biomatlante, Nantes, France	60/40
Tribone 80	Biomatlante, Nantes, France	20/80
Osteosynt	Einco Ltds, Belo Horizonte, Brazil	60/40

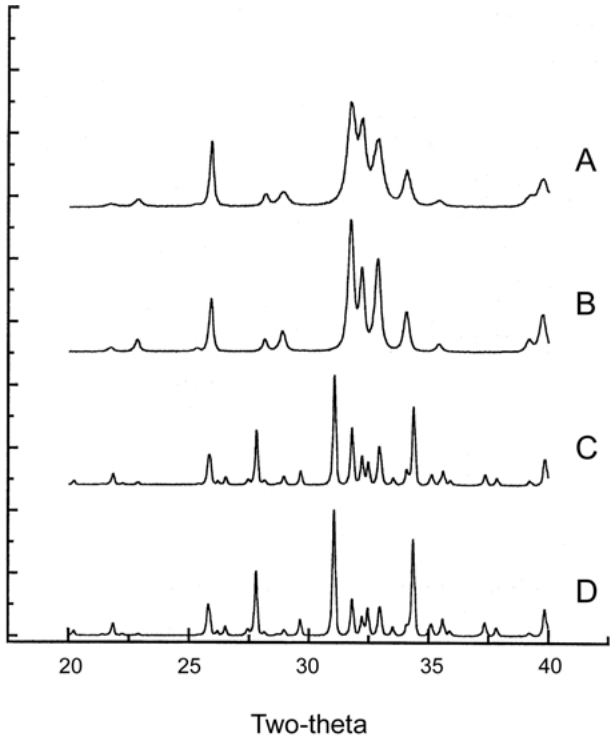

Figure 1 X-ray diffraction patterns profiles of CDA prepared by precipitation at (a) pH 4.5 and (b) pH 9. BCP obtained after sintering at 950 °C: (c) from CDA prepared at pH 4.5, and (d) from CDA prepared at pH 9. HA/β-TCP ratio: (c) 25/75; (d) 35/65.

The apatite is considered calcium deficient when the Ca/P ratio is lower than the stoichiometric value of 1.67 for pure calcium hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. Calcium deficient apatites (CDA) maybe represented by the formula, $Ca_{10-x}M_x(PO_4)_{6-y}(HPO_4)_y(OH)_2$ where M represents other ions (e.g. sodium, magnesium) which can substitute for calcium (Ca) ions [28, 29].

Calcium deficiency of the apatites depends on the synthesis conditions: precipitation or hydrolysis methods, reaction pH and/or temperature [28–34]. CDA may be obtained by precipitation method under different conditions of pH and temperature; the lower the pH, the higher the temperature required for the precipitation of apatite [29]. For example, CDA is obtained by precipitation at 80 to 100 °C even at low pH (pH 4–6). At lower temperatures and low pH, non-apatitic calcium phosphates (e.g. dicalciumphosphate dihydrate (DCPD), $CaHPO_4 \cdot 2H_2O$; or octacalcium phosphate (OCP), $CaH_2(PO_4)_6 \cdot 5H_2O$, is obtained [29]. The crystallinity (reflecting crystal size or perfection) of the CDA obtained at 80–100 °C depends on the pH of the preparation: the lower the pH, the higher the crystallinity, that is the bigger the crystals. This is demonstrated by the sharper diffraction peaks in the X-ray diffraction pattern of CDA obtained at pH 4.5 compared to that at pH 9 (Fig. 1(a) versus 1(b) indicating larger apatite crystals obtained at low pH compared to those obtained at at high pH (Fig. 2(a) versus 2(c)).

Another method of CDA preparation is by hydrolysis of non-apatitic calcium phosphates (Fig. 3(a)), including amorphous calcium phosphate (ACP), $Ca_x(PO_4, HPO_4)_y$ [29]; DCPD [29–31]; dicalcium phosphate anhydrous (DCPA), $CaHPO_4$ [29, 32]; OCP [29, 33]; or β-TCP, $Ca_3(PO_4)_2$ [29]. In the hydrolysis of DCPD in NaOH solutions, the calcium deficiency of the unsintered apatite and, subsequently, the HA/β-TCP ratio of the BCP obtained after sintering, can be controled by two variables: the concentration of the NaOH solution and

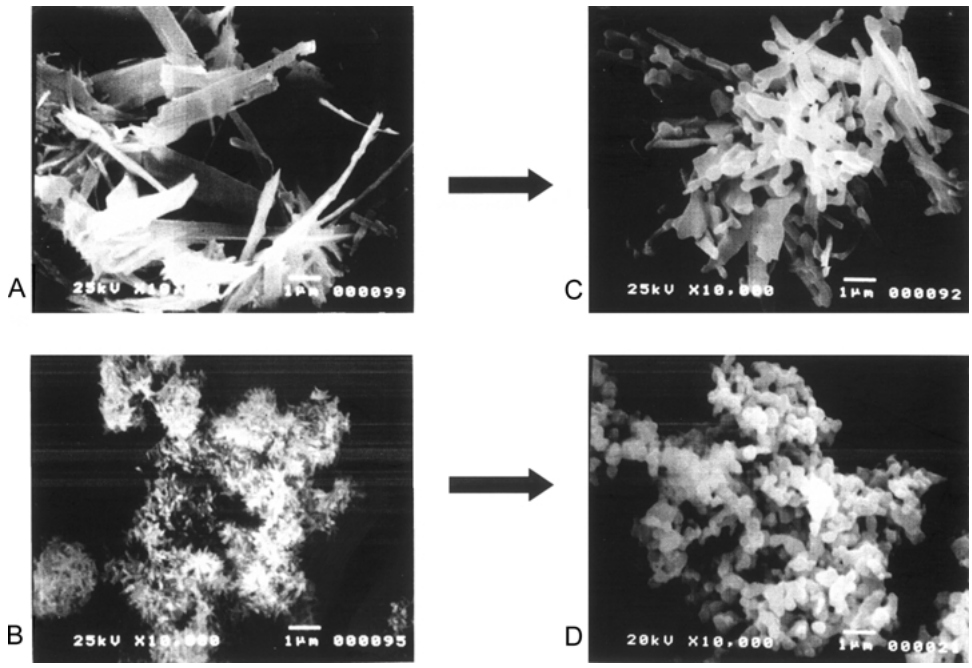

Figure 2 SEM micrographs of CDA prepared at pH 4.5: (a) before and (c) after sintering at 950 °C; and SEM micrographs of CDA prepared at pH 9: (b) before and (d) after sintering at 950 °C. Note that CDA crystals obtained at lower pH are much larger than those obtained at higher pH.

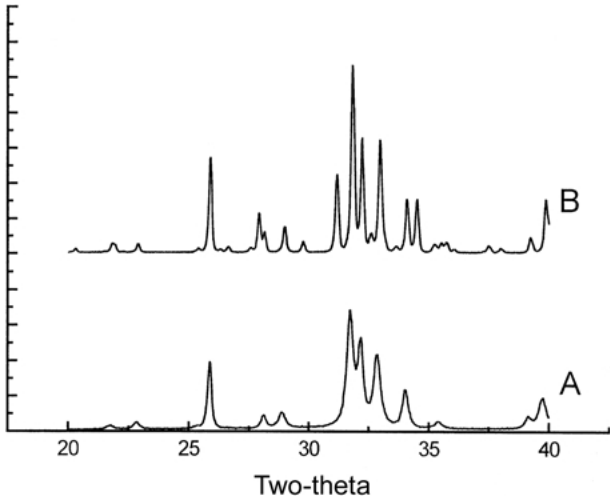

Figure 3 X-ray diffraction patterns of calcium-deficient apatite prepared by hydrolysis of dicalcium phosphate anhydrous (DCPA), $CaHPO_4$, in NaOH solution: (a) before sintering; (b) after sintering at 950 °C. The HA/β-TCP in the BCP in (b) is 70/30.

the ratio of the weight of the DCPD to the volume of the solution [31]. Hydrolysis of DCPD in phosphate solution results in apatite with greater calcium deficiency (LeGeros, unpublished).

2.2. Variation in HA/β-TCP ratio

Formation of pure HA, pure β-TCP or BCP (mixture of HA and β-TCP) is obtained after sintering biologic apatite or synthetic apatite obtained either by precipitation or hydrolysis methods (Fig. 4). The BCP composition (HA/β-TCP ratio) obtained after sintering depends on the calcium-deficiency of the unsintered biologic or synthetic apatite (Figs. 1, 3 and 4) and on the sintering temperature [29]. Presence of other ions during the preparation of the unsintered CDA can also affect the HA/β-TCP after sintering. For example, incorporation of carbonate or fluoride ions in the synthetic apatite results in a higher HA/β-TCP ratio in the BCP or even pure HA (or fluorapatite, FA, in the case of fluoride incorporation); while incorporation of magnesium (Mg) or zinc (Zn) results in a lower HA/β-TCP ratio in the BCP or even pure Mg- or Zn-substituted β-TCP, β-TC**M**P or β-TC**Z**P, respectively [28, 29, 35].

Sintering commercial calcium phosphate reagents (labeled as ''hydroxyapatite'' or ''calcium phosphate, tribasic'' or ''tricalcium phosphate'', for example, Baker or Fisher or Mallinckrodt, USA; or Merck, Darmstadt) above 900 °C was shown to result in pure HA, pure β-TCP, or BCP [36, 37]. BCP ceramic may be also prepared by mechanically mixing two types of synthetic apatites or commercial calcium phosphate reagents (e.g. Merck, Darmstadt, Germany): one resulting in β-TCP and the other, resulting in HA after sintering at temperatures above 900 °C [38].

Biologic apatite (e.g. enamel, dentin or bone mineral) can be approximated by the formula, $(Ca,Mg,Na)_{10}(PO_4,HPO_4,CO_3)_6(OH,Cl)_2$. After sintering at 900 °C or above, Mg-substituted β TCP, β-TCMP and carbonate-free HA are obtained from sintered enamel, dentin or young animal bones [28, 29, 39].

2.3. Introduction of macroporosity and microporosity

Macroporosity in the BCP ceramic is introduced by incorporating volatile materials (naphthalene, hydrogen peroxide or other porogens, for example, sugar etc.) heating at temperature below 200 °C and subsequent sintering at higher temperatures [40–42]. Macroporosity is formed resulting from the release of the volatile materials. Microporosity is a consequence of the temperature and duration of sintering.

3. *In vitro* properties of BCP ceramics

3.1. Crystal properties

The BCP crystals may be larger or smaller than the original unsintered CDA (Fig. 2), depending on the

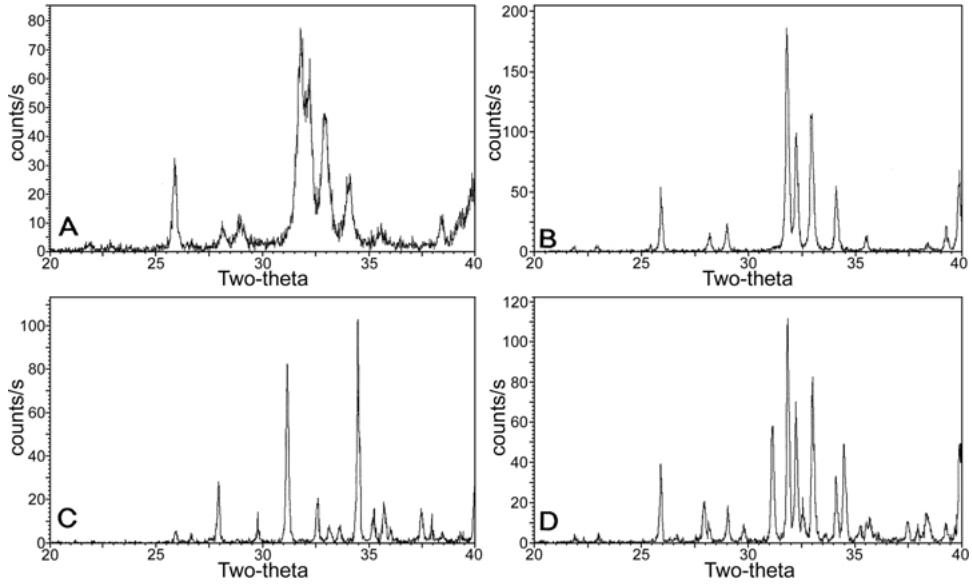

Figure 4 X-ray diffraction patterns: (a) synthetic CDA prepared by precipitation at 80 °C; (b) HA obtained by sintering synthetic apatite obtained under conditions of high pH; (c) β-TCP obtained by sintering apatite with high calcium-deficiency; (d) BCP, mixture of HA and β-TCP (HA/β-TCP = 65/35), obtained by sintering a precipated CDA.

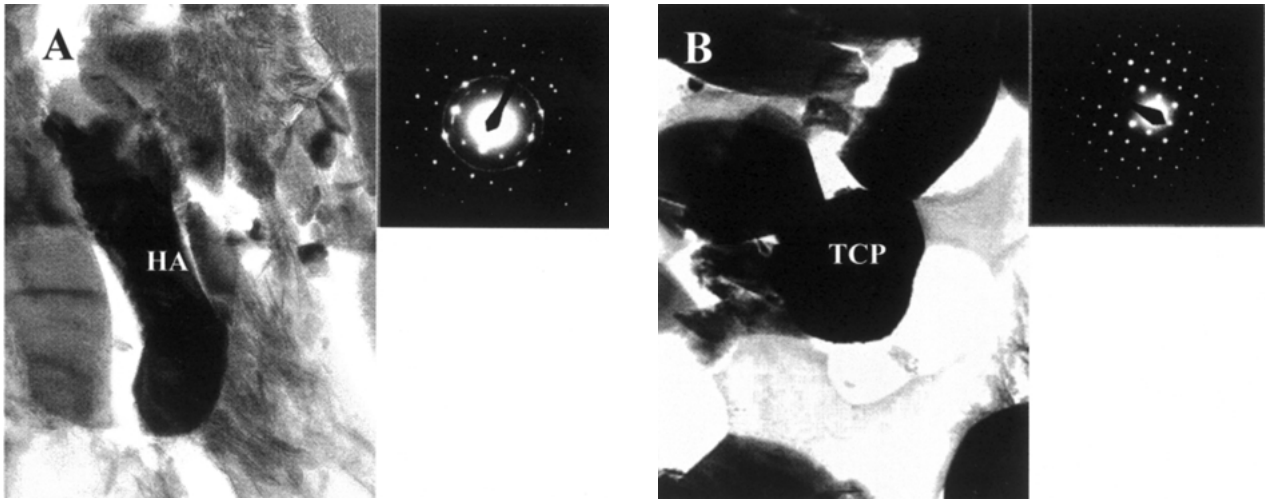

Figure 5 Transmission electron micrographs and corresponding electron diffraction patterns (insets) of (a) HA and (b) β-TCP crystals in the BCP implanted in rabbit [58].

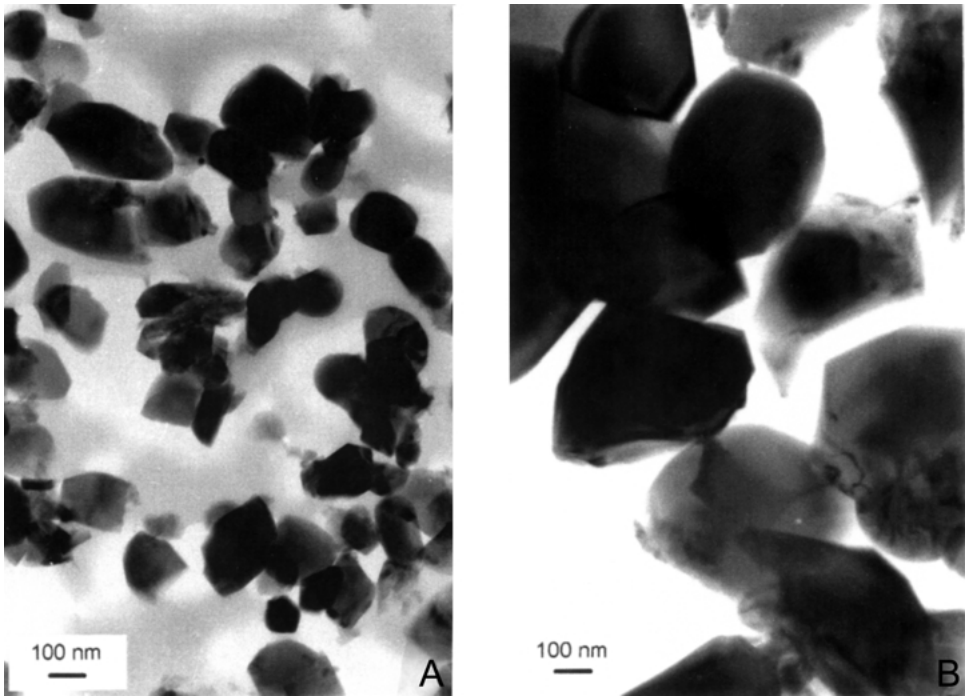

Figure 6 Transmission electron micrographs of biologic CDA (from dentin) after sintering at: (a) 800 °C and (b) 950 °C.

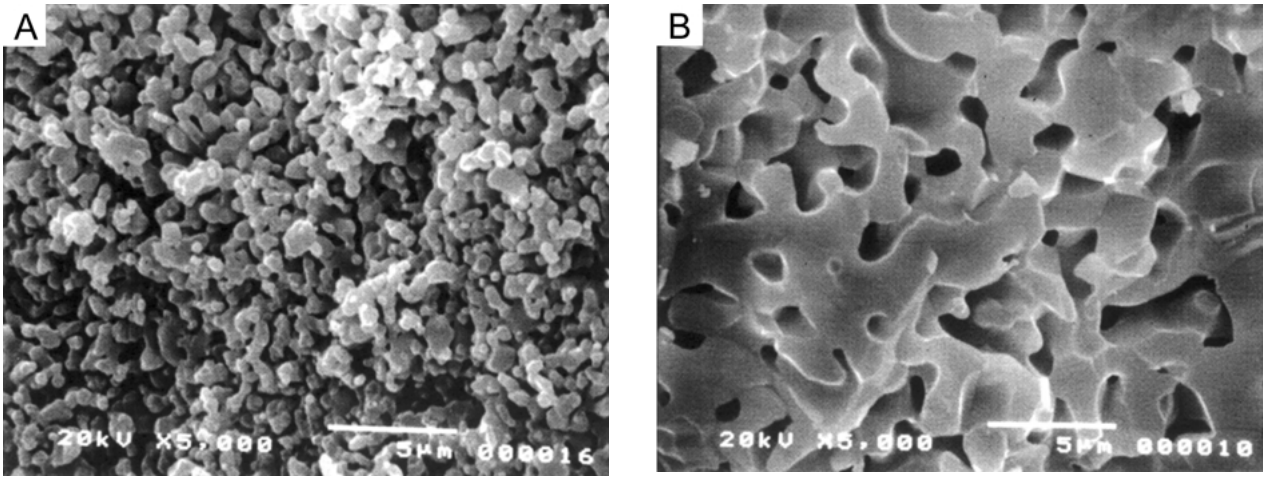

Figure 7 SEM of BCP sintered at: (a) 900 °C and (b) 1100 °C showing apparent fusion of smaller crystals in (b).

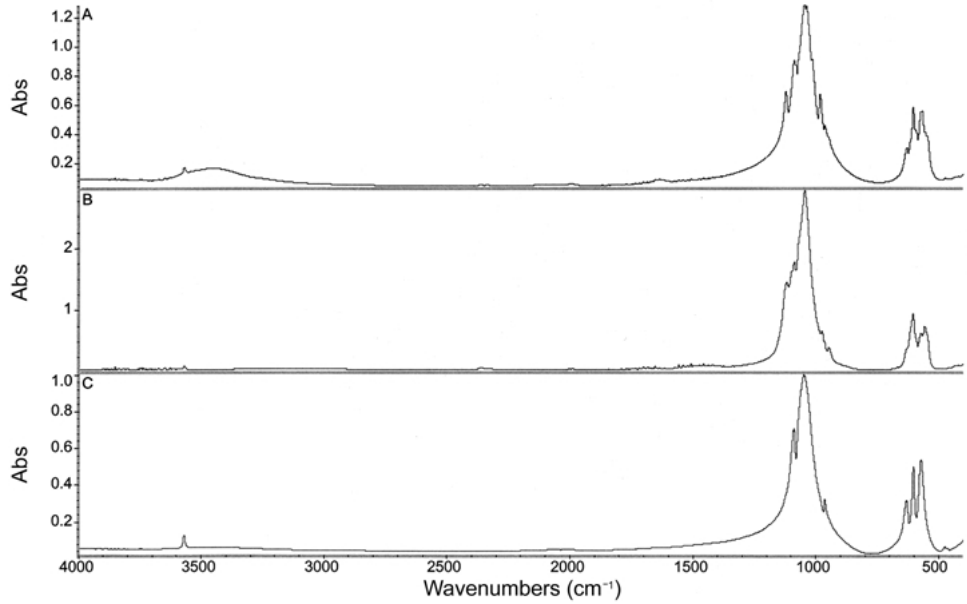

Figure 8 FTIR spectra of (a) HA, (b) BCP, consisting of 60HA/40β-TCP, and (c) β-TCP. (the corresponding XRD profiles are Figs. 4(b)–(d), respectively.

preparation conditions of the CDA. Using scanning electron microscopy, SEM, the HA crystals cannot be distinguished from the β-TCP crystals in the BCP sintered above 900 °C (Fig. 2). However, using transmission electron microscopy (TEM), electron diffraction is able to distinguish between HA and β-TCP in the BCP [43], as shown in Fig. 5. The crystallinity (reflecting crystal size and perfection) of the HA and β-TCP phases in BCP depends on the sintering temperature of CDA: the higher the sintering the temperature, the higher the crystallinity [29, 39]. For example, sintering temperature had a significant effect on BCP crystal size obtained by sintering dentin apatite (Fig. 6) and synthetic apatite (Fig. 7).

3.2. Variation in the BCP composition (HA/β-TCP ratio)

Infrared absorption analyzes reveals the composition of the BCP based on the absorption bands attributed to the HA and the β-TCP phases (Fig. 8) but cannot determine their relative amounts. Only X-ray diffraction analyzes can provides a means of approximating the HA/β-TCP ratio in the BCP (Figs. 1c, 1d, 3b and 4d). This ratio is determined using the ratio of the intensities of the most intense diffraction peaks of the HA phase to those of the most intense diffraction peaks of β-TCP phase compared with the ratios obtained from calibrated standard mixtures of pure HA and β-TCP [31, 34–36].

3.3. Macroporosity and microporosity

The ideal pore size for a bioceramic approximates that of bone (Fig. 9). It has been demonstrated that microporosity (diameter $< 10\,\mu m$) allows body fluid circulation whereas macroporosity (diameter $> 100\,\mu m$) provides a scaffold for bone–cell colonization [44–46]. It was reported that BCP ceramic with an average pore size diameter of 565 μm (compared to those with average pore size diameter of 300 μm) and 40% macroporosity (compared to 50% macroporosity) had greater bone

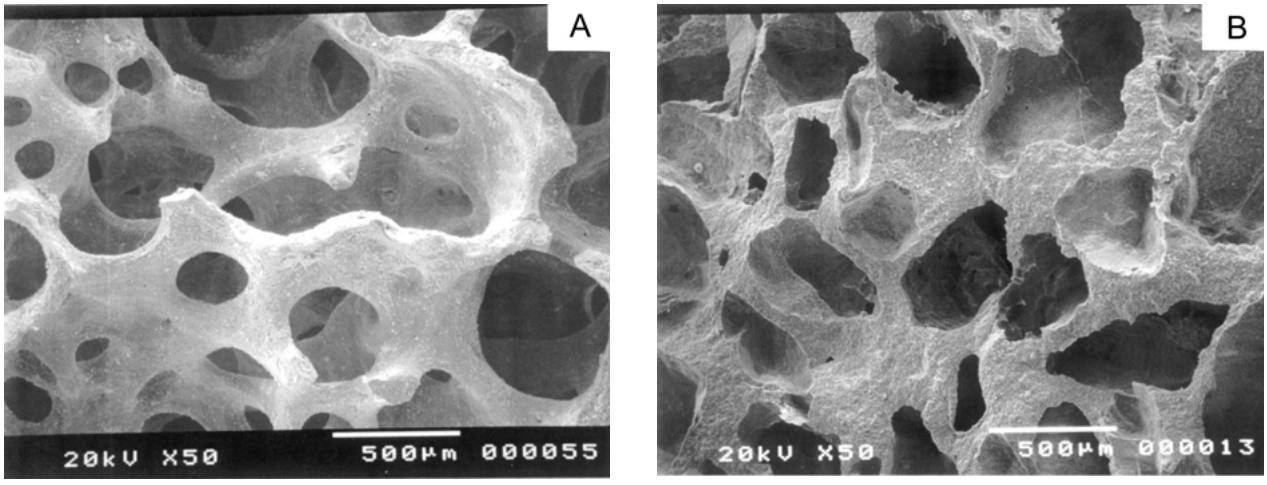

Figure 9 SEM: (a) bone and (b) BCP showing interconnecting macroporosity in both biologic and synthetic materials.

ingrowth [24]. Interconnecting macroporosity is observed in bone (Fig. 9(a)) and in a commercial macroporous BCP (Fig. 9(b)). Sintering temperature also affects macroporosty and microporosity (Fig. 8).

3.4. Mechanical properties

It is expected that the pore size and percent macroporosity of the BCP ceramic will affect the mechanical properties [34]. The preparation method also was found to have a significant influence on compressive strength. BCP ceramic prepared from a single calcium-deficient apatite phase was reported to exhibit higher compressive strength compared to BCP ceramic prepared by mixing two unsintered calcium phosphate, one which, after sintering at 1200 °C, resulted in only HA or only β-TCP [38].

3.5. Dissolution properties

The extent of dissolution in acidic buffer *in vitro* is much higher for the β-TCP ceramic compared to that for the HA ceramic [29, 47]. Thus, the extent of dissolution of BCP ceramic, of comparable macroporosity and particle size, will depend on the HA/β-TCP ratio: the higher the ratio, the lower the extent of dissolution [11, 12, 47] as shown in Fig. 10. The dissolution properties is also affected by the methods of obtaining BCP: whether from a single calcium-deficient apatite phase (BCP1) or from a mechanical mixture of two unsintered calcium phosphate preparations (BCP2): BCP2 exhibited higher extent of dissolution compared to BCP1 [37]. In some cases, BCP ceramic with similar HA/β-TCP ratios could present different dissolution rates (Fig. 11). This phenomenon may be caused by processing variables (sintering time and temperature) which could affect the total macroporosity and microporosity (Fig. 11): the greater the macroporosity and microporosity, the greater the extent of dissolution. *In vivo*, dissolution of BCP ceramics is manifested by a decrease in crystal size and increase in macroposity and microporosity [12, 13, 18].

3.6. *In vitro* cell/BCP ceramic interaction

BCP ceramics, like HA or β-TCP ceramics, present hospitable surfaces to monocytes [48] or osteoclasts [23, 47]. *In vitro* studies using commercial BCP ceramic with an HA/β-TCP ratio of 60/40 (*Triosite*®, Zimmer, Europe) showed less resorption pits on the BCP discs compared to those in dentin [49]. Yamada *et al*. [50] reported that osteoclastic resorption was highest for the BCP ceramic with HA/β-TCP ratio of 25/75 compared to those with higher ratios which seemed to indicate that osteoclastic resorption is a function of the solubility of the BCP, which in turn, is a function of the HA/β-TCP ratio: the lower the ratio, the higher the solubility (Fig. 10). However, the same study demonstrated that the BCP (HA/β-TCP ratio, 25/75) had a higher osteoclastic resorption than pure β-TCP in spite of the higher solubility of the latter. This may be related to the observation that the amount of calcium and phosphate ions released in the environment is critical to cellular activities [51].

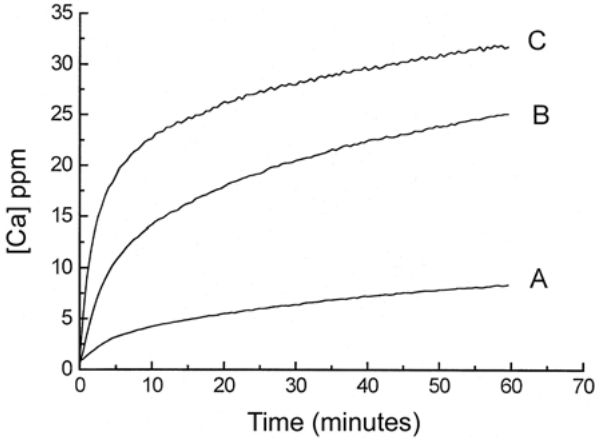

Figure 10 Effect of HA/β-TCP ratios on the dissolution properties of BCP: the higher the ratio, the lower the extent of dissolution. Dissolution experiments conducted in 0.1 M KAc, pH 6, 37 °C. The release of calcium ions to the acidic buffer was monitored using calcium-selective ion electrode. The HA/β-TCP ratios of the BCP samples: (a) 80/20; (b) 40/60 and 20/80.

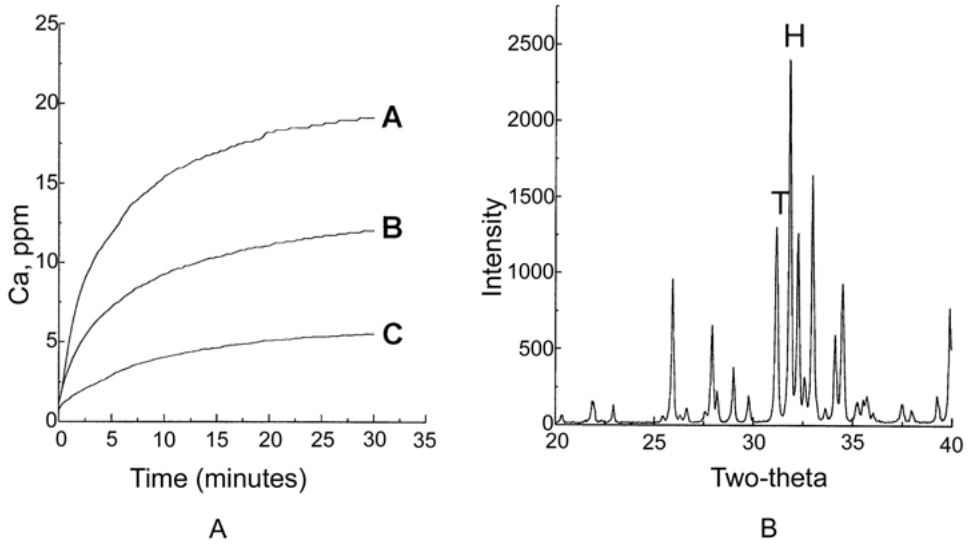

Figure 11 (a) Comparative extent of dissolution of BCP specimens in acidic buffer (0.1 M KAc, pH 6, 37 °C) with similar BCP composition shown in (b). The BCP specimens were sintered under different conditions.

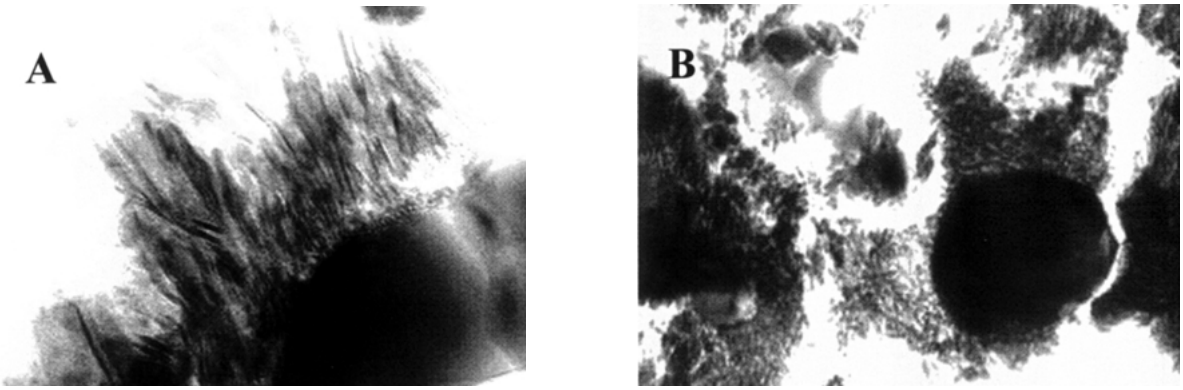

Figure 12 Formation of carbonate hydroxyapatite on surfaces of BCP crystals: (a) *in vitro* in simulated body fluid [43]; and (b) *in vivo* after implantation [58].

3.7. Bioactivity

A special characteristic of bioactive materials is their ability to form a direct bond between the tissues and the material resulting in a uniquely strong interface [52]. Bioactive materials include special glass formulations (bioglass) first developed by Hench [52] and calcium phosphate materials such as HA, β-TCP, BCP [4–8, 52–54]. Bioactivity has been associated with the formation of bone apatite-like (carbonate hydroxyapatite, CHA) on surfaces of the biomaterial *in vitro* when exposed to metastable calcium phosphate solution or simulated body fluid [43, 55] and *in vivo* after implantation in osseous and non-osseous sites [11–13, 29, 56–58] as shown in Fig. 12.

4. Tissue response: bone formation

4.1. BCP/tissue interface

While bioactive materials form a strong and direct bond between the bone and the material, non-bioactive materials usually form a weak and indirect bond between the tissues and the material, the interface consisting of non-mineralized tissues [53, 54, 56]. Transmission electron microscopy demonstrated the presence of bone apatite-like (CHA) crystals on the surfaces of BCP crystals (Figs. 5(b), 11). The abundance of the CHA crystals appeared to be inversely related to the HA/β-TCP ratio of the BCP ceramic [11, 12]: the lower the ratio, the greater the abundance of the CHA crystals on the surface. It was proposed that the formation of CHA results from the partial dissolution of the HA or β-TCP crystals increasing the supersaturation of the calcium and phosphate ions in the microenvironment causing the precipitation of CHA which incorporates the carbonate, magnesium ions present in the biological fluid [12, 29, 56].

While a difference in rate of biodegradation was observed for BCPs obtained from different methods, the rate of new bone formation associated with the BCP bioceramics were not significantly different [38]. One report suggested that BCP with HA/β-TCP ratio of 20/80 was most effective in eliciting a greater bone formation [59]. On the other hand, Nery *et al*. [14] reported that BCP with HA/β-TCP ratio of 80/20 compared to other ratios, was more efficient in repairing surgically created periodontal defects.

4.2. Osteoconductive and osteoinductive properties

BCP ceramics, like other bioactive bone graft materials (HA, β-TCP, bioglass, bone-derived or coral-derived HA), are osteoconductive but not osteoinductive [12, 60]. Osteoconductive materials provide the appropriate scaffold or template which would allow ''vascular ingress, cellular infiltration and attachment, cartilage formation and calcified tissue deposition'' [61]. Osteoinductive materials (e.g. bone morphogenetic proteins) ''stimulate uncommitted cells (e.g. mesenchymal stem cells) to convert phenotypically to chondroprogenetor and osteoprogenitor cells'' [61–63].

However, osteoinductive property has been attributed to BCP ceramic implanted subcutaneously [18, 64]. This observation may not be due to an inherent osteoinductive property of the BCP ceramic but may be a consequence of a critical geometry similar to that reported for porous HA ceramic [65, 66]. This critical geometry allows concentration of endogenous BMPs in sufficient amount to induce osteoinductivity [63, 66].

5. Present and future applications

5.1. Medical and dental applications of BCP bioceramics

Commercial BCP bioceramics of varying HA/β-TCP (Table I) are currently available in different forms: granules, blocks, specially designed forms for specific applications (wedges, cylinders, etc.), and in injectible form in a hydroxypropylmethyl cellulose carrier [67–69]. Dental and orthopedic applications of BCP bioceramics include: repair of large bony defects, periodontal defects, orthopedic lesions, lumbar spine fusion, correction of scoliosis, fillers for enchondroma of the metacarpals and phalanges of the hand, and ophthalmic implant [9, 10, 14, 15–27, 67, 70]. BCP has also been used a carrier for antibiotics related to repair of orthopedic lesions [27].

5.2. BCP for surface modification of implant substrate

A preliminary study reported that BCP used as an abrasive to roughen the surfaces of titanium alloy implant provided the following advantages compared to

other abrasives normally used (e.g. alumina or silica): clean surface, free of contamination from abrasives, and a more reactive surface [71].

5.3. Potential future applications of BCP

Studies on potential applications of BCP include: carriers for growth factors [72], hormones [73], and as scaffold for tissue engineering using stem cells [74–77].

6. Summary and conclusion

Biphasic calcium phosphate biomaterials are a group of bone substitute materials consisting of a mixture of HA and β-TCP. It is obtained by sintering calcium deficient apatites. These materials have the advantage of controled bioactivity by controlling the HA and β-TCP ratio. BCP, like other bioactive bone substitute materials, are osteoconductive and has the possibility of acquiring osteoinductive properties through appropriate critical geometry of macroporosity. Besides the medical and dental applications, BCP has a potential for other applications such as delivery system for drugs, antibiotics, hormones; carriers for growth factors; scaffolds for tissue engineering.

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