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BETA-TRICALCIUM PHOSPHATE RESORPTION BY MONOCYTES IN BIPHASIC CALCIUM PHOSPHATE: AN *IN VITRO* STUDY

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Abstract

A macroporous biphasic calcium phosphate (BCP) of 60.7% carbonated dense non-porous hydroxyapatite (HA)/39.3% dense microporous β -tricalcium phosphate (β -TCP) was immersed during 15 days in a supplemented α -MEM (minimal essential medium) solution with and without dog bone marrow cells obtained by puncture. The aim of the study was to demonstrate the time-related changes in the BCP fractions by means of X-ray diffraction, infrared spectroscopy and scanning electron microscopy, and to investigate the cell populations. The HA/ β -TCP ratios varied according to immersion duration and cell presence. If any, there was a slight preponderance of β -TCP dissolution over that of HA. The dissolution rate was much lower in the presence of cells. Cell-mediated resorption was observed only in β -TCP sites. The implicated small round-shaped cells of about 30 μ m were located in resorption lacunae, presented numerous short filopodia and a ruffled border faced towards the material to resorb. They did not undergo fusion with other cells but lost, on about day 15, their ruffled border. Then blast-cells highly involved in matrix secretion and mineralization appeared. There seems to be ample evidence that the clast-cells are specific monocytes which resorb β -TCP under these *in vitro* conditions. To elucidate their exact nature, functions and fate, further long-range *in vitro* studies conducted under standardized conditions are needed.

Key Words: Bone marrow, biphasic calcium phosphate, cell/substrate interactions, clast cells, blast cells.

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Introduction

Many *in vitro* and *in vivo* studies on hydroxyapatite (HA), β -tricalcium phosphate (β -TCP) and combinations of both, so-called biphasic calcium phosphates (BCP) have been published. BCP with various HA/ β -TCP ratios have become the material of choice for dental and medical applications (LeGeros, 1988; Passuti and Daculsi, 1989; Nery *et al.*, 1990; Weng *et al.*, 1991; Hamel, 1992; LeGeros, 1993; Ducheyne, 1994). In spite of the differences concerning results and their interpretation, there is a general consensus that the physico-chemical properties of BCP and cell-culture medium influence the behaviour of seeded cells and vice-versa.

Among the physico-chemical properties of BCP, porosity and solubility are important parameters in biomaterial degradation processus. Calcium phosphates are classified as "dense" or "porous," referring to the presence of micro- or macropores (LeGeros, 1988). Macropores are due to deliberate chemical processing (LeGeros, 1988), while sintering is responsible for dense non-porous or microporous material and for material transformation or decomposition. During sintering, HA can transform into metastable oxy-HA (OHA) or decompose into a multiphasic mixture, consisting of HA, OHA, α -TCP and tetracalcium phosphate (TTCP) (Ducheyne *et al.*, 1993); β -TCP can transform in part or in full to monoclinic α -TCP (Fowler and Kuroda, 1986; Binder-Royer, 1990; Bohne *et al.*, 1993; Doi *et al.*, 1993; Ducheyne *et al.*, 1993). α and β -TCP are known as rather unstable forms of calcium phosphate (Okasaki and Sato, 1990; Radin and Ducheyne, 1994). Nevertheless, when β -TCP is dense it can be as stable as HA because of its little specific surface area (Ducheyne *et al.*, 1993). On the other hand, dense stoichiometric HA, which is among the more stable of the calcium phosphates (LeGeros *et al.*, 1991; Ducheyne, 1994), becomes greatly resorbable when CO_3^{2-} is incorporated (Ong *et al.*, 1994). In biphasic ceramics, the dissolution rate is clearly affected by the presence of the more soluble component (Ducheyne *et al.*, 1993; Kohri *et al.*, 1993). So those HA/ β -TCP mixtures with similar

Table 1. Diffraction angles 2θ , peak intensities and hkl reflections of the HA fraction; SD = standard deviation.

2θ	SD (2θ)	Intensity	h k l
25.744	± 0.019	49.13	0 0 2
28.830	± 0.088	14.31	2 1 0
31.683	± 0.010	100.00	2 1 1
32.080	± 0.030	52.85	1 1 2
32.806	± 0.020	66.92	3 0 0
33.963	± 0.030	23.76	2 0 2
39.713	± 0.020	26.36	3 1 0
46.573	± 0.020	30.12	2 2 2
49.359	± 0.030	32.11	2 1 3

Table 2. Diffraction angles 2θ , peak intensities and hkl reflections of the β -TCP fraction; SD = standard deviation.

2θ	SD (2θ)	Intensity	h k l
13.531	± 0.101	11.6	1 0 4
16.896	± 0.061	14.27	1 1 0
27.758	± 0.041	38.55	2 1 4
29.568	± 0.128	9.78	3 0 0
30.975	± 0.025	61.29	0 2 10
32.406	± 0.142	11.16	1 2 8
34.329	± 0.018	45.01	2 2 0
46.923	± 0.044	13.29	4 0 10
47.985	± 0.043	21.61	2 3 8

Table 3. HA/ β -TCP ratios related to time of immersion and medium composition.

	Day 0	Day 7	Day 14
medium only	1.21	1.63	1.51
medium + cells	1.21	1.31	1.46

micro-porosity are directly proportional to the β -TCP content (LeGeros, 1988; Daculsi *et al.*, 1989; Nery *et al.*, 1990; Ducheyne *et al.*, 1993). As the composition, porosity, crystal structure and ultrastructure control the kinetic reactions on BCP surfaces (Radin and Ducheyne, 1994), samples should be characterized thoroughly before use.

In vitro and *in vivo* studies show that ceramics undergo gradual changes at their surfaces by dissolution, precipitation and/or ion-exchange reactions (Ducheyne, 1994; Radin and Ducheyne, 1994; de Bruijn *et al.*, 1995; Suzuki *et al.*, 1996), which have been related to the composition of the employed culture medium, and on the physico-chemical properties of the biomaterial (Nery *et al.*, 1990; Kohri *et al.*, 1993; Radin and Ducheyne, 1994; Soueidan *et al.*, 1995).

Clast and blast-cells are other actors which participate in resorption and apposition mechanisms. Osteoclasts, odontoclasts and monocytes are related to hard tissue resorption (Bagi and Miller, 1989; Baslé *et al.*, 1993; Gomi *et al.*, 1993; Benahmed *et al.*, 1994; Kadoya *et al.*, 1994; Blottière *et al.*, 1995; Dersot *et al.*, 1995; Sidqui *et al.*, 1995; Sahara *et al.*, 1996a,b). Their biochemical identification seems to be extremely difficult: all stain TRAP \oplus (Bagi and Miller, 1989; Ugadawa *et al.*, 1990; Gomi *et al.*, 1993; Kadoya *et al.*, 1994; Dersot *et al.*, 1995) and do not possess exclusive antibody markers (Kadoya *et al.*, 1994). So, morphological features, such as cell size, nuclei number and cell membrane specializations, are commonly used (Baslé *et al.*, 1993; Dersot *et al.*, 1995; Guillemain *et al.*, 1995; Piper *et al.*, 1995; Väänänen and Horton, 1995; Sahara *et al.*, 1996a,b). Osteoclast-like cell formation *in vitro*, osteoclast formation *in vivo*, and odontoclast formation are the result of the fusion of the "late monocytic" cell types, both with each other (Sahara *et al.*, 1996a,b; Solari *et al.*, 1996). Mature multinucleate osteoclasts and odontoclasts actively recruit and shed nuclei (Parfitt, 1994; Sahara *et al.*, 1996a,b; Solari *et al.*, 1996). Osteoclasts (Segawa *et al.*, 1989; Baslé *et al.*, 1993; Martin and Ng, 1994; Dersot *et al.*, 1995; Sidqui *et al.*, 1995; Väänänen and Horton, 1995; Athanasou and Path, 1996; Quinn *et al.*, 1996; Solari *et al.*, 1996), odontoclasts (Sahara *et al.*, 1996a,b) and hard-tissue resorbing monocytes (Martin and Ng, 1994; Sahara *et al.*, 1996a,b) have dorsal microvilli, a ruffled border and a clear zone facing the tissue to resorb. Resorption lacunae formed in dentin by monocytes and fused monocytes (multinucleate odontoclasts), for example, resemble those of osteoclasts (Sahara *et al.*, 1996a,b). In spite of the difficulty in characterizing clast-cells, we think that small, hard tissue resorbing cells are monocytes, rather than osteoclasts or odontoclasts.

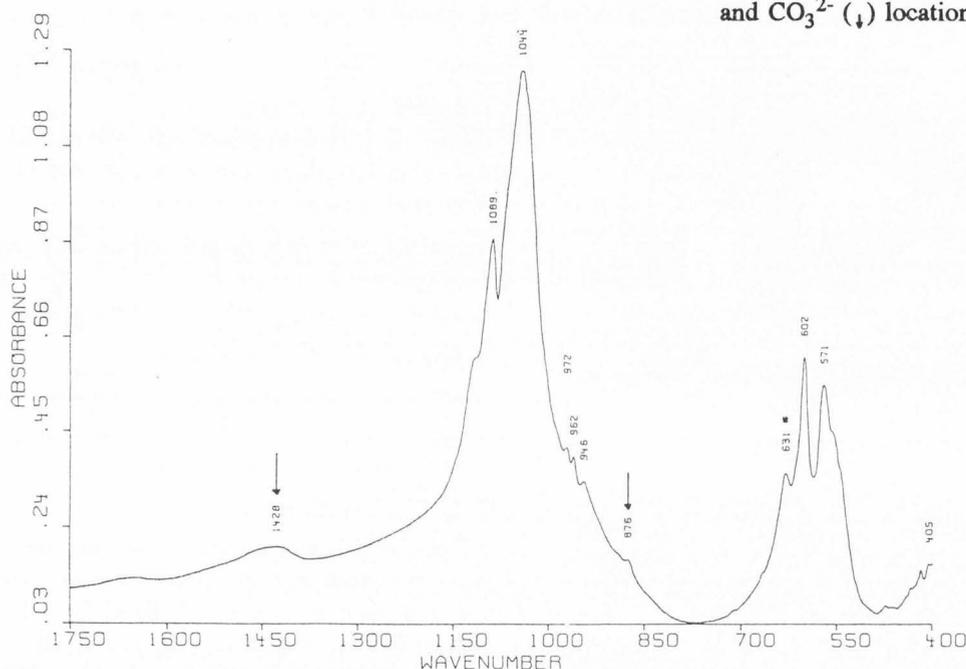


Figure 1. FTIR scan of BCP; water (*) and CO_3^{2-} (↓) locations.

Compared to clast-cell identification, that of blast-cells is easy: morphological and ultrastructural features are generally sufficient (Ishaug *et al.*, 1994).

The aim of this study was to demonstrate the changes in a well-defined BCP exposed during 15 days to a standardized cell culture medium containing dog bone marrow cells and to the culture medium alone using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) techniques, and to investigate the cell populations.

Materials and Methods

Culture medium, cells and BCP

α -MEM (minimal essential medium) supplemented with respectively 1% glutamine, sodium pyruvate, penicillin and streptomycin, with 10^{-6} M OH-cortisone and, respectively, 12.5% foetal calf and horse serum, dog bone marrow cells obtained by puncture and 3 series of 3 samples each of BCP blocks 5 to 6 mm in diameter and about 3 mm in height made of synthetic HA and β -TCP, compacted and sintered at 1100°C into a 40 to 50% macroporous BCP were used. Characterized by XRD, FTIR and SEM investigations as described below, the biomaterial was a macroporous BCP of 60.7% carbonated dense non-porous HA/39.3% dense microporous β -TCP (weight ratio). The cell culture solution was

maintained at 37°C and, referring to Ducheyne *et al.* (1993), not changed during the immersion period. The initial mean pH of BCP samples wetted in distilled and sterilized water was 8.28 ± 0.15 .

XRD investigations

The BCP samples immersed in culture medium alone or in culture medium containing marrow cells, were removed at days 7 and 14 respectively, dried in pure ethanol, ground, passed through a $100 \mu\text{m}$ sieve and pooled. Exposed during 30 minutes to a Cu K α radiation ($\lambda = 1.54059 \text{ \AA}$), they were examined using an INEL XRG 3000 CPS diffractometer (INEL, Arltenay, France) under 40 kV, 30 mA, $1^\circ/\text{min}$ in 2θ , connected to a PC-card multianalyzer. The 2θ reflections of HA and β -TCP were identified, and the relative peak intensities calculated. The relative amounts of the HA fractions were obtained using the following standardized method {Association Française de Normalisation (AFNOR) 594-066}: $\text{HA}/\beta\text{-TCP} = 6.83 (I_1/I_2) - 0.38$, where I_1 was the relative peak intensity of the HA peak at hkl reflection 2.1.0., and I_2 that of the β -TCP peak at hkl reflection 0.2.10.

FTIR investigations

The BCP blocks were removed, dried, ground, sieved and pooled as described above. Pellets of 300 mg KBr/1 mg BCP were examined by means of a Nicolet 20 SXC FTIR spectroscope (Nicolet, Trappes, France) with 4 cm^{-1} resolution.

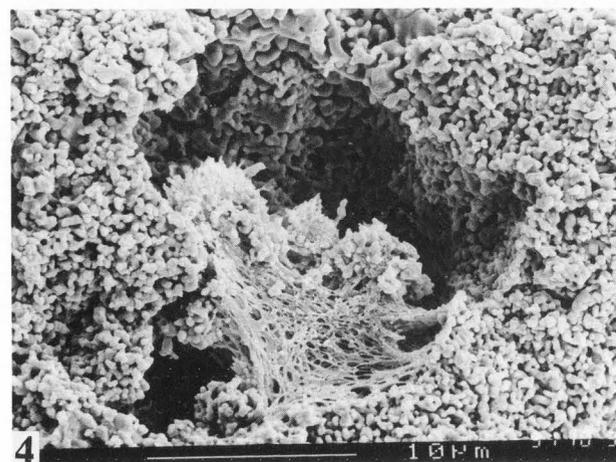
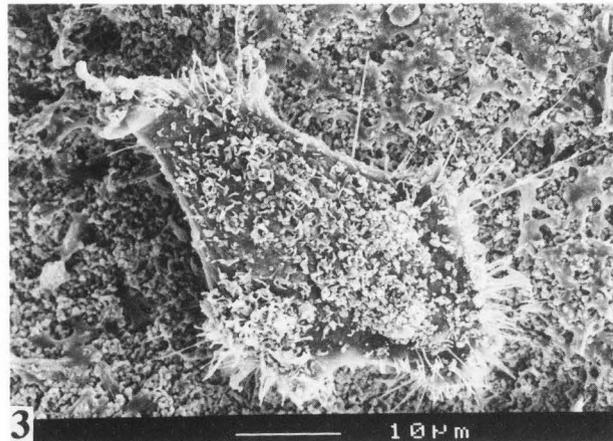
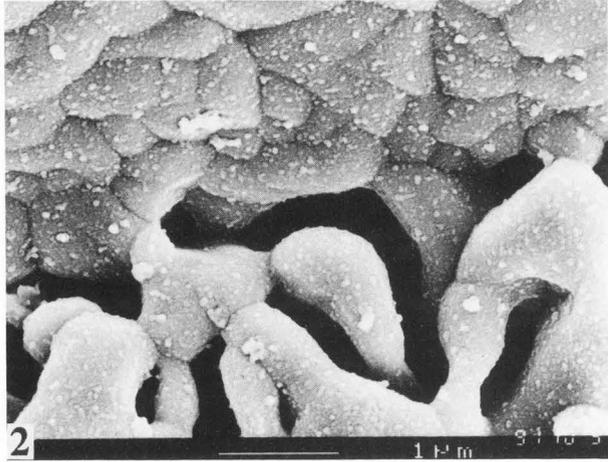


Figure 2. Junction between HA (above) and β -TCP (below). Bar = 1 μ m.

Figure 3. Cell with short filopodia and ruffled border in β -TCP site. Bar = 10 μ m.

Figure 4. Ruffled border in resorption lacunae in β -TCP site. Bar = 1 μ m.

(Figures 5-10 on facing page)

Figure 5. Detail of Figure 3. Bar = 1 μ m.

Figure 6. Empty resorption lacunae in β -TCP site. Bar = 1 μ m.

Figure 7. Round-shaped cell without filopodia and ruffled border in a resorption lacuna in β -TCP site. Bar = 10 μ m.

Figure 8. A spindle-shaped cell on BCP surface. Bar = 10 μ m.

Figure 9. Star-like cells in β -TCP site interconnected by cell processes. Bar = 10 μ m.

Figure 10. Monolayer of interconnected flattened cells in β -TCP site. Bar = 10 μ m.

SEM investigations

The BCP samples immersed into the cell culture medium containing dog bone marrow cells were removed at days 6, 10 and 15, fixed for 1 hour in 2.5% glutaraldehyde, buffered with cacodylate at pH 7.2, rinsed in the same buffer, post-fixed with 1% osmium tetroxide during 1 hour at room temperature, dehydrated by ethanol, then acetone, treated by the critical point method (Balzers, Liechtenstein) and examined in a JEOL (Tokyo, Japan) JSM 6300 Scanning Electron Microscope operating at 7 kV.

Results

XRD investigations

All diffraction spectra showed HA and β -TCP lines exclusively (Tables 1 and 2). The initial 60.7% HA/39.3% β -TCP ratio varied according to the immersion duration and to the presence or absence of cells (Table 3). In other terms, the relative HA amounts of BCP exposed to culture medium solely were 60.7, 81.5 and 75.3% at respectively zero, 7 and 14 days. When exposed to culture medium containing bone marrow cells, they increased from 60.7 to 65.5 and reached 73.2% at day 14.

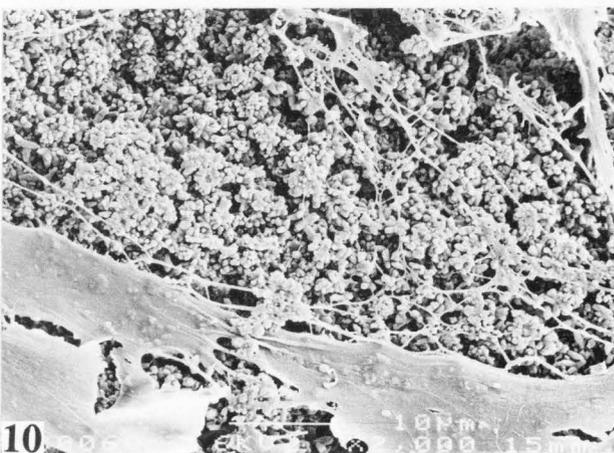
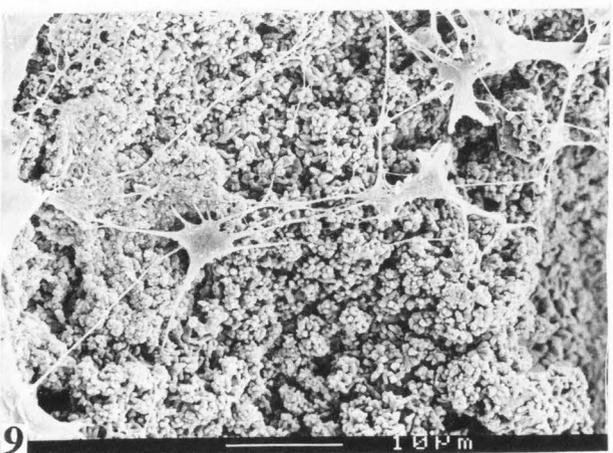
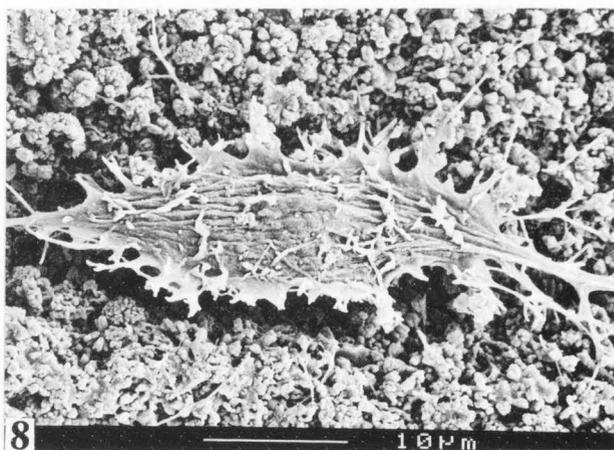
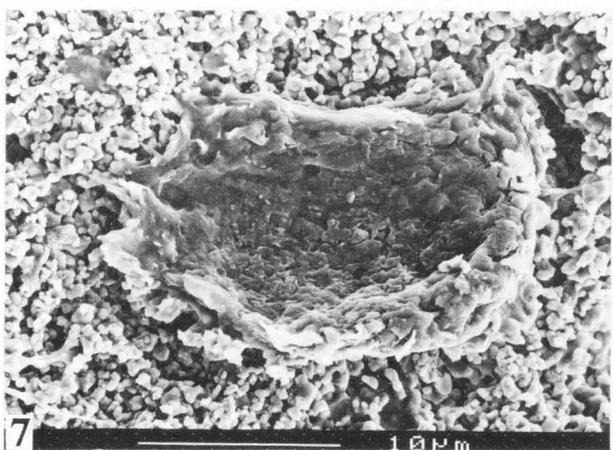
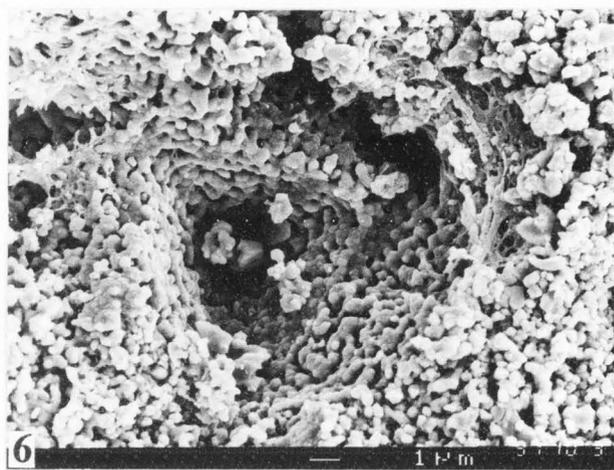
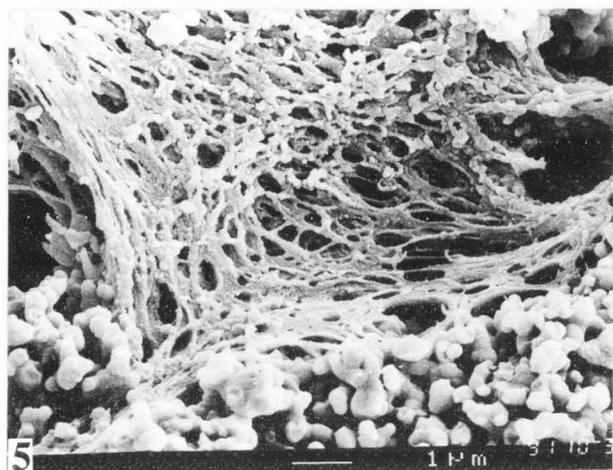
FTIR investigations

All FTIR scans showed a well crystallized carbonated BCP (Fig. 1). A water band was seen at 631 cm^{-1} , CO_3^{2-} bands at 1432-1426 cm^{-1} , a CO_3^{2-} band type B at 876 cm^{-1} , PO_4^{3-} bands at 1200-900 and 620-550 cm^{-1} . The PO_4^{3-} bands found at 1125 and 571 cm^{-1} and the CO_3^{2-} band at 876 cm^{-1} were identified to correspond to the non-apatitic environment (Rey *et al.*, 1989).

SEM investigations

The samples presented a large number of macropores of 300 to 800 μ m. Compact, polyhedric and soft-angled HA blocks of about 7 to 12 μ m were embedded

In vitro β -TCP resorption by monocytes in BCP



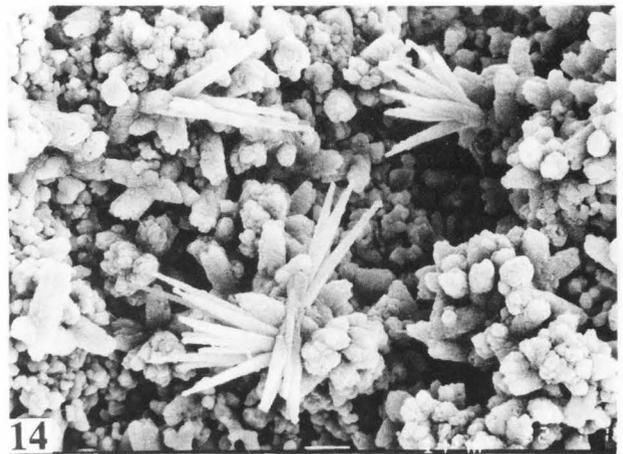
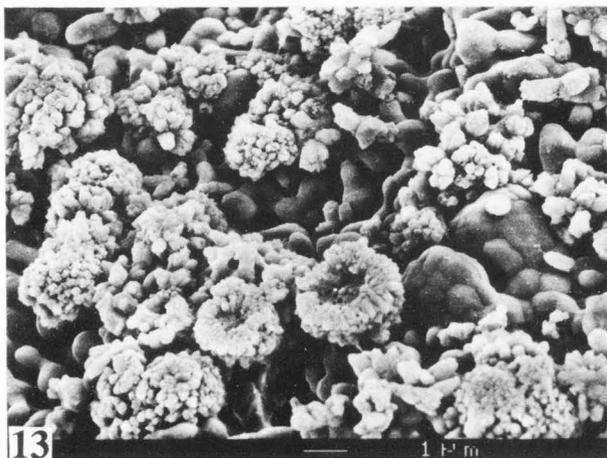
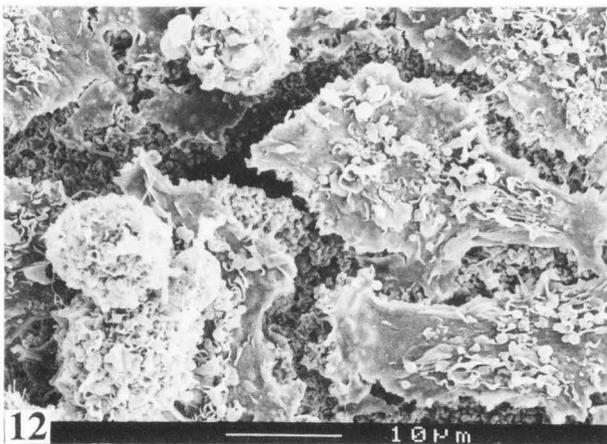
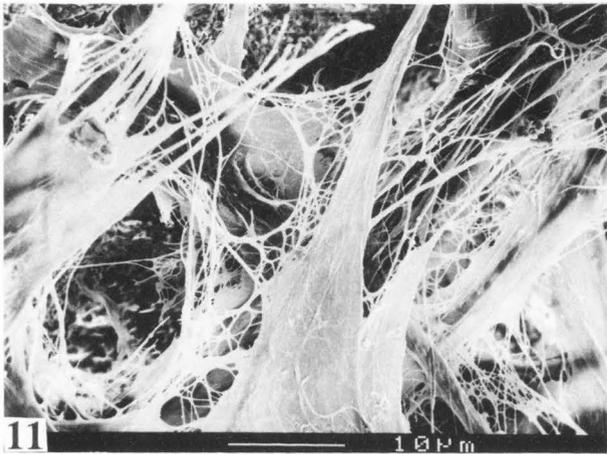


Figure 11. Overlapping and interconnected cells in β -TCP site. Bar = 10 μm .

Figure 12. Cells presenting ridges, ruffles, blebs or globules on their dorsal surface. Bar = 10 μm .

Figure 13. Round-shaped, polymorphous and flower-like accretions in HA and β -TCP site. Bar = 1 μm .

Figure 14. Needle-like accretions in β -TCP site. Bar = 1 μm .

in microporous β -TCP. The entirely coalesced HA powder grains had persisting grain boundaries. The β -TCP powder grains of 0.5 to 2 μm had fused by bridging and presented micropores of about 1 to 2 μm . Bridging was also stated between HA and adjacent β -TCP. When HA was located at the sample surfaces, however, it was bridge-free on its external face (Fig. 2). At day 6, HA and β -TCP surfaces were scattered with precipitations of 0.1 to 0.2 μm in diameter. In β -TCP sites, groups of round-shaped or oblong cells of about 25 to 40 μm presenting numerous short filopodia and a ruffled border were observed within resorption lacunae located on the floor of the macropores or on the surfaces (Fig. 3). In some lacunae, ruffled borders without cell bodies were observed (Figs. 4 and 5), others were empty (Fig. 6). About day 15, some resorption lacunae were inhabited by round-shaped cells of the same size but without ruffled borders (Fig. 7). HA sites were devoid of cells and resorption lacunae. At day 15, round or spindle-shaped cells were seen on the surfaces. Some of these were 30 μm in length and 10 μm in width, presented many filopodia of various lengths (Fig. 8) or had a long one of about 30 μm like a tail. Others were star-like with a little round-shaped cell body of 5 μm (Fig. 9) or polyhedral, flattened, 45 μm in length and 15 μm in width. Some cell processes had invaded deep resorption lacunae. Cells formed a monolayer (Fig. 10), were overlapped (Fig. 11) or seemed to be in a so-called "stand-off" position (Fig. 8). Many of the cells had ridges, ruffles, blebs or globules on their dorsal surface (Figs. 8 and 12). Neighbouring cells were connected via filopodia (Figs. 9, 10 and 11). Between cells a network of fine fibrils could be seen (Figs. 9, 10 and 11). Numerous round-shaped, polymorphous, needle and flower-like accretions of 1 to 3 μm were observed in HA and β -TCP sites (Figs. 13 and 14).

Discussion

In this *in vitro* study, a well defined BCP was immersed during 15 days into a supplemented standard α -MEM solution, with or without dog bone marrow cells. Besides the dissolution-reprecipitation phenomena and the cell-mediated resorption-apposition activities commonly observed in such studies, it was interesting to note that the dissolution rate was much lower in the presence of β -TCP resorbing cells resembling monocytes, that these cells became inactive 10 days later and that blast cells appeared at day 15, flattened and started secretory activity.

With regard to the physico-chemical properties of the BCP and the composition of the culture medium used in our study, the important increase of the HA/ β -TCP ratio observed after immersion into the culture medium solely was not surprising and is in accordance with the literature (LeGeros *et al.*, 1991; Kohri *et al.*, 1993; Radin and Ducheyne, 1993). The XRD investigations showed clearly that there was β -TCP resorption or preponderance of the β -TCP resorption over that of HA, if there was any at all. The needle-like, round shaped, polymorphous or flower-like accretions of varying diameters observed at day 15 correspond to findings related in other studies, namely that β -TCP dissolution is followed by reprecipitation (Daculsi *et al.*, 1989; Kohri *et al.*, 1993; Radin and Ducheyne, 1993; Ducheyne, 1994; Ishikawa *et al.*, 1994; Soueidan *et al.*, 1995; Davies, 1996) which undergo conversion to apatite (Kohri *et al.*, 1993; Radin and Ducheyne, 1993; Ishikawa *et al.*, 1994; Soueidan *et al.*, 1995; Davies, 1996), something that we did not verify in the present study.

The differences in time-related of the HA/ β -TCP ratios found between culture medium without and with cells, were interpreted as the results of interactions between cells, secreted matrix and the substrate, other than chemical reaction and physical interaction (Kohri *et al.*, 1993).

The striking fact in this study, however, was the observation, up to day 15, of small round shaped cells presenting ruffled cell borders facing the lacunae and implicated in β -TCP resorption, exclusively. They presented osteoclast and odontoclast features, except for size. This raises a series of questions concerning their nature and functions. Are these cells "small osteoclasts" as suggested (Soueidan *et al.*, 1995), monocytes or "activated" or "late monocytes" as described in *in vivo* studies on bone (Martin and Ng, 1994) and on deciduous tooth dentin, cementum and enamel (Sahara *et al.*, 1996a,b)? Are they β -TCP specific because of the lack of HA resorption? In *in vivo* studies "activated" or "late monocytes" usually fuse to form osteoclasts or odontoclasts. In our study, however, the clast-cells did not do

this during their life-span of about 10 to 15 days, at the end of which they had lost their ruffled border. There seems to be much evidence that these cells are specific β -TCP resorbing monocytes which lose their functions about 10 days after the beginning of their resorption activity. This is supported by studies on similar cells found in dentin of deciduous teeth where they were identified as hard-tissue resorbing monocytes at the end of their resorbing period before vanishing (Sahara *et al.*, 1996a,b).

As in bone the resorption period lasts for about 10 days (Mundy, 1991), it was not surprising to observe blast-cells at day 15 in our study. Their morphology and their secretory activity corresponded to those described by many authors in comparative studies. They were spindle shaped, polygonal (Courtenay-Harris *et al.*, 1995; Solari *et al.*, 1996), and star-like (Bagambisa and Joos, 1990; Meyer *et al.*, 1993). They had many filopodia and lamellipodia (Bagambisa and Joos, 1990; Meyer *et al.*, 1993; Ben-Bassat *et al.*, 1994; Ishaug *et al.*, 1994) and had a flattened shape (Bagambisa and Joos, 1990) interpreted as intimate contact with the substrate (Meyer *et al.*, 1993). Blast-cells constituted a monolayer (Bagambisa and Joos, 1990; Meyer *et al.*, 1993) or were partially overlapping (Bagambisa and Joos, 1990). They often had a so-called "stand-off position" (Meyer *et al.*, 1993) and were in contact with neighbouring cells via cellular extensions (Bagambisa and Joos, 1990; Meyer *et al.*, 1993; Ishaug *et al.*, 1994). They presented ridges, microvilli, ruffles, blebs and globules on their dorsal surface (Bagambisa and Joos, 1990; El-Ghannam *et al.*, 1995; Stanford *et al.*, 1995), which reflected their secretory activity, and showed extracellular fibrils and fibrillar matrix (Sammons *et al.*, 1994). Taking in consideration these features, the observed cells might be osteoblast-like.

Conclusions

In the present *in vitro* study resorption/-reprecipitation phenomena depending on BCP/culture medium interactions and on cell mediated and/or matrix-bound protein expression were observed. The striking fact, however, was that small clast-cells which did not fuse both with each other resorbed exclusively β -TCP before losing their ruffled borders about day 15. This raises some questions concerning the nature, functions and fate of these cells. Are they "small osteoclasts" as suggested (Soueidan *et al.*, 1995)? Are they "activated" or "late" monocytes, or specific clast-cells which resorb β -TCP? In our view, there is ample evidence that these cells are specific monocytes which resorb β -TCP under *in vitro* conditions. To elucidate their exact nature, functions and fate, further long-range *in vitro* studies conducted under standardized conditions are needed.

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Discussion with Reviewers

J.D. de Bruijn: The authors mention that small cells seem to resorb the material and speculate on their origin such as "small osteoclasts," activated or late monocytes, etc. Since there are many cells present in the bone marrow that do not survive in culture, and the authors seemingly do not refresh the culture medium during the experiment, could it be that these small cells are simply dying cells that, due to acidification in the culture medium, non-specifically create resorption areas in the ceramic?

Authors: In many BCP surface cavities, we found small polarized cells which had a well developed "ruffled border" facing the cavity surfaces and numerous dorsal microvilli, both signs of cell activity. So we did not think that these cells were dying. Some inactive cells, however, were observed at day 15 (Fig. 7).

J.D. de Bruijn: Can the small resorbing cells also produce lacunae in dentin or bone slices?

Authors: We only studied surface changes of BCP, but as mentioned in the text, Martin and Ng (1994) observed bone resorbing monocytes and Sahara *et al.* (1996a,b) dentin and enamel resorbing monocytes.

P. Ducheyne: Please describe the relationship of the present *in vitro* study to the other *in vitro* experiments from their laboratory (Blottière *et al.*, 1995): what characteristics do the cells in these studies have in common, were the results similar, etc.?

Authors: The characteristics of the resorbing cells in our study were different from those observed by Benahmed *et al.* (1994) and Blottière *et al.* (1995). We found little cells of 20 to 30 μm presenting a ruffled border located in resorption lacunae of β -TCP sites. Benahmed *et al.*, who used human peripheral blood monocytes activated by 1,25 (OH)₂ Vitamin D₃ and IFN γ (interferon- γ), stated monocyte transformation in polykation macrophage-type cells of 50 to 150 μm in diameter without ruffled borders which showed phagocytosis. Blottière *et al.* using U 937 monocytic leukemia cells activated with 1,25 (OH)₂ Vitamin D₃ and PDBu (phorbol 12,13-dibutyrate) observed their transformation in polykation macrophage-type cells of 50 to 100 μm showing phagocytosis.

P. Ducheyne: You mention that the pH at day zero is 8.28 ± 0.15 . This pH is too high for normal cell via-

bility. One can wonder, though, whether the authors adjusted the pH prior to cell seeding? Alternatively, did the pH drift down during the cell culture experiment?

Authors: The value given is the initial pH measured after wetting of the BCP in distilled sterilized water. We did not measure the pH during the cell culture experiment.

P. Ducheyne: Please include the standard deviation of the data, and also the number of independent observations (specimen) used for the ratios reported.

Authors: The triplicate samples were removed, dried, ground, passed through a sieve and pooled. So standard deviation values cannot be given.

H. Ben-Bassat: Why not use pre-isolated and well-characterized monocytes for these experiments in addition to bone marrow?

Authors: The aim of the study was to observe "the changes in a well-defined BCP exposed during 15 days to a standardized culture medium containing dog bone marrow cells." Bone marrow seemed interesting because it includes precursor cells responsible for resorption and secretion activities which in our study happened in an analogous sequence described in bone.

H. Ben-Bassat: Can the cultured cells be removed from/taken off the material and then characterized?

Authors: Indeed, the cultured cells could have been removed from the material and then characterized. The aim of our study was, however, to observe "the changes in a well-defined BCP..."

H. Ben-Bassat: What are the growth characteristics of the cells (monocytes?) on the material? Do they replicate?

Authors: Up to 15 days, no fusion or replication of monocytes was observed.

H. Ben-Bassat: Why are the osteoblasts observed late, and not from the start?

Authors: Bone marrow do not contain osteoblasts but precursor cells. In bone, matrix secretion followed by matrix mineralization is always preceded by a resorption period of about 10 days. Our study showed the first steps of such a sequence.