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Cellular response to calcium phosphate ceramics implanted in rabbit bone

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Two hydroxyapatite ceramics, synthesized by sintering from bovine bone and from a mixture of phosphate tricalcium and natural hydroxyapatite, were implanted in bone sites in rabbits. From day 7 after implantation, osteoblast-like cells were visible with thin layers of new bone on both biomaterials. Histomorphometry showed progressive increase in volume and surface of newly formed bone. Signs of cell-dependent resorption were visible at the surface of biomaterials and newly formed bone. There was a progressive decrease in relative volume and trabecular thickness of the biomaterials. Resorption of biomaterials appears to involve two cell types: multinucleated giant cells and osteoclast-like cells. The multinucleated giant cells observed had neither tartrate resistant acid phosphatase activity (TRAP) nor a ruffled border. Vesicles and vacuoles containing crystals observed in these cells suggest phagocytosis of biomaterials. The number of these cells decreased after day 14 following implantation. The osteoclast-like cells were TRAP positive. The structured modification and the TRAP activity demonstrated in the subjacent biomaterial suggest that the dissolution of the implant may be associated to an extracellular enzymatic activity of these cells. Electron microscopy revealed a clear zone and cytoplasmic membrane infolding in these cells, suggesting a ruffled border differentiation. The number of these cells increased with delay after implantation. It was concluded that the implantation of calcium phosphate ceramics in bone leads to new bone formation as well as to resorption of the biomaterials. The mechanism of resorption appears to associate crystal endocytosis by multinucleated giant cells and more classical resorption by osteoclast-like cells.

1. Introduction

Calcium and phosphate ceramics (Ca–P ceramics) are being increasingly used as bone substitutes in orthopaedic surgery, stomatology and dental surgery [1–4]. It is imperative that such ceramics be perfectly tolerated by the organism and lead to local osteogenesis. New bone, laid down by osteoblasts, has been observed at the surface of biomaterials implanted in bone sites [5–8]. However, it was reported that Ca–P ceramics implanted in extra-osseous sites did not induce bone formation, but when the ceramics were associated with bone marrow cells, local osteogenesis was effected by osteoblasts, differentiated from medullar precursor cells [9–12].

It is also important to determine the reliability and the future of the implanted biomaterial, i.e. whether it is progressively resorbed or incorporated into the newly formed bone [13]. Resorption of the biomaterial is usually associated with the presence of multinucleated cells. The nature and origin of these multinucleated cells are still under discussion and results seem to differ according to the receiving tissue and to the species. Devitalized particles of mineralized bone, subcutaneously implanted in the rat, elicit the recruitment of osteoclast-like multinucleated cells, which show tartrate resistant acid phosphatase activity and ultrastructural evidence of a differentiated ruffled border [14, 15]. However, in the rabbit, such subcutaneous implants elicit multinucleated giant cells lacking the enzymatic activity, cell surface aspects and functional features of osteoclasts [16]. Furthermore, hydroxyapatite ceramics grafted in soft tissue [10] or onto chick chorioallantoic membranes [17] lead to the formation of multinucleated cells with incomplete ruffled border differentiation [17].

The aim of this work was to study the bone formation occurring inside macroporous calcium–phosphate ceramics implanted in rabbit bone and

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resorption processes of both biomaterials and newly formed bone. Two types of Ca–P ceramics, synthetic hydroxyapatite and transformed bovine bone, were used to test the specificity of rabbit bone tissue response to implantation. Kinetics and mechanisms of cellular colonization of the implants were studied using histomorphometry to investigate quantitative changes in the biomaterials and in the newly formed bone, histochemistry to demonstrate the tartrate resistant acid phosphatase activity, and electron microscopy to search for signs of resorption and differentiation of a ruffled border in multinucleated cells.

2. Materials and methods

2.1. Biomaterials

Two calcium phosphate ceramics were implanted: MBCP (macroporous biphasic calcium phosphate), a mixture of 40% tricalcium phosphate ($Ca_3(PO_4)_2$) and 60% hydroxyapatite ($Ca_{10}(PO_4)_6OH_2$). The powders were compacted and sintered to obtain a macroporous structure (400 to 600 μm), as previously described [18]; and BONAP, a bovine bone hydroxyapatite, prepared by deproteinization of trabecular bone tissue and hydrothermal transformation of the inorganic phase into hydroxyapatite. This method conserves the general organization of the trabecular network and transforms the inorganic phase into hydroxyapatite crystals associated to ions such as Mg^{++}, CO_3^{--} and HPO_4^{--}.

The ceramics thus obtained were milled into cylindrical samples, 4 mm in length and 2.5 mm in diameter. Samples were sterilized in an autoclave before implantation.

2.2. Implantation

Sixty ceramic samples were implanted in bone sites in 15 young adult New Zealand white rabbits, weighing 2.5 to 3.0 kg, each rabbit receiving four implants (two MBCP implants in the left tibia and two BONAP implants in the right tibia). All operations were carried out under sterile conditions, using general anaesthesia. After a small incision of superficial tissues and periosteum, 2.5-mm holes were drilled, at low speed under sterilized saline coolant, through the cortex to the medullar zone in the diaphysea of the tibia. After installation, each implant was covered with its periosteal flap. The animals were maintained in separate cages but without immobilization. Post-operative follow-up revealed no inflammatory or septic complications. The animals were killed 3, 7, 14 and 21 days after implantation and large bone samples containing the implants were taken.

2.3. Light microscopy

Forty-four specimens (22 MBCP and 22 BONAP) were prepared for light microscopic examination. They were fixed in 4% neutral buffered paraformaldehyde, for 48 h, at + 4 °C, rinsed in phosphate buffer pH 7.4, for 12 h, decalcified in 7.5% ethylene diamine tetra acetic acid (EDTA) in neutral buffer for 10 days, and then embedded in paraffin. The 7-μm sections were used for histological observation, acid phosphatase detection and histomorphometric analysis.

2.4. Tartrate resistant acide phosphatase (TRAP)

Histochemical assays for acid phosphatase activity, in the presence of 50-mM tartrate, were performed using naphtol AS BI phosphate (Sigma) as substrate with freshly diazotized Fast Garnet GBC (Sigma). Deparaffined slides were incubated for 1 h at 37 °C and counterstained with hematoxylin. The number of stained cells was determined by examination of the whole section of the implant at a magnification of × 250, using at least 15 contiguous fields, each with an area of 0.65 μm^2. Cells were considered to be positive when they showed strong staining with numerous red granulations spread through the cytoplasm. Multinucleated positive cells were classified as being in contact with the biomaterial (BM) or with newly formed bone (NB).

2.5. Histomorphometry

Histomorphometric analysis was carried out, on four non-contiguous sections of each implant, by the point-and-intercept counting method [19] using a Zeiss microscope equipped with an integratory eyepiece reticule (100 points and 10 parallel lines). The following measurements were performed using the nomenclature previously proposed [20]: relative volume of the biomaterial, BMV/IV (biomaterial volume BMV, expressed as a percentage of the implant volume IV); relative volume of the newly formed bone, NBV/IV (newly formed bone NBV, expressed as a percentage of the implant volume); thickness of the biomaterial trabeculae (BMTh, μm); and relative surface of the biomaterial covered with newly formed bone (NBS/BMS, %). However, at day 3 and day 7, decalcification led to partial dissolution of the MBCP biomaterial and did not allow measurements of BMV and BMTh; from day 14, the organization of the ceramic was better preserved because of the development of a biological matrix invading the intercrystalline spaces and BMV was calculated. Although the samples were also decalcified, the structure of the BONAP biomaterial was visible, even 3 days after implantation. Before implantation measurements of BMV were made on undecalcified and methylmetacrylate embedded samples of MBCP and BONAP.

2.6. Electron microscopy

Sixteen implants (eight MBCP and eight BONAP) were prepared for electron microscopy. Each implant was minced into 1 mm^3 fragments and fixed for 90 min, at + 4 °C, in a 2% paraformaldehyde and 0.1% glutaraldehyde solution, phosphate buffered to pH 7.4. After post-fixation in 2% osmium tetroxide, followed by dehydration, the non-decalcified bone samples were processed for embedding in 812 resin epoxy. Ultra-thin sections, 40–60 nm, were obtained

using a diamond knife and stained with uranyl acetate and lead citrate. Observation was carried out using a 100C Jeol electron microscope.

3. Results

3.1. Morphological observations

Clinically and histologically, the implanted biomaterials were well tolerated. There were no signs of rejection, necrosis or infection. Bone marrow around the implant showed normal morphology.

Under light microscopy, similar tissue reactions were observed with implants of MBCP and BONAP. On samples taken 3 days after implantation, loose connective tissue containing a few inflammatory cells was observed within the superficial lacunae of the implants. Globular osteoblast-like cells with spherical nuclei were found at the surface of both biomaterials. However, at day 3 no newly formed bone was observed around or within the implants.

At day 7 and day 14, dense connective tissue was found in all the lacunae of the biomaterials and thin layers of woven bone had formed around the implants and inside of most of the superficial lacunae. Lines of osteoblasts, with large Golgi zones, were visible lying on both biomaterials and on newly formed bone (Fig. 1). As early as day 7, signs of resorption were seen at the surface of newly formed bone and biomaterials, with the presence of small multinucleated cells (Fig. 2). At day 14, examination showed that the resorption of the biomaterials and of the new formed bone increased and that newly formed lamellar bone was deposited near the woven bone (Fig. 3).

At day 21, bone formation was greatly intensified (Fig. 4). Numerous layers of plump osteoblasts were apposed to bone and biomaterial surfaces. There were many multinucleated cells among which some were very large, reaching 100–200 μm in length, with several dozen nuclei (Fig. 5). These giant multinucleated cells were found exclusively in contact with MBCP and BONAP biomaterials and never against newly formed bone.

3.2. Tartrate resistant acid phosphatase (TRAP)

Tartrate resistant acid phosphatase activity (TRAP +) was detected in the smaller multinucleated cells

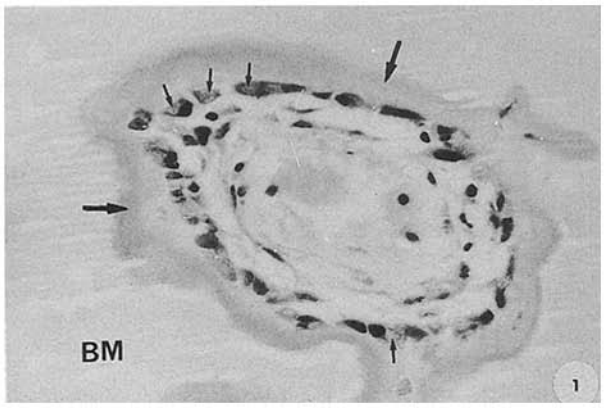

Figure 1 New bone formation in MBCP biomaterial. At day 7 after implantation osteoblasts are clearly differentiated and thin layers of newly formed bone (←) are visible at the surface of lacunae in MBCP biomaterial. Note the presence of large Golgi zones in osteoblasts (←) (BM = biomaterial). Hematoxylin (× 264).

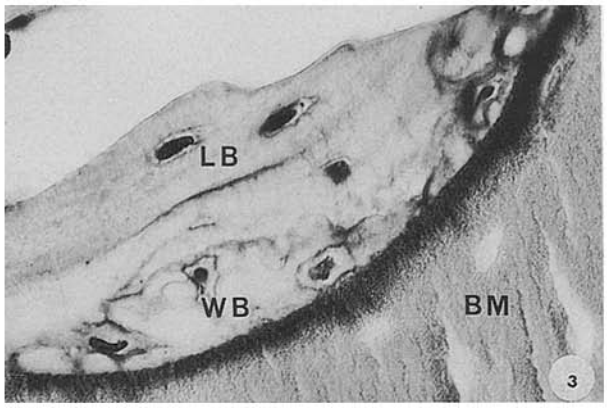

Figure 3 Newly formed bone at the surface of BONAP biomaterial 14 days after implantation. In contact with the biomaterial the new bone tissue is of the woven type (WB). At the surface, the bone tissue is of the lamellar type (LB). (BM = biomaterial). Hematoxylin (× 171).

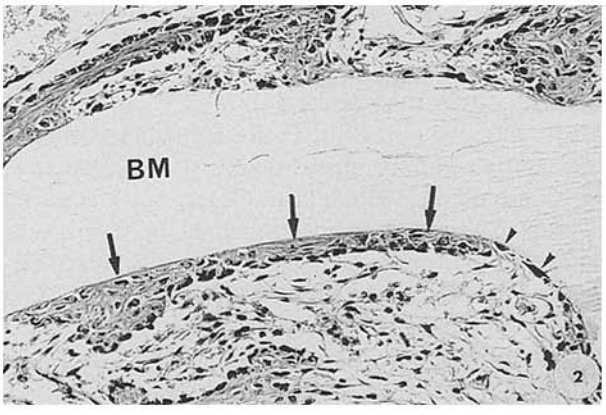

Figure 2 New bone formation in BONAP biomaterial. At day 14 after implantation a newly formed bone (←) is observed at the surface of the trabeculae of the biomaterial. Note the presence of small multinucleated cells (◀). (BM = biomaterial). Hematoxylin (× 114).

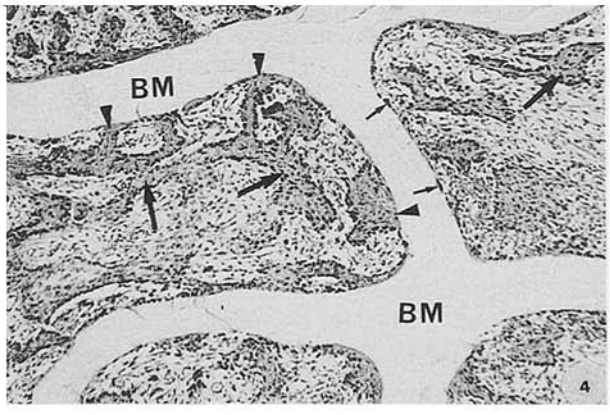

Figure 4 Bone formation in BONAP biomaterial implant at day 21 after implantation. Note the presence of newly formed bone bound to the surface of the biomaterial (◀) and inside the intertrabecular spaces of the biomaterial (←). Some osteoclast-like cells can be seen (←). (BM = biomaterial). Hematoxylin (× 46).

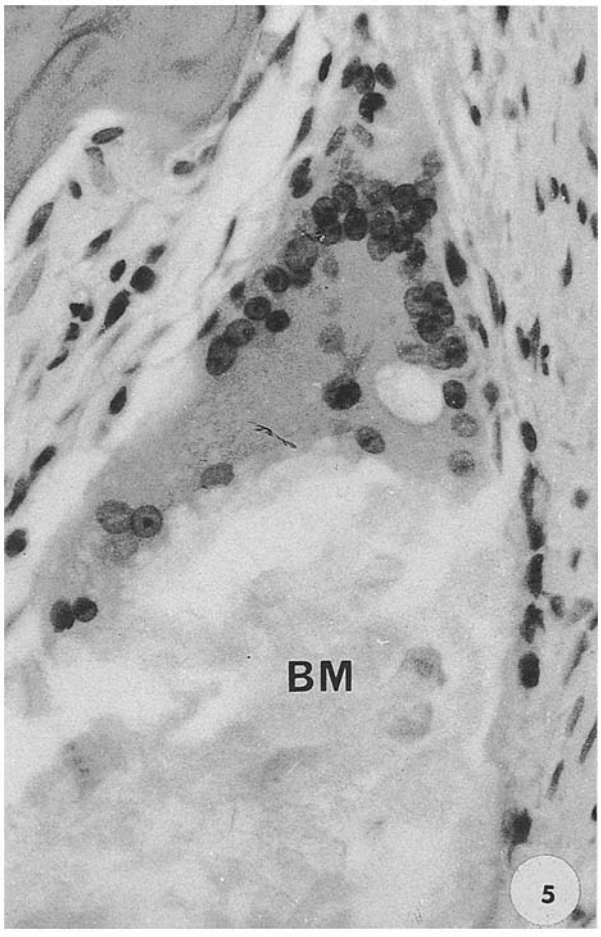

Figure 5 Decalcified section, 7 um, of a giant multinucleated cell observed at the surface of MBCP biomaterial, at day 14 after implantation. Note the semi-circular disposition of the nuclei. (BM = biomaterial). Hematoxylin (× 200).

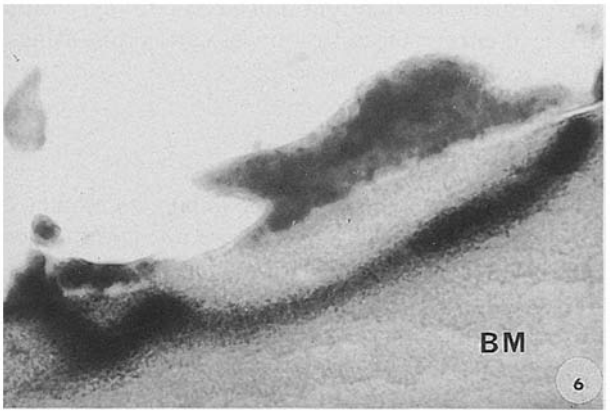

Figure 6 Tartrate resistant acid phosphatase activity (TRAP). Tartrate resistant acid phosphatase activity is detected in this small multinucleated cell and within the underlying MBCP biomaterial 14 days after implantation (× 280).

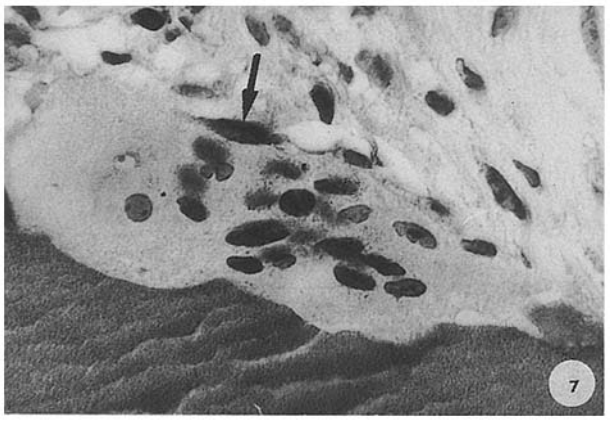

Figure 7 Tartrate resistant acid phosphatase activity (TRAP). This giant multinucleated cell shows, 14 days after implantation of a BONAP bioceramic, no TRAP activity. Note the close contact between the biomaterial and the cell surface. A TRAP + mononucleated cell (←) is in close contact with the giant multinucleated cell suggesting a fusion process. TRAP + activity would appear to be lost after fusion (× 300).

and was also observed within the biomaterials facing the TRAP + multinucleated cells (Fig. 6), whereas all the giant cells were negative (TRAP −) (Fig. 7). A few mononucleated cells in connective tissue or in contact with the biomaterials were TRAP + , as were host osteoclasts in the epiphyseal plate. All other cell types were TRAP − .

Table I shows that: (1) the number of TRAP + multinucleated cells in contact with MBCP is greater than that in contact with BONAP; (2) the number of TRAP + multinucleated cells in contact with newly formed bone is similar in both biomaterials, and increases with delay after implantation; (3) the number of TRAP − giant cells, per unit volume of biomaterials, is similar in both biomaterials and decreases after day 14 following implantation.

3.3. Histomorphometry

Table II shows that: (1) the relative volume of the biomaterials (BMV/IV) decreases with delay after implantation. In the case of BONAP, there is a 26% decrease from before implantation to day 21; (2) the mean thickness of biomaterial trabeculae (BMTh) also decreases. For BONAP there is a 44.7% decrease from before implantation to day 21; (3) the volume of newly formed bone and the surface of both biomaterials covered by the newly formed bone increases gradually following implantation. However, bone formation appeared to occur much faster in BONAP than in MBCP.

3.4. Electron microscopy

Most of the mononucleated cells in contact with both biomaterials had an ultrastructure similar to that of active osteoblasts, with a large Golgi zone and a well developed rough endoplasmic reticulum. These cells had long, thin processes penetrating the intercrystalline spaces (the micropores) of the biomaterials. Osteocytes observed in the newly formed bone were similar to those found typically in woven bone.

Multinucleated cells in contact with biomaterials showed numerous intracytoplasmic vesicles and vacuoles containing crystals from the implant (Fig. 8). These cells were poor in organelles. In some multinucleated cells the cytoplasm close to the biomaterial was marked by a clear zone, free of organelles, lying

TABLE I Multinucleated cell determination in MBCP and BONAP biomaterials 3, 7, 14 and 21 days after implantation. The total number of multinucleated cells was determined and expressed per mm^2 of the total area of the implant. Separate evaluations were made for TRAP + multinucleated cells lying against biomaterials (BM) or against newly formed bone (NB) and for TRAP − multinucleated cells. The number of TRAP positive cells is consistently greater with MBCP than with BONAP. Statistical analysis were carried out using the *t* student test. NS: not significant.

	Day 3		Day 7		Day 14		Day 21	
	MBCP	BONAP	MBCP	BONAP	MBCP	BONAP	MBCP	BONAP
Number of implants (*n*)	$n = 4$	$n = 4$	$n = 6$	$n = 6$	$n = 6$	$n = 6$	$n = 6$	$n = 6$
TRAP + (BM)	0	0	10.8 ± 2.5	5.5 ± 2.4	18.7 ± 4.2	6.9 ± 2.4	15.3 ± 3.4	7.2 ± 2.6
MBCP vs BONAP			$p < 0.005$		$p < 0.0005$		$p < 0.002$	
TRAP + (NB)	0	0	1.0 ± 0.6	0.8 ± 0.7	3.7 ± 1.6	3.8 ± 1.3	7.5 ± 2.8	8.8 ± 2.5
MBCP vs BONAP			NS		NS		NS	
TRAP −	0	0	1.3 ± 1.0	0.6 ± 0.5	3.6 ± 1.6	5.7 ± 1.2	1.8 ± 0.9	1.7 ± 0.8
MBCP vs BONAP			NS		$p < 0.05$		NS	

TABLE II Histomorphometric determinations of relative biomaterial volume (BMV/IV), biomaterial trabecular thickness (BMT), relative volume of the newly formed bone (NBV/IV) and of the surface of biomaterial covered with newly formed bone (NBS/BMS). Greater quantities of newly formed bone were observed with BONAP than with MBCP in the early stages. There was no significant difference at day 21. Statistical analysis were carried out using the *t* student test. ND: not determined.

	Before implantation (Day 0)		Day 3		Day 7		Day 14		Day 21	
	MBCP	BONAP	MBCP	BONAP	MBCP	BONAP	MBCP	BONAP	MBCP	BONAP
Number of implants (*n*)	$n = 4$	$n = 4$	$n = 4$	$n = 4$	$n = 6$	$n = 6$	$n = 6$	$n = 6$	$n = 6$	$n = 6$
BMV/IV (%)	49.6 ± 4.1	48.1 ± 3.2	ND	47.9 ± 3.6	ND	43.0 ± 3.4	42.8 ± 4.3	40.4 ± 4.6	36.2 ± 4.8	35.6 ± 3.8
versus Day 0				NS		$p < 0.05$	$p < 0.04$	$p < 0.02$	$p < 0.003$	$p < 0.001$
BMT (μm)	ND	295 ± 35	ND	288 ± 41	ND	233 ± 27	ND	170 ± 31	ND	163 ± 26
versus Day 0				NS		$p < 0.02$		$p < 0.001$		$p < 0.0001$
NBV/IV (%)	0	0	0	0	2.7 ± 0.9	7.2 ± 2.1	6.3 ± 1.3	9.9 ± 2.3	13.3 ± 2.9	12.9 ± 2.7
MBCP vs BONAP					$p < 0.001$		$p < 0.01$		NS	
NBS/BMS (%)	0	0	0	0	ND	29.1 ± 4.8	58.0 ± 7.4	34.7 ± 5.3	62.0 ± 5.1	58.5 ± 6.2
MBCP vs BONAP							$p < 0.001$		NS	

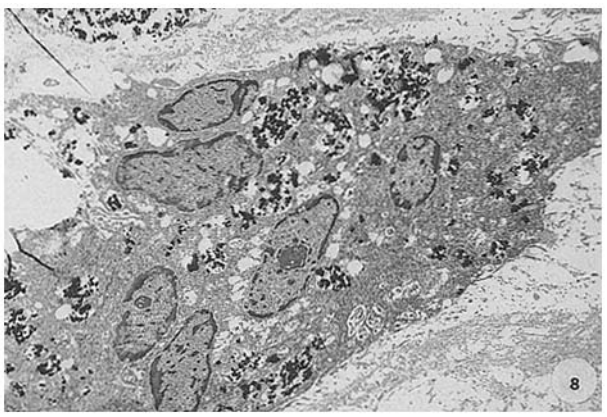

Figure 8 Transmission electron microscopy (TEM) of a giant multinucleated cell. The numerous vacuoles and vesicles contain crystals from the MBCP biomaterial 14 days after implantation (× 1600).

against surface irregularities of the biomaterials. Infolding of the cytoplasmic membrane, resembling a ruffled border, was found near the clear zone (Fig. 9). In the zone away from contact with the implant, the cytoplasmic membrane had numerous microvillosities.

Before implantation, the Ca–P ceramic structure consisted of spherical or polygonal crystals, 0.1 to 0.2 μm in diameter, with a microporosity of < 10 μm between particles. The increased microporosity observed after implantation indicated the dissolution of some crystals, as previously described [21]. After implantation, the collagen fibrils observed in the newly formed bone, at the surface and in the micropores of the superficial regions of the biomaterials, revealed the characteristic 67 nm striation of collagen (Fig. 10). Needle-shaped hydroxyapatite crystals, 2–5 nm thick, were found closely associated to the collagen network in newly formed bone and in the interstitial spaces of the biomaterials (Fig. 10).

4. Discussion

These findings show that macroporous Ca–P ceramics, implanted in bone sites, lead to rapid invasion of the intertrabecular spaces of the biomaterials by loose connective tissue with no sign of inflammatory pro-

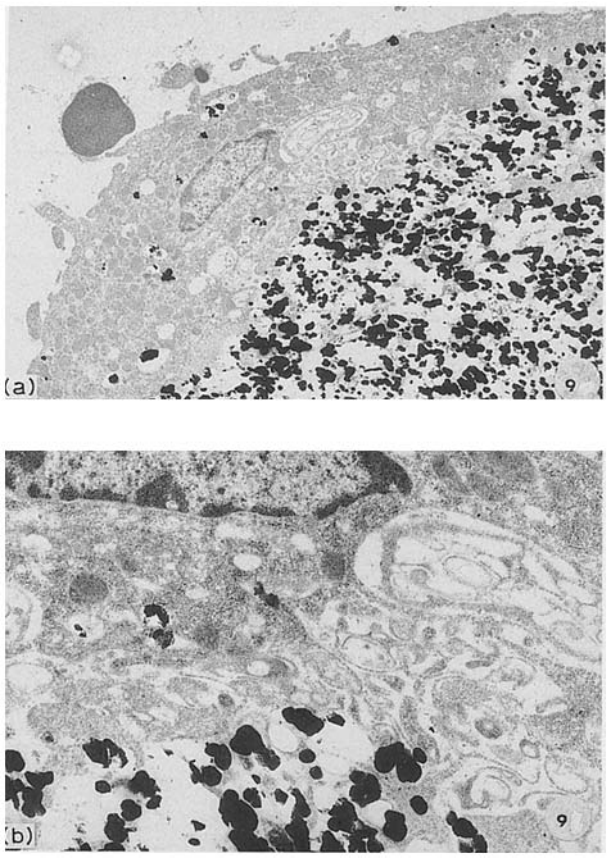

Figure 9 Transmission electron microscopy (TEM) of an osteoclast-like cell in contact with MBCP biomaterial 7 days after implantation. (**a**) the cell, multinucleated on serial sections, shows a clear zone lying against the biomaterial and infoldings of the cytoplasmic membrane (Magnification: X:8500). (**b**) detail of the same cell showing the ruffling of the cytoplasmic membrane near the clear zone (× 18 000).

Figure 10 Transmission electron microscopy (TEM) of the interface between the Bonap biomaterial and the newly formed bone matrix. At day 14 needle-shaped hydroxyapatite crystals (◄) are closely associated to collagen fibrils in newly formed bone. Note the presence of crystals of the biomaterial (←) (× 44 000).

cesses. This is in agreement with previous reports showing that Ca–P ceramics induce little or no inflammation [22–25]. Newly formed bone appears shortly after implantation together with signs of cellular resorption of the newly formed bone as well as the biomaterials. Woven bone is elaborated prior to lamellar bone deposition by osteoblasts.

However, in this experiment, the origin of the osteoblasts in the implant remains uncertain. It has been observed that bone formation and deposition directly onto an implant require a non-toxic surface that allows cell attachment and proliferation [26]. The binding of various host tissues to implanted sintered hydroxyapatite has been demonstrated [26–28]. Osteoblastic activity, particularly migration, was reported to be influenced by the presence of protein adsorbed on the surface of the biomaterials [29]. Thus, the biomaterial itself may guide the migration of these cells from the bordering bone. However, the factors involved in the osteoblastic migration remain to be determined.

Ca–P ceramics implanted in bone sites may also act as a support for the local differentiation of osteoblasts from medullar precursor cells. Indeed, it has been reported that subcutaneously implanted Ca–P ceramics induce bone formation only when implanted in association with bone marrow cells among which osteoprogenitor cells are present [9, 10, 12].

There are few transmission electron microscopic observations of the interface between implants and the matrix of newly formed bone [30–32]. The results of this present study show that collagen microfibrils, closely associated with needle-shaped hydroxyapatite crystals, invade the intercrystalline spaces (micropores) inside the biomaterial. Similar findings have been reported at the interface between a glass ceramic and bone [30]. Newly formed bone appears to be not merely deposited on the surface but actually anchored into the biomaterial. Thus, the implant acts as a support for the deposition of newly formed bone. The spatial organization of the trabeculae of the biomaterial within implanted ceramics would seem to influence the arrangement of newly formed bone.

Resorption leads to a decrease in relative volume and to a thinning of trabeculae of the biomaterials. Are these modifications due to mere dissolution of apatite crystals or to cellular resorption such as that caused by osteoclasts in bone remodelling?

Osteoclasts can be identified by their morphology, enzymic activity and functional aspects [33–38]. There is considerable evidence to suggest that these multinucleated cells originate from hematopoietic stem cells [39–46]. TRAP + activity is generally considered as a marker for osteoclast identification [47] although the specificity of the reaction is still under discussion [48, 49]. A ruffled border, composed of a clear zone and a region of cytoplasmic membrane infolding lying against the resorption surface, is a classical ultrastructural element of osteoclasts [38, 50]. Multinucleated giant cells which appear in reaction to foreign bodies show certain morphological features similar to those of osteoclasts, but they have little or no TRAP + activity [16] and present no signs of ruffled border differentiation [51].

In vitro observations have shown that other various types of cell, such as synovial cells [52], fibroblasts [53] and monocytes [53–55] can phagocytose and dissolve calcium phosphate crystals. It has also been demonstrated that endocytosis precedes the solubilization of the calcium phosphate crystals by macro-

phages [53, 54, 56], and that solubilization of the crystals seems to be required for the induction of a mitogenic effect on fibroblasts [56].

Our results suggest that two types of multinucleated cells are elicited by contact with the biomaterials implanted in bone site. The first type consists of huge cells, containing more than 20 nuclei and resembling giant cells. These cells have neither TRAP activity nor a ruffled border, but some have vacuoles or vesicles with crystals, suggesting phagocytosis of biomaterial. Similar cells have also been observed in contact with subcutaneous bone implants in rabbits, and these multinucleated giant cells do not have the specific enzymatic activity, cell surface aspects and functional features of osteoclasts [16]. The second type consists of multinucleated cells with some characteristics similar to those of osteoclasts. These cells are smaller, containing less than 10 nuclei, and are TRAP + . The enzyme activity of the cells appears to provoke extracellular dissolution of the implant as demonstrated by the TRAP + reaction and structural modification in the biomaterial subjacent to the multinucleated cells. Electron microscopy reveals that these cells have a clear zone and some cytoplasmic membrane infolding, resembling a ruffled border, were observed.

Similar observations have been described in previous reports, using other experimental procedures. Grafted on the chick chorioallantoic membrane, hydroxyapatite [17, 57] or mineralized bone particles [57], induced multinucleated cell formation. In these cells the presence of a well-defined ruffled border would appear to be associated with the presence of specific bone matrix proteins, particularly osteocalcin [57]. However, the osteoclastic differentiation appears to depend not only on the composition of the implant but possibly on the nature of the receiving tissue and of the receiving species. Indeed, in the rabbit, the multinucleated giant cells recruited to subcutaneous implants of mineralized bone particles did not stain with an osteoclast monoclonal antibody and showed considerably weaker TRAP activity when compared to host osteoclasts [16]. In the rat, bioceramic (80% tricalcium phosphate and 20% hydroxyapatite) implanted into muscle pouches, was not resorbed and did not induce bone formation [58].

Our results show that bone formation and resorption of the biomaterial occur within Ca–P ceramics when implanted in bone sites in rabbits. Similar results were observed in both biomaterials used in this experiment, showing the specificity of the response to Ca–P ceramic bone implantation. However, the degree of resorption and of bone formation differed according to the nature of the ceramic. The number of TRAP positive cells in contact with MBCP, a composite of synthetic hydroxyapatite and tricalcium phosphate, was greater than that in contact with BONAP, a bovine bone hydroxyapatite. This appears to suggest that MBCP favours the migration or local differentiation of osteoclast-like cells. However, with BONAP, bone formation is faster in the first 2 weeks after implantation, suggesting that this biomaterial is a better support for osteoblastic activity, at least in the short term.

The sequence of events observed following implantation of biomaterials; connective tissue proliferation, formation of woven bone and, later, deposition of lamellar bone, is similar to that typical of the early stages of embryonic bone formation or of bone fracture healing [59–62]. Bone formation is associated with numerous plump osteoblasts and resorption appears to involve two cell types, multinucleated giant cells and osteoclast-like cells, the origin of which remains to be determined. It is obviously important to understand the cellular events involved in the *in situ* bone formation and resorption of biomaterials so as to evaluate the quality and fate of hard tissue substitutes. We now aim to study the migration of differentiated cells and/or local differentiation of precursor cells from the bordering bone and medullar spaces after implantation of Ca–P macroporous bioceramics in bone.

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