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Ultrastructural evidence *in vitro* of osteoclast-induced degradation of calcium phosphate ceramic by simultaneous resorption and phagocytosis mechanisms

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Summary. Osteoclasts are physiological polykaryons specialized in the resorption of calcified tissue. In the context of the clinical use of calcium-phosphate (CaP) ceramics as bone substitutes, this study used transmission electron microscopy to investigate the *in vitro* mechanisms of CaP ceramic degradation by osteoclastic cell types. Osteoclasts cultured on CaP ceramic developed typical ultrastructural features of bone osteoclasts, such as a polarized dome shape, a clear zone and a ruffled border. Modification of the shape and density of CaP crystals under the ruffled border indicated an acidic microenvironment. Moreover, osteoclasts were able to degrade ceramic by simultaneous resorption and phagocytosis mechanisms. Phagocytosis did not alter the ability of osteoclasts to resorb CaP ceramic. The phagocytosis mechanism consisted of three steps: crystal phagocytosis, disappearance of the endophagosome envelope membrane and fragmentation of phagocytosed crystals within the cytoplasm. The common mechanism of phagocytosis described here is similar to that observed with the monocyte/macrophage lineage, confirming that osteoclasts are part of the mononuclear phagocyte system. Osteoclasts are thus clearly involved in CaP degradation by means of resorption and phagocytosis.

Key words: Osteoclast, Resorption, Phagocytosis, Calcium-phosphate ceramic, Biomaterial

Introduction

Calcium-phosphate (CaP) ceramics have been used for bone substitution in dental, stomatological and orthopedic surgery as an alternative to autogenous bone grafts (Passuti et al., 1989; Daculsi et al., 1990). Immediately after implantation, numerous cellular events (phagocytosis by monocytes/macrophages) and chemical processes (dissolution/reprecipitation mechanisms) occur before the replacement of material by new bone (see review, Heymann et al., 1999). Monocytes/macrophages, which are among the first cells to colonize CaP ceramic implantation sites, are highly involved in phagocytosis of ceramics (Eggli et al., 1988; Ikami et al., 1990; Benahmed et al., 1996a). During these stages, numerous growth factors released by the cells localized in inflammatory foci influence the cellular behavior of monocytes/macrophages, osteoclasts or osteoblastic cells (Roodman, 1993; Benahmed et al., 1996b; Heymann et al., 1998). Thus, human peripheral blood monocytes and histiocytic cells may contribute to the degradation of calcified tissue through their growth factor secretions, sensitivity to growth factors, and influence on bone cells (osteoclasts and osteoblasts).

Two types of multinucleated giant cells could also be involved in the degradation of bone tissue: inflammatory ones known as macrophage-polykaryons and osteoclasts which correspond to physiological polykaryons. The first cells degrade calcified matrix by a phagocytosis mechanism (Athanasou et al., 1991) and are able to resorb calcified tissues weakly [considered as "low-grade resorption" (Athanasou et al., 1992)]. The latter, the main cell type implicated in calcified tissue resorption, rely on an acidic mechanism, using a proton pump to reduce pH under the ruffled border and resorb the calcified matrix (Suda et al., 1997).

Though bone resorption mechanisms have been extensively studied, few works have focused on osteoclastic resorption of biomaterial surface *in vitro* and *in vivo* (Jones et al., 1984; Legeros, 1991; Baslè et al., 1993; Davies et al., 1993; Gomi et al., 1993; de Bruijn et al., 1994; Yamada et al., 1997a,b). Recent data have shown that inflammatory giant cells and osteoclasts may be involved in the degradation of CaP ceramic (Baslè et al., 1993; Yamada et al., 1997a,b). Baslé et al. (1993) have demonstrated that macroporous CaP biomaterials implanted in bone elicit the recruitment of a double

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multinucleated cell population possessing degrading/resorbing activities. The first population consists of inflammatory giant cells with a large number of nuclei, no tartrate-resistant acid phosphatase (TRAP) activity, no ruffled border and a large accumulation of mineral crystals in vacuoles. These multinucleated cells originate by fusion of mature monocytes/macrophages. The second population is composed of osteoclasts exhibiting TRAP activity and displaying a well-delineated ruffled border. Osteoclasts differentiate from mononuclear medullar precursors.

Yamada et al. (1997a,b) used scanning electron microscopy to show that osteoclasts isolated from neonatal rabbit bone cells are able to perform lacuna resorption on CaP ceramic. The degraded crystals inside the lacunae appear to be dissolved by an acid extracellular process. However, the closed mechanisms of osteoclast degradation of CaP ceramics were not clearly defined.

The purpose of the present study was to clarify the *in vitro* mechanisms of calcium ceramic degradation by osteoclastic cell types. Electron microscopy was used to analyze the resorption activity of osteoclasts cultured on CaP ceramic and to show that osteoclasts are capable of phagocytosing CaP ceramic particles.

Materials and methods

Preparation of biphasic CaP ceramic

The biphasic CaP ceramic (BCP) used in this study consisted of a 40/60 weight mixture of hydroxyapatite (HA) $[Ca_{10}(PO_4)_6(OH)_2]$ and beta-tricalcium phosphate (ß-TCP) $[Ca_3(PO_4)_2]$ obtained by calcinating (950 °C for 2 h) a calcium-deficient apatite prepared by an aqueous precipitation method (Legeros, 1991). BCP particles 40-80 mm in size were selected. Pellets 10 mm in diameter and 1 mm thick were manufactured by compacting (Specac, Kent, UK) 200 mg of particles at 130 MPa/20 s. Pellet cohesion was increased during a sintering stage (1,000 °C for 4 h). The specimens thus produced were sterilized by heating at 180°C for 2 h. Before cell-seeding, each specimen was preincubated in 2 ml of α-MEM medium (Gibco, Eragny, France) in one well of a 24-well plate (Nunc, Roskild, Denmark) for 18 h at 37 °C.

Rabbit osteoclast preparation and culture

Neonatal rabbit bone cells were isolated using a previously reported method (Tetzuka et al., 1992; Yamada et al., 1997a,b; Guicheux et al., 1998). The long bones of a 10-day-old rabbit were minced with scissors and agitated in a-MEM medium in a vortex for 30 s. After sedimentation for 2 min, the cell suspension was harvested (repeated twice). The cells were washed in a centrifuge (400 g, 3 min) and suspended in α-MEM supplemented with 10% fetal bovine serum (Gibco) and antibiotics (100 U/ml penicillin G, 100 mg/ml streptomycin, Gibco). After a BCP pellet was placed at the bottom of each well of a 24-well plate, one milliliter of the cell suspension containing about 10^7 cells (mixture of stromal cells, osteoclasts, etc.) was seeded in each well. The cultures were maintained in a humidified 95% air, 5% CO_2 atmosphere at 37 °C. The medium was changed the day after seeding to remove non-adherent cells. The experiments were done in duplicate.

Scanning electron microscopy (SEM)

The specimens were removed from the cell culture after 4 days, washed in phosphate-buffered saline and treated with 0.001% pronase E (Sigma, Saint-Quentin Fallavier, France) plus 0.02% ethylenediaminetetraacetic acid (EDTA) for 5 min to remove most of the cells but not the osteoclasts (Tetzuka et al., 1992; Yamada et al., 1997a,b; Guicheux et al., 1998). Tetzuka et al. (1992) have reported that more than 95% of the attached cells after this treatment are multinucleate, responsive to calcitonin, and positive for TRAP activity and monoclonal antibody 23C6 (which recognizes vitronectin receptors) and negative for alkaline phosphatase and non-specific esterase activities. These cells were considered to be osteoclasts. The specimens were then fixed with 4% glutaraldehyde in cacodylate buffer (pH 7.4) for 30 min at 4 °C, given three 10-min washings in 0.1M sodium cacodylate buffer, postfixed with 1% osmium tetroxide in 0.1M sodium cacodylate buffer (pH 7.4) for 1 h at room temperature and given three 10-min washings in buffer. After dehydration in graded alcohols, they were critical-point-dried and sputter-coated with gold-palladium. The surface of the specimens was examined using a JEOL 6300 scanning electron microscope (JEOL, Tokyo, Japan) operating at 15 kV.

Transmission electron microscopy (TEM)

Similar fixation and alcohol dehydration were done for SEM and TEM. After dehydration, samples were embedded in Epon and polymerized at 60 °C. Ultrathin sections were prepared on a Reichert microtome (Reichert, Vienna, Austria) and then contrasted with uranyl acetate and lead citrate before examination with a JEOL 200 CX transmission electron micrsocope (JEOL) at 100 kV.

Results

After 4 days of culture, many osteoclasts expressing typical morphological features (polarization, fine dense peripheral filopodia, dorsal microvilli) were observed on the ceramic surface (Fig. 1a,b). Scanning electron microscopy showed that the osteoclasts were associated with degraded areas (Fig. 1b,c) easily distinguishable from non-degraded surface. The resorption lacunae displayed by rabbit osteoclasts had well-defined margins, and the crystals inside the lacunae were smaller

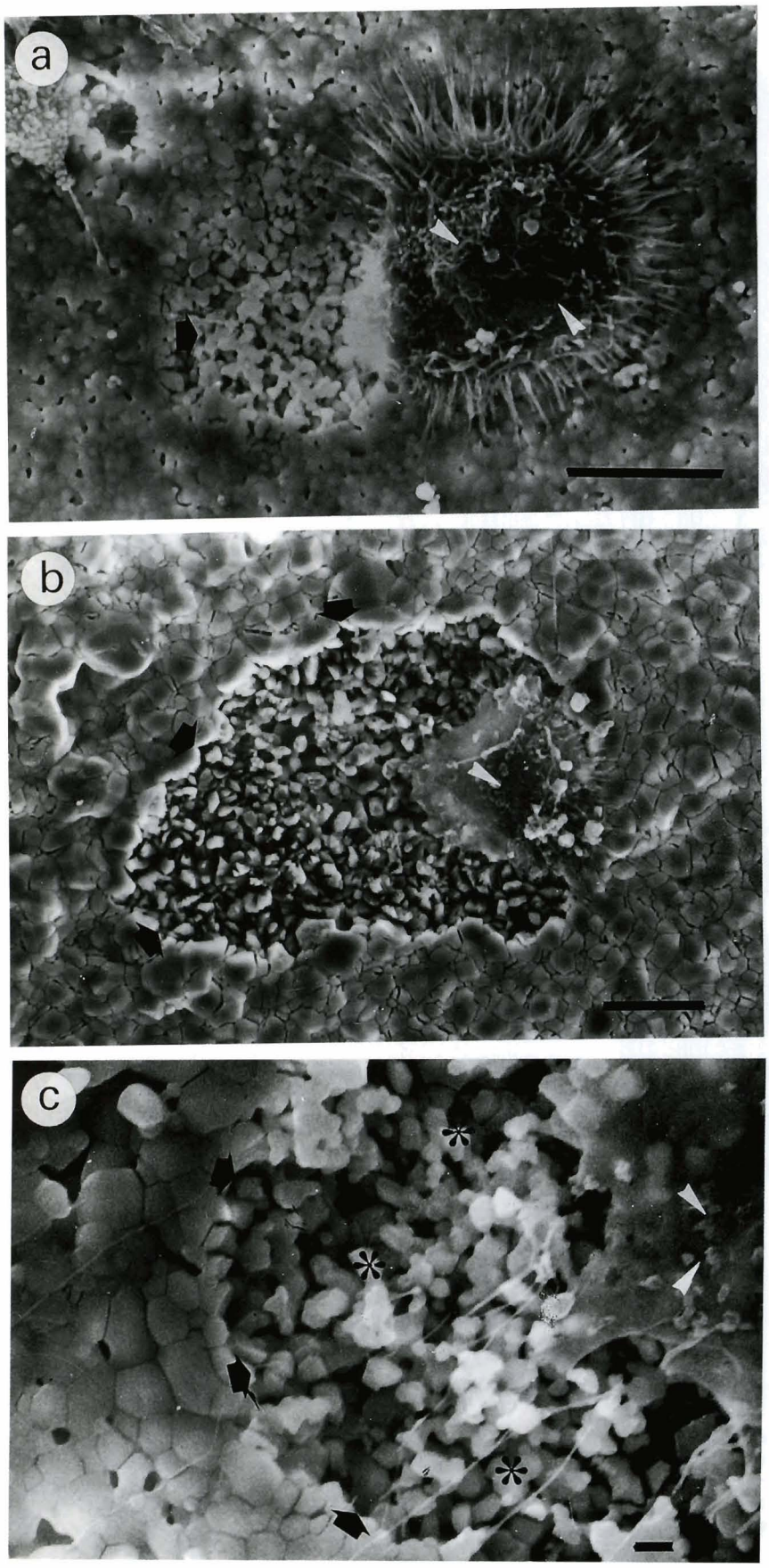

Fig. 1. SEM morphology of rabbit osteoclasts degrading CaP ceramic. After 4 days of culture on CaP ceramic, total rabbit cells are treated by pronase E and EDTA to remove stromal cells and allow observation of osteoclasts. Osteoclast (white arrowhead), limit of the lacuna (black arrow), crystals inside the lacuna (black asterisk). a, x 2,000, bar: 10 μm. b, x 1,500, bar: 10 μm. c, x 5,000, bar: 1 μm.

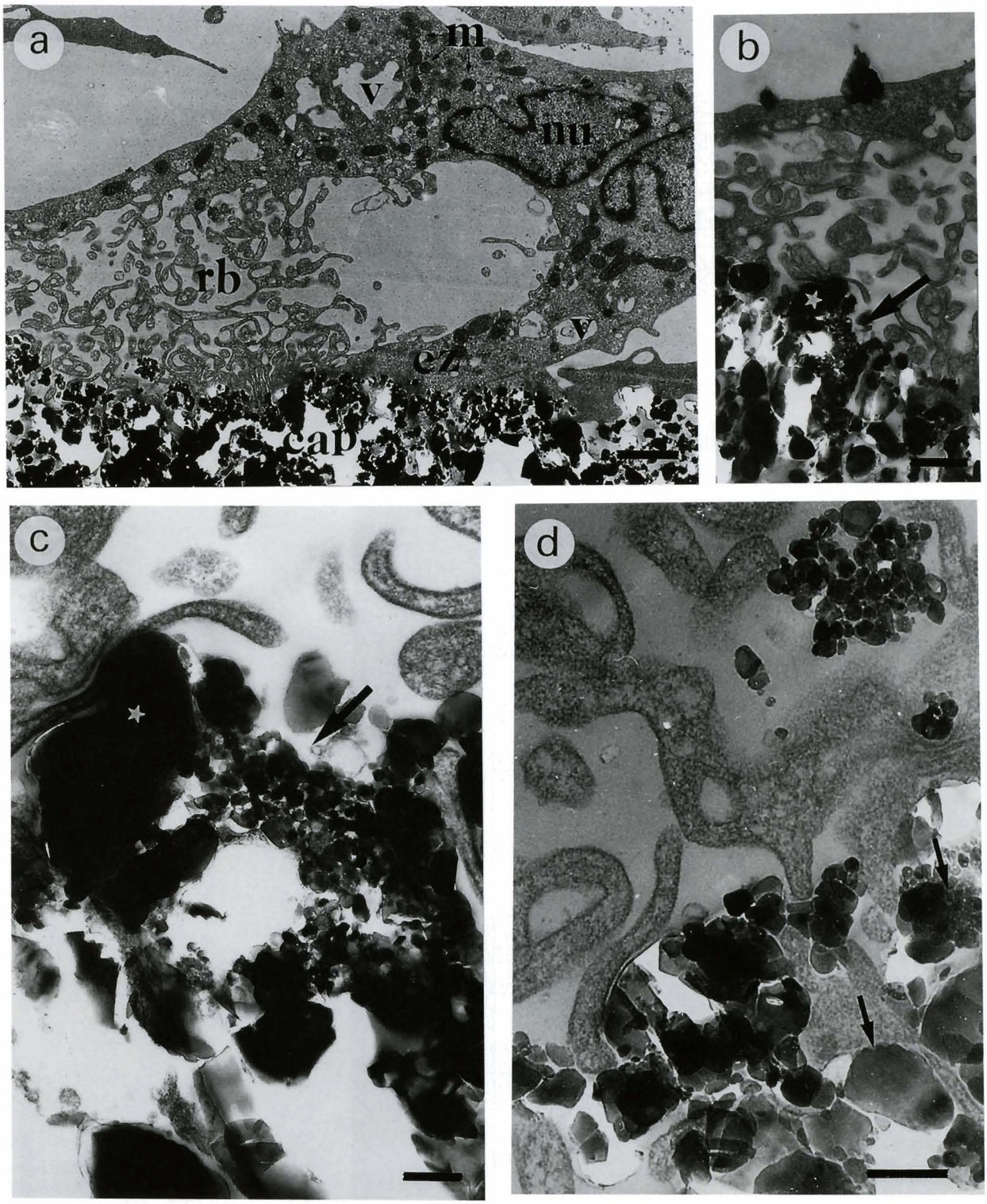

Fig. 2. TEM analysis of CaP ceramic resorption by rabbit osteoclasts. After 4 days of culture on CaP ceramic, total rabbit cells are treated by pronase E and EDTA to remove stromal cells and allow observation of osteoclasts. **a.** Typical osteoclasts resorbing the ceramic surface. cap: calcium phosphate ceramic; cz: clear zone; m: mitochondria; nu: nucleus; rb: ruffled border; v: vacuole. x 3,000, bar: 1.5 μm; **b.** CaP crystals resorbed in contact with rabbit osteoclasts (arrow). The resorbed crystals are smaller than the original crystals (white asterisk). x 7,300, bar: 3 μm. **c.** Higher magnification of figure b. x 30,000. bar: 0.25 μm. **d.** Resorption area of CaP crystals in contact with osteoclast ruffled border (arrow). Note the loss of electron density. x 15,000, bar: 0.25 μm.

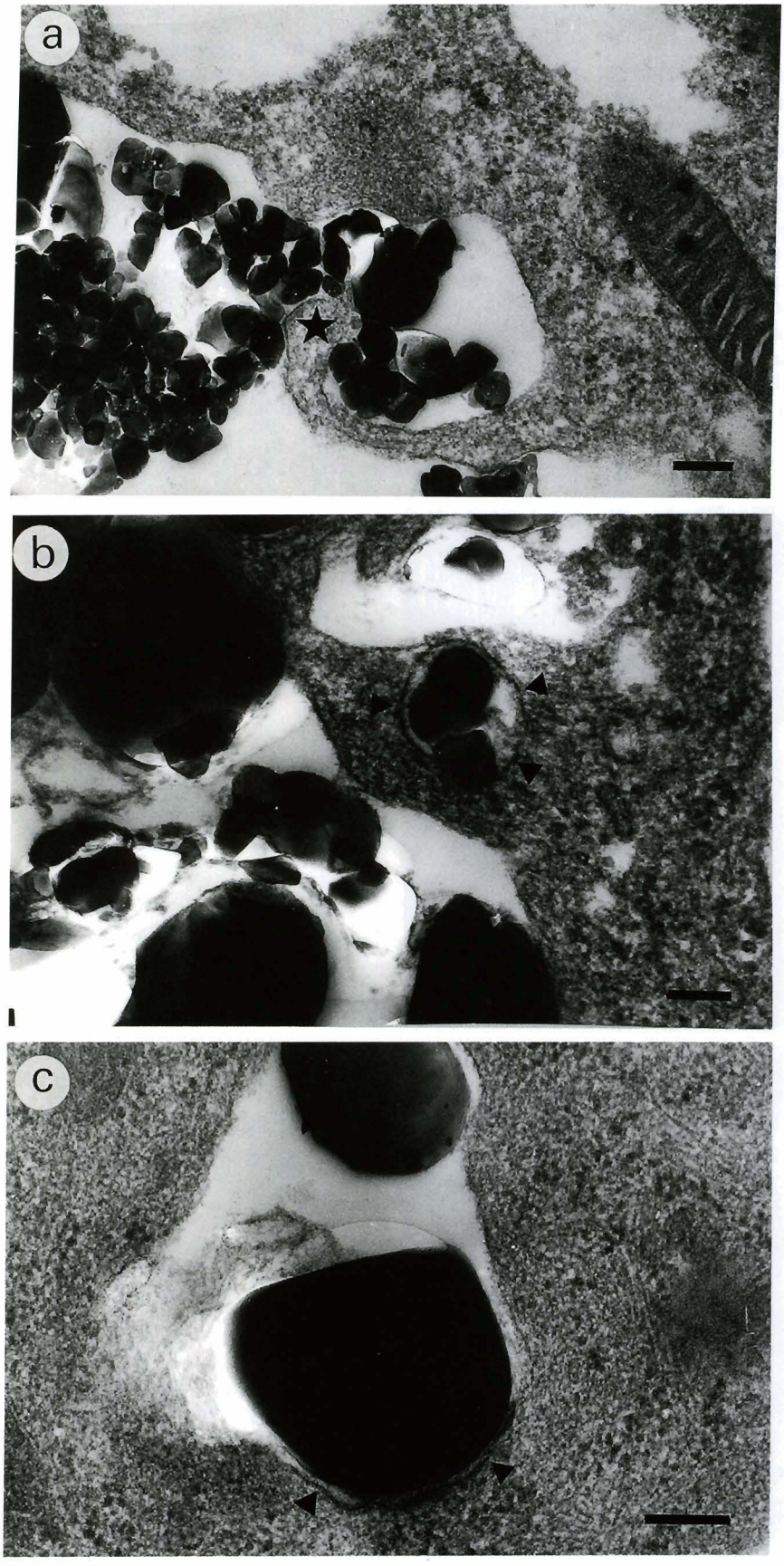

Fig. 3. Capture of CaP crystals by osteoclastic phagocytosis. **a.** A cytoplasmic wave (asterisk) of rabbit osteoclasts surrounding small CaP crystals. x 37,000, bar: 0.15 μm. **b and c.** Small endophagosomes surrounded by their envelope membrane (arrowhead). x 50,000, bar: 0.15 μm.

than the grains outside (Fig. 1c).

Ultrastructural study was carried out by TEM to determine the nature of the degradation mechanism. Rabbit osteoclasts cultured on the ceramic surface had a well-developed ruffled border indicative of the intense resorption activity of cells and a clear zone closely adherent to the material (Fig. 2a). There were many mitochondria around the ruffled border, and many vacuoles and free ribosomes in the cytoplasm. Rough-surfaced endoplasmic reticulum was present in small quantity. The resorption mechanism was evidenced by small crystals in contact with the cellular membrane of the ruffled border. The CaP crystals under the ruffled border became small and rounded, and their opacity to electrons decreased in comparison with original crystals (Fig. 2b,c). The surface of the resorbed crystals was less smooth because of the many resorption areas (Fig. 2d). Similar amounts of small rounded crystals are never observed in CaP ceramic and can be related only to osteoclatic activities.

Osteoclasts degraded CaP ceramic *in vitro* via simultaneous resorption and phagocytosis mechanisms (Figs. 3, 4). The cytotic mechanisms consisted of two steps. The first related to the capture of resorbed CaP crystals by osteoclasts. Osteoclasts developed a cytoplasmic wave closely adherent to the ceramic and surrounding small CaP crystals (Fig. 3a). Encircling of CaP crystals with a small amount of culture medium led to the formation of endophagosomes (Fig. 3b,c). The cytotic vesicle membrane was always observed at this

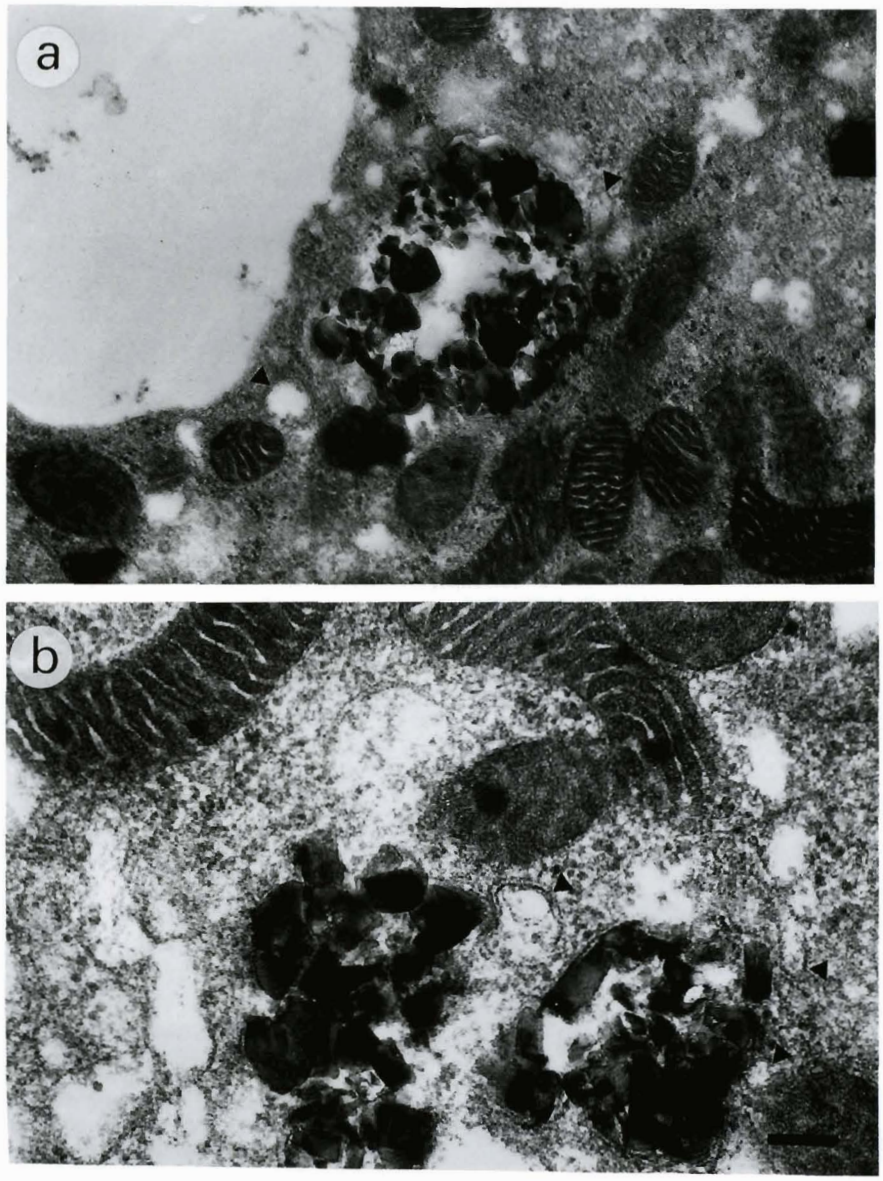

Fig. 4. Fragmentation and dissolution of CaP crystals phagocytosed by rabbit osteoclasts. Dissolution is perceptible by the loss of electron density and the modification of the original shape of CaP crystals. Vesicles of agranular endoplasmic reticulum can be seen near the phagocytosed crystals (arrowhead). a, x 20,000, bar: 0.3 μm; b, x 37,000. bar: 0.15 μm.

stage. The second step related to the disappearance of the endophagosome envelope membrane, leading to the release of the phagocytosed crystals into the cytoplasm (Fig. 4a,b). The crystals then underwent *in situ* fragmentation into very small pieces, facilitating interactions between organelles and crystals (numerous agranular endoplasmic reticulum vesicles were seen near the small crystal fragments).

Discussion

This study demonstrates that Ca-P ceramic does not alter the behavior of osteoclasts as compared to those cultured on bone slices. They develop typical ultrastructural features of bone osteoclasts such as a polarized dome shape, a clear zone and a ruffled border. The shape and density of Ca-P crystals are modified under the ruffled border, indicating an acidic microenvironment. Osteoclasts are able to degrade Ca-P ceramic by simultaneous resorption and phagocytosis mechanisms. The phagocytosis does not alter osteoclastic ability to resorb Ca-P ceramic.

There has been controversy as to whether the multinucleated giant cells involved in the degradation of CaP ceramic are osteoclasts. Yamada et al. (1997a) used a neonatal rabbit bone cell model to show that osteoclasts are able to resorb CaP ceramic. Their SEM studies indicated that cellular degradation of the ceramic was due to the extracellular process characteristic of osteoclastic resorption. These data confirmed the results obtained on other ceramics (Jones et al., 1984; Davies et al., 1993; Gomi et al., 1993). Numerous parameters influence the capability of osteoclasts to resorb ceramic. For example, the extent of osteoclastic resorption of CaP ceramic would appear to be fairly proportional to the solubility of the biomaterial (Yamada et al., 1997b). All parameters influencing the solubility of materials, such as their composition and porosity, can modify osteoclastic activity. Some authors consider that multinucleated cells at the implantation site do not possess osteoclast features but only the specificity of macrophage-polykaryons (Wada et al., 1989; Dersot et al., 1995). Finally, Baslé et al. (1993) observed two different multinucleated cell populations (osteoclasts and inflammatory giant cells) after CaP implantation which were closely associated with the ceramic surface. It is apparent that CaP ceramics can be degraded by phagocytosis induced by inflammatory giant cells and by resorption mechanisms induced by osteoclasts, although the precise effect of each of these cell populations is difficult to assess.

Osteoclasts are physiological polykaryons which carry out the highly specialized functions of lacuna bone resorption. These cells share numerous functional, cytochemical and morphological features of macrophage-polykaryons, including the capacity to phagocytose inert particles (Chambers, 1979; Wang et al., 1997a,b). They are able to phagocytose latex and polymethylmethacrylate particles in a similar manner to that of monocytes/macrophages. However, this point is also controversial since Willert et al. (1990a,b) did not observe polymeric and metallic particles in the cytoplasm of osteoclasts at the bone-implant interface of cemented and uncemented arthroplasties.

CaP ceramics are bioactive biomaterials. The image sequences observed in this study clearly indicate that osteoclasts are able to degrade CaP ceramic by producing ultrastructural features similar to those of osteoclasts cultured on bone slices and involving simultaneous resorption and phagocytosis mechanisms. The phagocytosis mechanism did not alter the resorption activity of osteoclasts, which remained functionally competent and capable of resorbing the ceramic surface by an acidic extracellular mechanism. This process is similar to that observed on inert particles which can be phagocytosed by osteoclasts without any alteration in their bone resorption ability (Wang et al., 1997a,b). Osteoclasts are thus able to phagocytose bioactive bone substitutes similarly to inert biomaterials. The three-step phagocytosis mechanism described in the present study, consisting of crystal phagocytosis, disappearance of the endophagosome envelope membrane and fragmentation of the phagocytosed crystals, was similar to those observed with the monocyte/macrophage lineage (Benahmed et al., 1996a). Yet only one mechanism, involving a small amount of culture medium, was observed in the present study with rabbit osteoclasts, whereas two phagocytic phenomena have been described for monocytes/macrophages. The first concerns CaP crystals detached from the ceramic surface and internalized by chance with large amounts of medium. The second concerns the internalization of CaP crystals with a small amount of culture medium in close relation with the ceramic surface. These observations indicate that crystals phagocytosed by osteoclasts are certainly from ceramic resorbed simultaneously by an acidic extracellular mechanism under the ruffled border. Multinucleated cells at the bone-implant interface which contain particles of cytoplasmic biomaterial are usually regarded as foreign-body giant cells or macrophage-polykaryons rather than osteoclasts (Willert et al., 1990a,b). Our study indicates that multinucleated giant cells can also be considered as osteoclasts and that the presence of particles in cells is not a discriminant criterion for distinguishing between these two cell populations.

Osteoclasts are formed by the fusion of medullar mononuclear precursors and have common precursors with the monocyte/macrophage lineage (Heymann et al., 1998). Thus, osteoclasts share numerous functional, cytochemical and morphological features with monocytes/macrophages. Accordingly, osteoclasts have been classified as a part of the mononuclear phagocyte system. The common mechanism of phagocytosis described here reinforces this notion. However, further experiments are required to determine the relative contributions of these two degradation mechanisms *in vivo* after implantation of CaP ceramics.

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References

Athanasou N.A., Quinn J., Ferguson D.J.P. and McGee J.O'D. (1991). Bone resorption by macrophage polykaryons of giant cell tumour of tendon sheath. Br. J. Cancer 63, 527-533.

Athanasou N.A., Quinn J. and Bulstrode C.J.K. (1992). Resorption of bone inflammatory cells derived from the joint capsule of hip arthroplasty. J. Bone Joint Surg. 74B, 57-62.

Baslé M.F., Chappard D., Grizon F., Filmon R., Delecrin J., Daculsi G. and Rebel A. (1993). Osteoclastic resorption of Ca-P biomaterials implanted in rabbit bone. Calcif. Tissue Int. 53, 348-356.

Benahmed M., Heymann D., Berreur M., Cottrel M., Godard A., Daculsi G. and Pradal G. (1996a). Ultrastructural study of degradation of calcium phosphate ceramic by human monocytes and modulation of this activity by HILDA/LIF cytokine. J. Histochem. Cytochem. 44, 1131-1140.

Benahmed M., Bouler J.M., Heymann D., Gan O. and Daculsi G. (1996b). Biodegradation of synthetic calcium phosphate by human monocytes *in vitro*: a morphological study. Biomaterials 17, 2173-2178.

Benahmed M., Heymann D., Pilet P., Bienvenu J. and Daculsi G. (1997). LPS increases biomaterial degradation by human monocytes *in vitro*. J. Biomed. Mater. Res. 34, 115-119.

Chambers T.J. (1979). Phagocytosis and tryspin-resistant glass adhesion by osteoclasts in culture. J. Pathol. 127, 55-60.

Daculsi G., Passuti N., Martin S., Deudon C., LeGeros R.Z. and Raher S. (1990). Macroporous calcium phosphate ceramic for long bone surgery in humans and dogs. Clinical and histological study. J. Biomed. Mat. Res. 24, 379-396.

Davies J.E., Shapiro G. and Lowenberg B.F. (1993). Osteoclastic resorption of calcium phosphate ceramic thin film. Cells Mater. 3, 245-256.

de Bruijn J.D., Bovell Y.P., Davies J.E. and van Blitterswiijk C.A. (1994). Osteoclastic resorption of calcium phosphate is potentiated in postosteogenic culture conditions. J. Biomed. Mater. Res. 28, 105-112.

Dersot J.M., Colombier M.L., Lafont J., Baroukh B., Septier D. and Saffar J.L. (1995). Multinucleated giant cells elicited around hydroxyapatite particles implanted in craniotomy defects are not osteoclasts. Anat. Rec. 242, 166-176.

Eggli P.S., Muller W. and Schenk R.K. (1988). Porous hydroxyapatite and tricalcium phosphate cylinders with two different pore size ranges implanted in the cancellous bone of rabbits. A comparative histomorphometric and histologic study of bone ingrowth and implant substitution. Clin. Orthop. 232, 127-138 .

Gomi K., Lowenberg B., Shapiro G. and Davies J.E. (1993). Resorption of synthetic hydroxyapatite by osteoclasts *in vitro*. Biomaterials 14, 91-96.

Guicheux J., Heymann D., Rousselle A.V., Gouin F., Pilet P., Yamada S. and Daculsi G. (1998). Growth hormone stimulatory effects on osteoclastic resorption are partly mediated by insulin-like growth factor I: an *in vitro* study. Bone 22, 25-31.

Heymann D., Guicheux J., Gouin F., Passuti N. and Daculsi G. (1998). Cytokines, growth factors and osteoclasts. Cytokine 10, 155-168.

Heymann D., Pradal G. and Benahmed M. (1999). Cellular mechanisms of calcium phosphate ceramic degradation. Histol Histopathol. 14, 871-877.

Ikami K., Iwaku M. and Ozawa H. (1990). An ultrastructural study of the process of hard tissue formation in amputated dental pulp dressed with alpha-tricalcium phosphate. Arch. Histol. Cytol. 53, 227-243.

Jones S.J., Boyde A. and Ali N.N. (1984). The resorption of biological and non-biological substratess by cultured avian and mammalian osteoclast. Anat. Embryol. 170, 247-256.

LeGeros R.Z. (1991). Calcium phosphates in oral biology and medicine. In: Monographs in oral sciences. Vol. 15. Myers H. and Karger S. (eds). Basel.

Passuti N., Daculsi G., Rogez J.M., Martin S. and Bainvel J.V. (1989). Macroporous calcium phosphate ceramic performance in human spine fusion. Clin. Orthop. Rel. Res. 248, 12-19.

Roodman G.D. (1993). Role of cytokines in the regulation of bone resorption. Calcif. Tissue Int. 53, 594-598.

Suda T., Nakamura I., Jimi E. and Takahashi N. (1997). Regulation of osteoclast function. J. Bone Miner. Res. 12, 869-879.

Tetzuka K., Sato T., Kamioka H., Nijweide P.J., Tanaka K., Matsuo T., Ohta M., Kuhihara N., Hakeda Y. and Kumegawa M. (1992). Identification of osteopontin in isolated rabbit osteoclasts. Biophys. Biochem. Res. Commun. 186, 911-917.

Wada T., Hara K., Quian H.Y., Wang F. and Rosenzweig S.A. (1989). Ultrastructural and histochemical study of ß-tricalcium phosphate resorbing cells in periodontium of dog. J. Period. Res. 24, 391-401.

Wang W., Ferguson D.P.J., Quinn J.M.W., Simpson A.H.R.W. and Athanasou N.A. (1997a). Osteoclasts are capable of particle phagocytosis and bone resorption. J. Pathol. 182, 92-98.

Wang W., Ferguson D.P.J., Quinn J.M.W., Simpson A.H.R.W. and Athanasou N.A. (1997b). Biomaterial particle phagocytosis by bone-resorbing osteoclasts. J. Bone Joint Surg. 79-B, 849-856.

Willert H.G., Bertram H.B. and Buchhorn G.H. (1990b). Osteolysis in alloarthroplasty of the hip: the role of bone cement fragmentation. Clin. Orthop. 258, 108-121 .

Willert H.G., Bertram H.B. and Buchhorn G.H. (1990a). Osteolysis in alloarthroplasty of the hip: the role of ultra-high molecule weight polyethylene wear particles. Clin. Orthop. 258, 95-107.

Yamada S., Heymann D., Bouler J.M. and Daculsi G. (1997a). Osteoclastic resorption of biphasic calcium phosphate ceramic *in vitro*. J. Biomed. Mat. Res. 37, 346-352.

Yamada S., Heymann D., Bouler J.M. and Daculsi G. (1997b). Osteoclastic resorption of calcium phosphate ceramics with different hydroxyapatite/ß-tricalcium phosphate ratios. Biomaterials 18, 1037-1041.

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