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Hydroxyapatite biomaterial implanted in human periodontal defects: an histological and ultrastructural study

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SUMMARY

The purpose of the present work was to study the response of human periodontium to hydroxyapatite biomaterial particles (180-200 μm). The biomaterial was implanted in two infraosseous periodontal defects (two patients) after clearing of the granulation tissue. At two months post-surgery, biopsies were studied using light and electron microscopy. No sign of inflammation was observed, the biomaterial aggregates were surrounded either by typical fibroblasts or larger phagocytotic cells with phagocytosis vesicles containing biomaterial crystals. These intracellular crystals were noticeably smaller than the non-phagocytized ones. Some of the phagocytized crystals showed morphological signs of intracellular dissolution. The spaces between the crystals constitutive of the aggregates were filled with organic substance containing collagen fibers.

KEY WORDS: hydroxyapatite, biomaterials, periodontal defect, implant, microscopy

RÉSUMÉ

Le présent travail a pour but d'étudier la réponse du parodonte humain à des particules d'hydroxyapatite de 180 à 200 μm. Le biomatériau a été implanté dans deux poches parodontales infraosseuses (deux patients) après élimination du tissu de granulation. Deux mois après l'intervention, des biopsies ont été étudiées en microscopie photonique et en microscopie électronique. Aucun signe d'inflammation n'a été décelé, les agrégats de biomatériau sont bordés soit de fibroblastes, soit de cellules phagocytaires, de taille plus importante, avec des vésicules de phagocytose renfermant des cristaux de biomatériau. Ces cristaux intracellulaires sont notablement plus petits que les cristaux non phagocytés. La morphologie de certains cristaux phagocytés traduit l'existence d'une dissolution intracellulaire. Les espaces entre les cristaux constitutifs des agrégats sont comblés par une substance organique contenant des fibres de collagène.

MOTS-CLES: hydroxyapatite, biomaterials, periodontal defect, implant, microscopy

INTRODUCTION

Several surgical procedures are in use today for the treatment of infrabony defects resulting from chronic periodontitis (Schallhorn, 1977). Among them, autografts seem to have the greater bone regenerating properties (Cushing, 1969; Bowers et al., 1982), however routine use of this procedure is not feasible since a previous surgical operation is needed. Since autogenous donor bone obtention is limited, attempts have been made to find synthetic substitute materials.

In the last decades, efforts have been made to develop bone implant materials composed of hydroxyapatite and related calcium phosphates whose crystalline and chemical composition is closely allied to the mineral component of bone. Two major calcium phosphate ceramics are currently investigated, hydroxyapatite (HAP) $Ca_{10}(PO_4)_6(OH)_2$ and β-tricalcium phosphate (β-TCP) $Ca_3(PO_4)_2$. They have been characterized as biocompatible materials (de Groot, 1983) but the biological events occurring after implantation of a calcium phosphate biomaterial are not well understood yet. Even the simple documentation concerning the cell types involved in the host response, which depend on the implantation sites, is far from complete. Histologic (Froum et al., 1982; Moskow and Lubar, 1983; Baldock et al., 1985; kenney et al., 1986; Stahl and Froum, 1986; Bowers et al., 1986; Carranza et al., 1987; Stahl and Froum, 1987) and ultrastructural (Ganeles et al., 1986; Frank et al., 1987; Ogilvie et al., 1987) data concerning the implantation of calcium phosphate biomaterials in infrabony defects in human periodontium (although available), are still uncomplete and controversial. Moreover, data obtained from experimental bone defects induced in animals (Nery et al., 1975; Boyne and Fremming, 1982; Misiek et al., 1984; Boetto and Freeman, 1984; Drobeck et al., 1984) cannot easily be compared to those concerning chronic progressive periodontitis in human.

The present study was undertaken to evaluate some of the histologic and ulstrastructural features consecutive to the implantation of HAP particles in patients presenting various degrees of periodontal involvement.

MATERIALS AND METHODS

Implant material

HAP was prepared through a double decomposition method already described elsewhere (Bonel et al., 1989). Briefly, a solution of diammonium hydrogen phosphate at pH>9 was slowly poured in a boiling calcium nitrate solution. The hot precipitate was filtered, dried at 70°C for nearly 12 hours and air calcinated at 900°C for 3 to 4 hours. The resulting material (Ca/P = 1.65 ± 0.01) was identified as hydroxyapatite by X-ray diffraction and infrared spectroscopy analysis.

The material was powdered in a mortar and sieved in order to select particles ranging from 180 to 200 μm.

Surgical procedure

Two volunteer patients presenting infraosseous maxillary defects ranging from 8 to 12 mm deep were involved in the study. Two sulcular incisions for mucoperiostal flaps were performed under local anaesthesia to expose the defect. The defect was cleared of granulation tissue and the biomaterial particles were placed within the confines of the osseous walls.

At two months following surgery, the implanted site underwent surgical re-entry, biopsies were preserved and prepared for light and electron microscopy.

Samples preparation

Light microscopy: part of the specimens were fixed in Carnoy's fluid, decalcified, embedded in paraplast, sectioned and stained with hematoxylin-erythrosin.

Transmission electron microscopy: specimens were fixed in 2% glutaraldehyde solution in cacodylate buffer, post-fixed in osmium tetroxide and embedded in methacrylate. Undecalcified ultrathin sections performed with a Sorval Porter ultramicrotome were examined with a Jeol 200 CX TEM.

RESULTS

Light microscopy findings

Decalcified sections observed in light microscopy showed no evidence of inflammatory response of the gingival corium. The aggregates were found surrounded either by lining fibroblasts either by larger cells packed along the surface of the biomaterial. In some cases, both kinds of cells were observed lining different areas of the particle surface (Figs 1-2). In the vicinity of gingival epithelial crests, the bioceramic aggregates were surrounded by epithelial cells (Fig. 3).

Electron microscopy findings

In agreement with the histological findings, different kinds of cells were seen lining the biomaterial aggregates:

— in places, the aggregates surface or part of it was in contact with typical elongated fibroblasts presenting rough endoplasmic reticulum. The cells were separated by an organic substance containing collagen fibers. The fibroblasts were not observed in direct contact with the aggregate but were always separated from it by an intervening layer of dense, granular organic material (Figs 4-5);

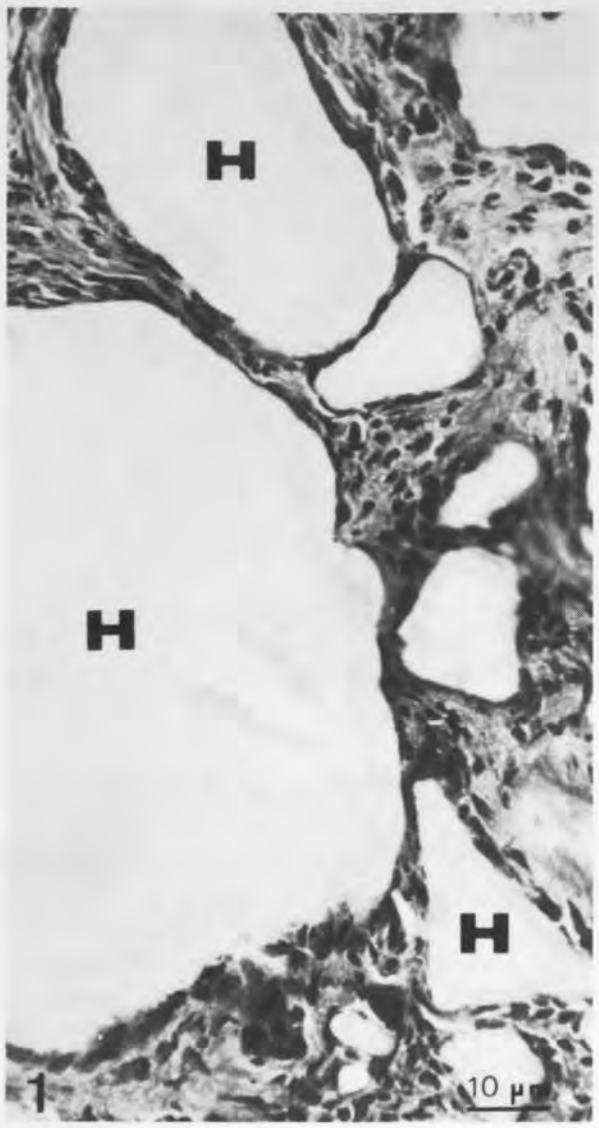

Fig. 1: Connective tissue surrounding hydroxyapatite aggregates or fragments of different sizes (H). No inflammatory cells are present.
(Decalcified section. × 800).
Fig. 1: Tissu conjonctif entourant des agrégats d'hydroxyapatite (ou des fragments) de différentes tailles (H). Noter l'absence de cellule inflammatoire.
(Coupe décalcifiée. × 800).

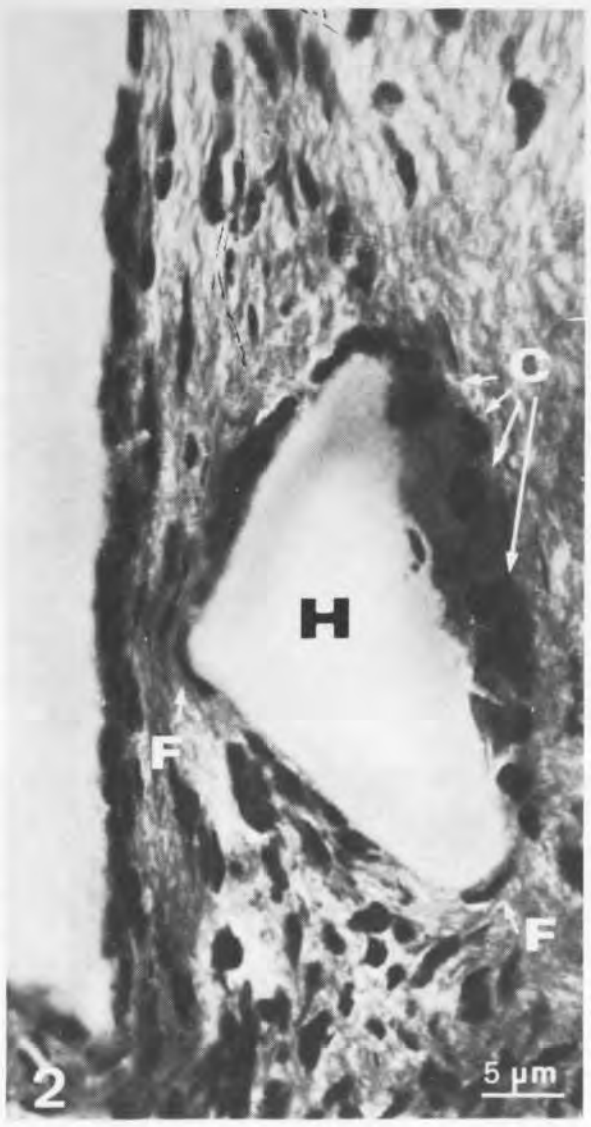

Fig. 2: Elongated fibroblasts (F) and larger cells (C) covering an hydroxyapatite aggregate (H).
(Decalcified section. × 1,600).
Fig. 2: Fibroblasts fusiformes (F) et cellules de plus grande taille (C) bordant un agrégat d'hydroxyapatite (H).
(Coupe décalcifiée. × 1.600).

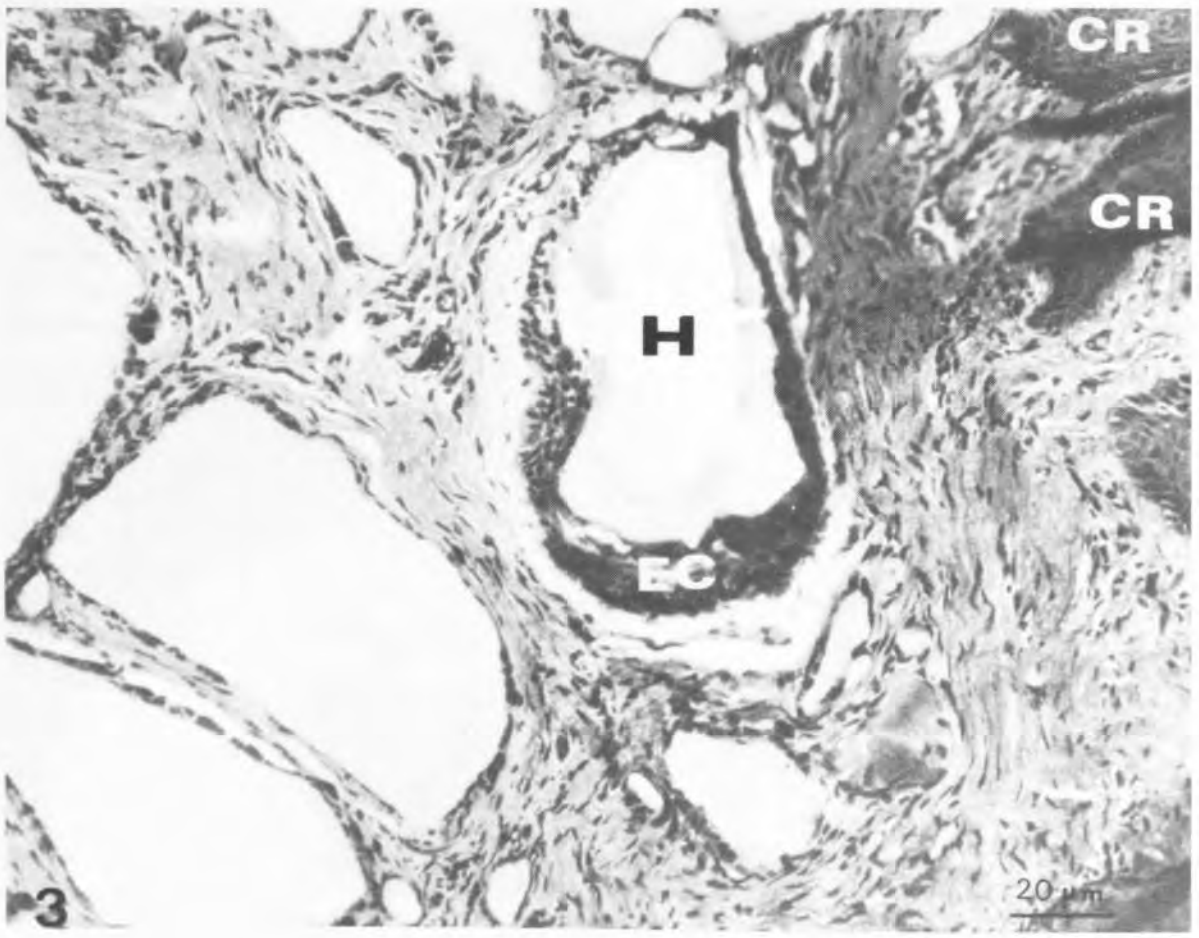

Fig. 3: Hydroxyapatite aggregate (and fragments) in the vicinity of epithelial crests (CR). Epithelial cells (EC) surrounding an aggregate (H).
(Decalcified section. × 500).
Fig. 3: Agrégats (et fragments) d'hydroxyapatite au voisinage des crêtes épithéliales (CR). Cellules épithéliales (EC) bordant un agrégat (H).
(Coupe décalcifiée. × 500).

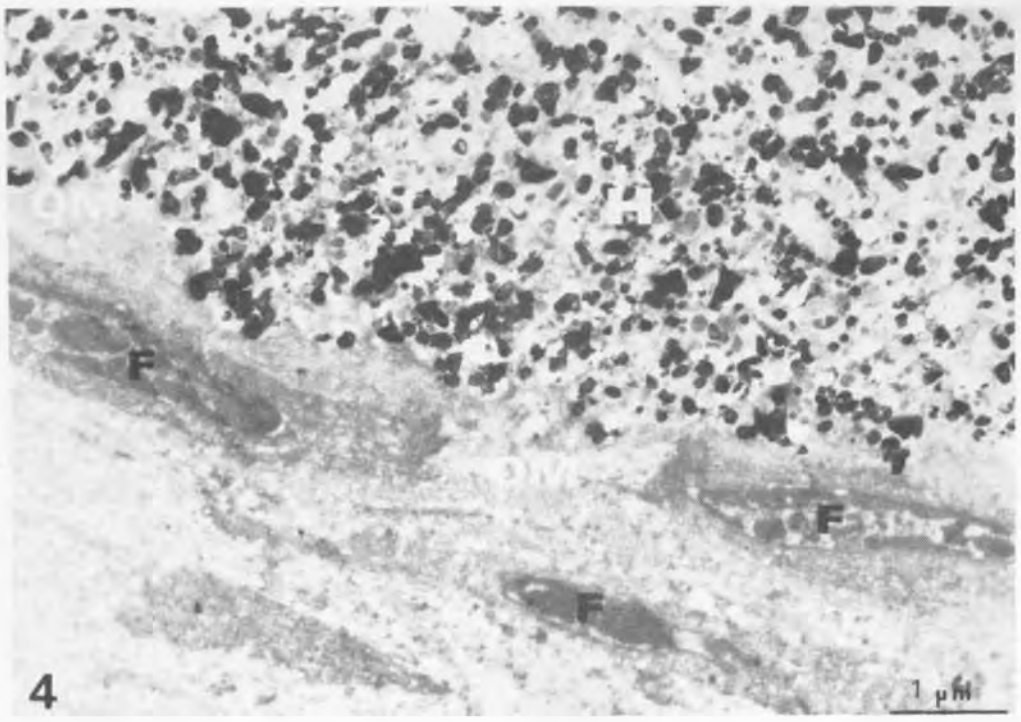

Fig. 4: Fibroblasts (F) lining the surface of an hydroxyapatite aggregate (H). Fibroblasts are separated by an organic material (OM).
(TEM. Undecalcified section. × 13,000).
Fig. 4: Fibroblastes (F) bordant la surface d'un agrégat d'hydroxyapatite (H). Les fibroblastes sont séparés les uns des autres par du matériel organique (OM).
(MET. Coupe non-décalcifiée. × 13.000).

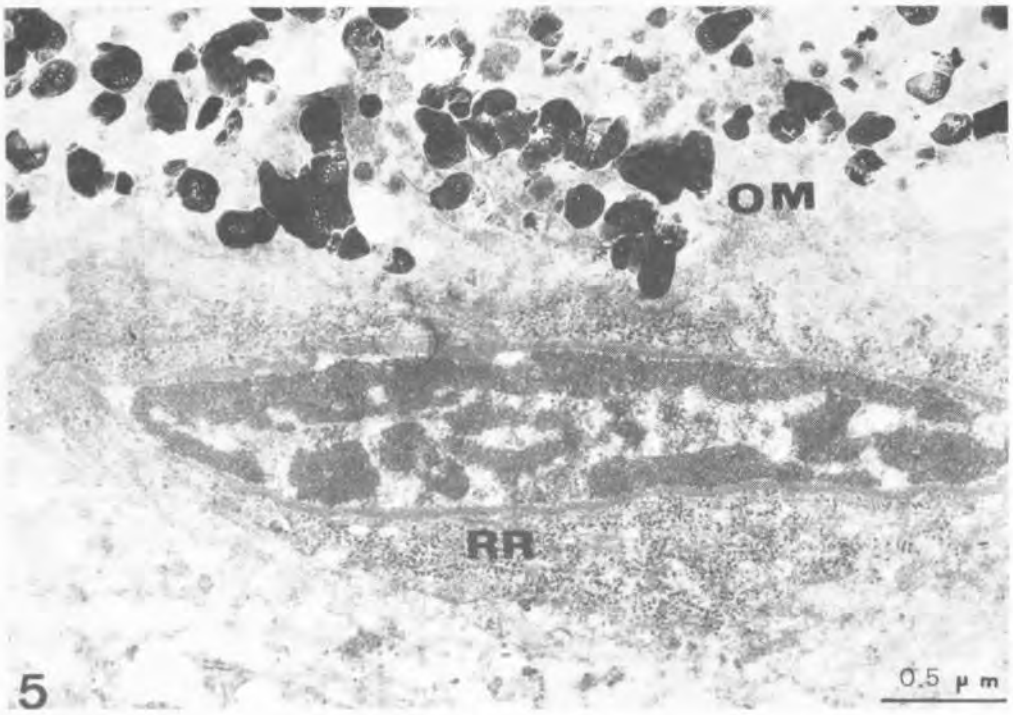

Fig. 5: Fibroblasts exhibiting abundant rough reticulum (RR). Note the intervening layer of organic material (OM) between the cell and the aggregate surface.
(TEM. Undecalcified section. × 30,000).
Fig. 5: Fibroblaste présentant un réticulum rugueux (RR) développé. Noter la présence d'une couche de matériel organique (OM) entre la cellule et la surface de l'agrégat.
(MET. Coupe non-décalcifiée. × 30.000).

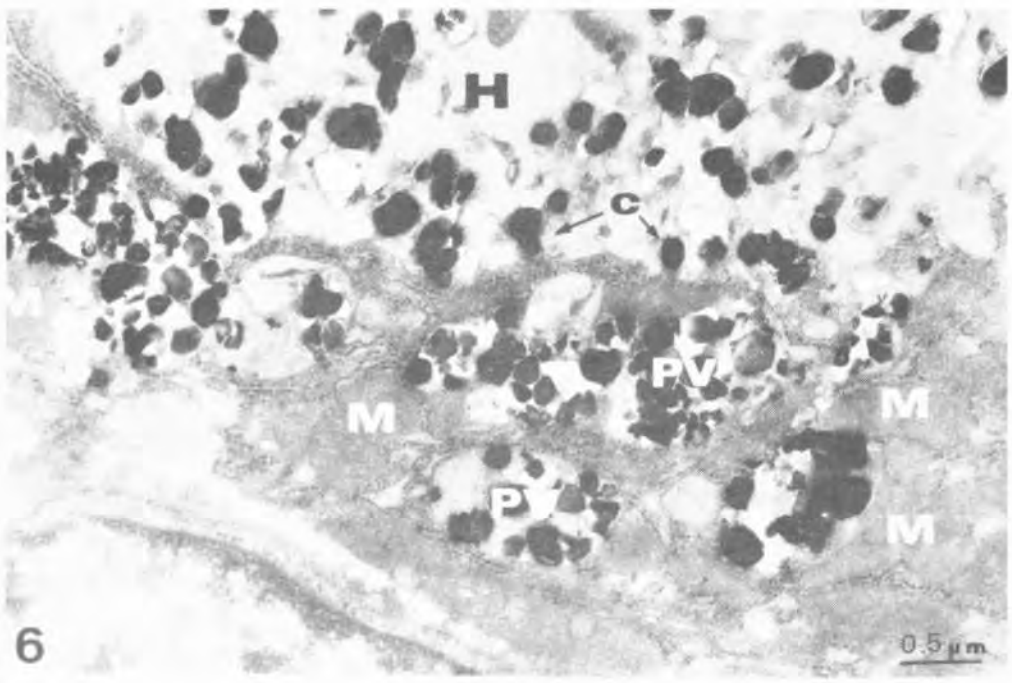

Fig. 6: Phagocytotic cell at the surface of an hydroxyapatite aggregate (H). The cytoplasm is packed with mitochondria (M) and contains numerous phagocytosis vesicles (PV) enclosing hydroxyapatite crystals. Some crystals (C) are sticked to the cell membrane.
(TEM. Undecalcified section. × 20,000).
Fig. 6: Cellule phagocytaire à la surface d'un agrégat d'hydroxyapatite (H). Le cytoplasme est envahi de mitochondries (M) et contient de nombreuses vésicules de phagocytose (PV) renfermant des cristaux d'hydroxyapatite. Quelques cristaux (C) adhèrent à la membrane.
(MET. Coupe non-décalcifiée. × 20.000).

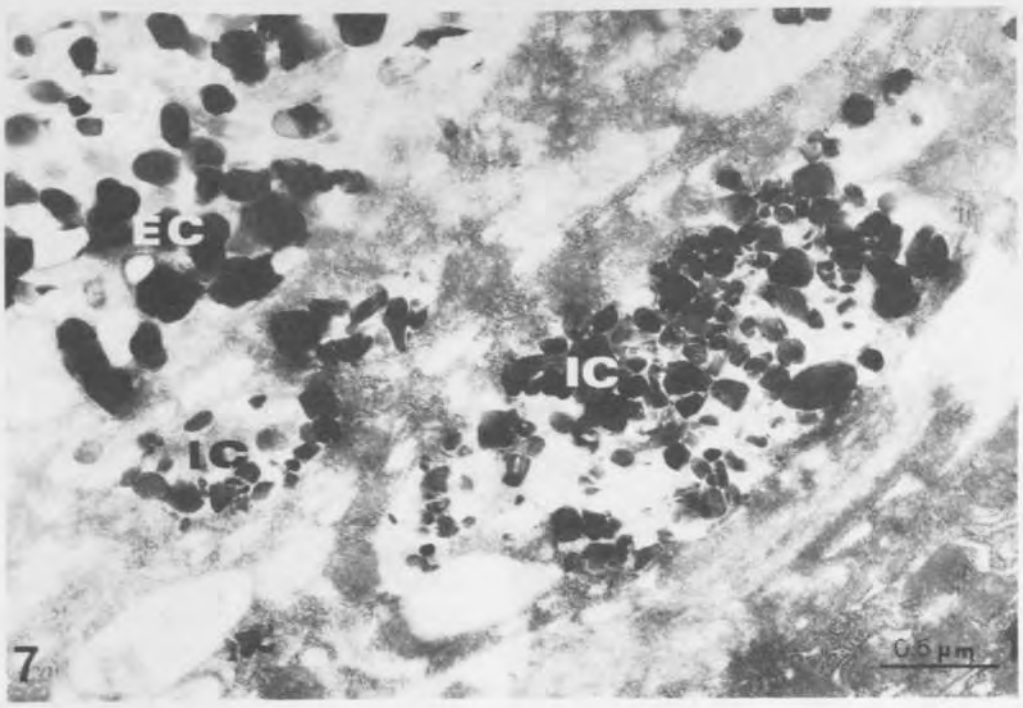

Fig. 7: Hydroxyapatite crystals within phagocytosis vesicles. Note the smaller size of the intraphagosomal crystals (IC) compared to the non-phagocytized ones (extracellular crystals: EC).
(TEM. Undecalcified section. × 25,000).
Fig. 7: Cristaux d'hydroxyapatite à l'intérieur d'une vésicule de phagocytose. Noter la taille réduite des cristaux intraphagosomaux (IC) par rapport aux cristaux non-phagocytés (cristaux extracellulaires: EC).
(MET. Coupe non-décalcifiée. × 25.000).

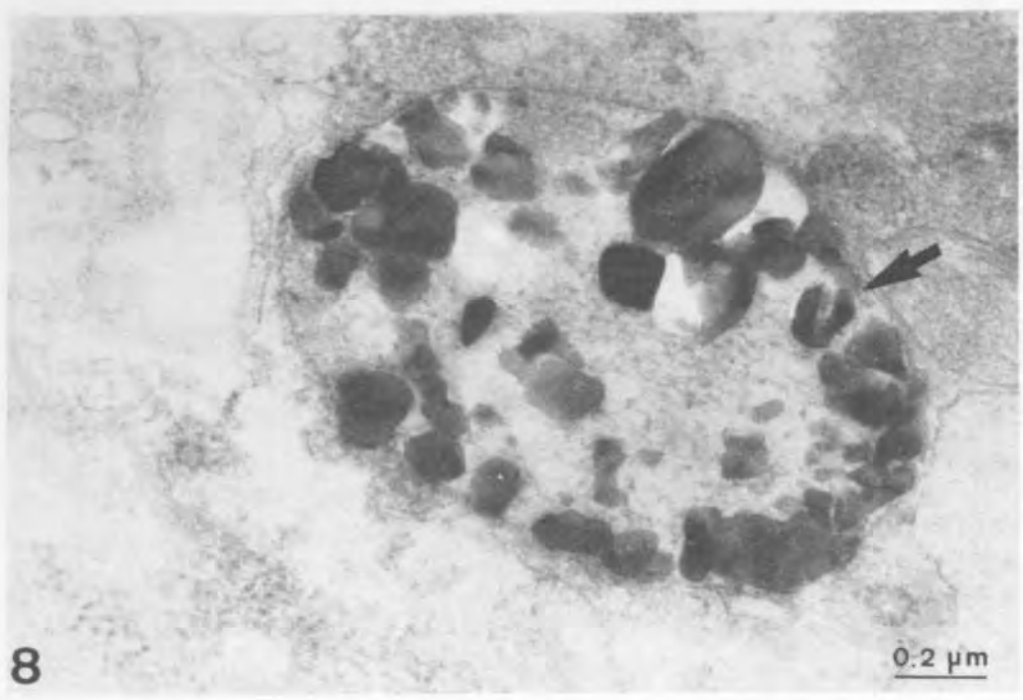

Fig. 8: Phagocytotis vesicle containing crystals of different sizes.
(TEM. Undecalcified section. × 50,000).
Fig. 8: Vésicule de phagocytose renfermant des cristaux de taille différente.
(MET. Coupe non-décalcifiée. × 50.000).

Fig. 9: Enlargement of the crystal indicated by an arrow on figure 8. This crystal is undergoing an intracellular dissolution.
(TEM. Undecalcified section. × 800,000).
Fig. 9: Agrandissement du cristal indiqué par une flèche sur la figure 8. Ce cristal subit une dissolution intracellulaire.
(MET. Coupe non-décalcifiée. × 800.000).

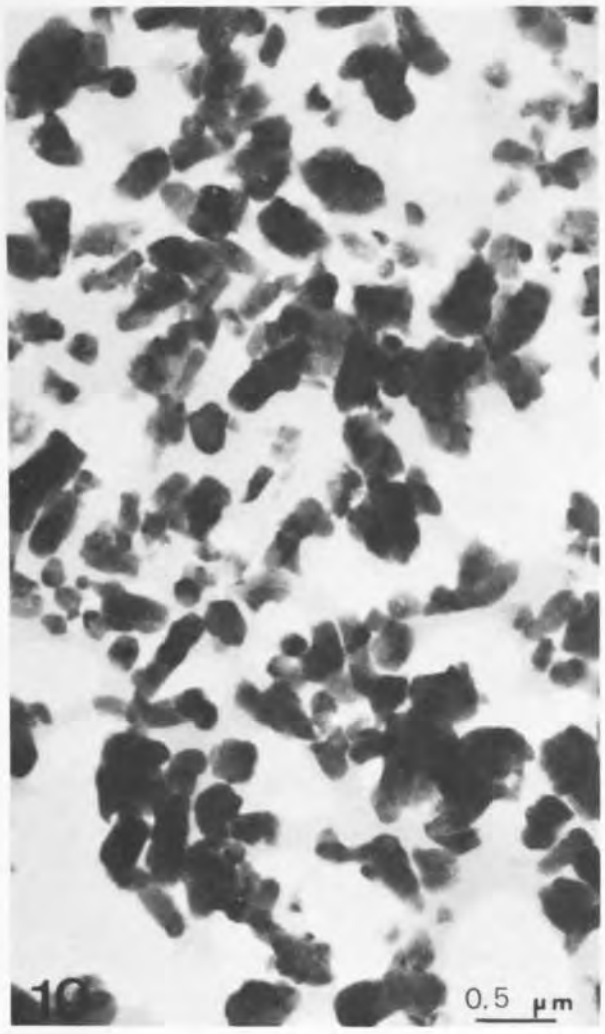

Fig. 10: Hydroxyapatite aggregate prior implantation.
(TEM. Undecalcified section. × 16,500).
Fig. 10: Agrégat d'hydroxyapatite avant implantation.
(MET. Coupe non-décalcifiée. × 16.500).

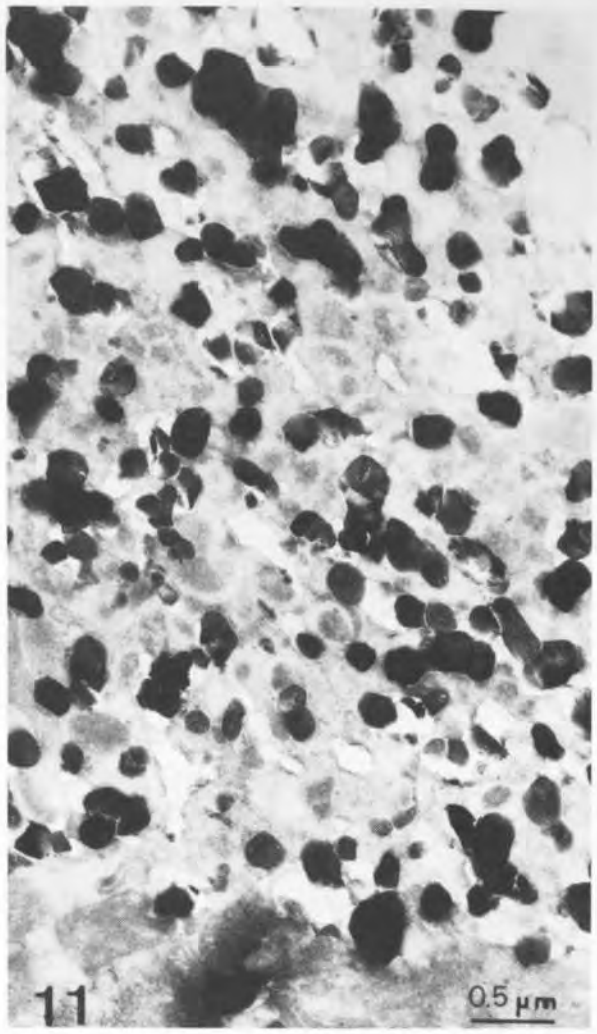

Fig. 11: Peripheral part of an implanted aggregate. Note the loosening of the crystals when compared to figure 9 and the presence of invading organic substance.
(TEM. Undecalcified section. × 16,500).
Fig. 11: Partie périphérique d'un agrégat implanté. Noter la densité réduite des cristaux par rapport à la figure 10 et l'infiltration des espaces intercristallins par une substance organique.
(MET. Coupe non-décalcifiée. × 16.500).

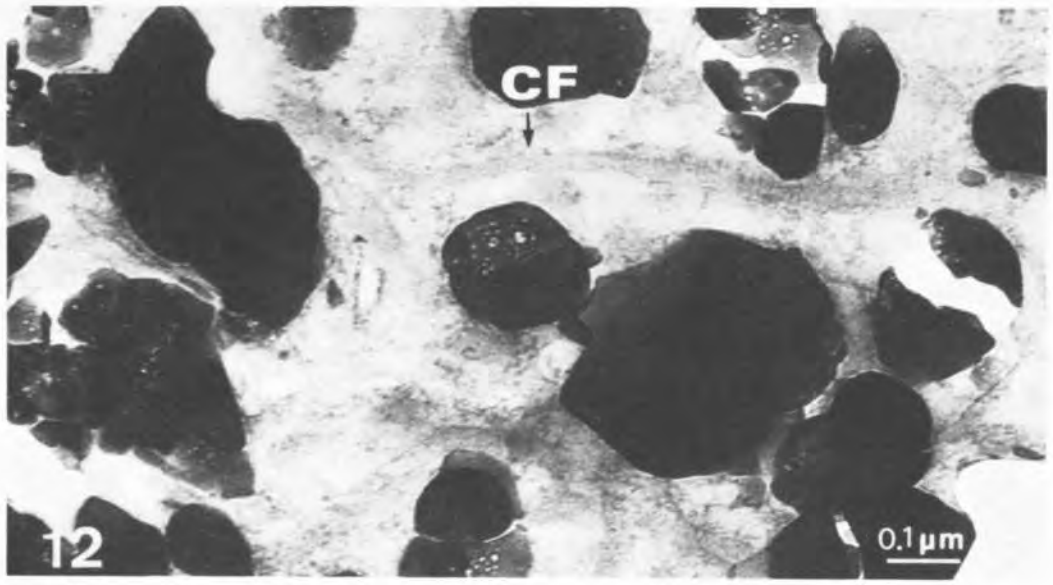

Fig. 12: Granular organic substance and collagen fibers (CF) invading the spaces between the aggregate crystals.
(TEM. Undecalcified section. × 65,000).
Fig. 12: Substance organique granuleuse et fibres de collagène (CF) envahissant les espaces intercristallins.
(MET. Coupe non-décalcifiée. × 65.000).

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— in other places, the aggregate was lined by larger cells with a great number of mitochondria packed in the cytoplasm, some vacuoles and numerous vesicles containing ceramic crystals. The cells were observed closely layered onto the surface of the aggregate without any intervening organic material. In some places, crystals at the surface of the aggregate were sticked to the cell membrane, visibly undergoing an internalization process (Fig. 6). Most of the crystals present within the phagocytosis vesicles were noticeably smaller than the crystals of the adjacent aggregate (Fig. 7), some of them showed signs of intracellular dissolution (Figs 8-9).

Whatever the implantation site area, the packing of crystals at the periphery of the aggregate was looser than that observed on non-implanted aggregates (Figs 10-11). Spaces between crystals were filled with an organic substance containing collagen fibers (Fig. 12).

DISCUSSION

It is admitted today that calcium phosphate bioceramics are clinically well tolerated (Nery and Lynch, 1978; Yukna et al., 1984; Meffert et al., 1985; Kenney et al., 1985; Greenstein et al., 1985; Louise et al., 1987). Histological controls in human have demonstrated that the inflammatory response to the implants may be disregarded (Froum et al., 1982; Moskow and Lubarr, 1983; Piecuch and Fedorka, 1983; Baldock et al., 1985; Kenney et al., 1985; Kenney et al., 1986; Stahl and Froum, 1986; Bowers et al., 1986; Carranza et al., 1987; Stahl and Froum, 1987). Our results corroborate these data and emphasize a great diversity of the interface between the biomaterial aggregates and the living tissues.

One of the most interesting features emerging from the present study is the presence of phagocytotic cells which seem to be very specific from both morphological and functional points of view. In spite of their intensive phagocytosis-turned activity, on a morphological basis, these cells cannot be identified as cells currently involved in phagocytosis processes such as macrophages or giant cells.

The cells observed in our study seem to be morphologically quite similar to those already described as osteoclasts by Ogilvie et al, 1987. However, in our opinion, they do not fit several criterions considered as typical features for osteoclasts (Bourne, 1972; Holtrop and King, 1977): they were sometimes located in areas quite distant from bone while regular osteoclasts are always found in close vicinity to bone; they did not have any brush border; multinucleated cells were not observed; the number of crystal-containing phagocytosis vesicles was unusual since the osteoclastic function is more especially aimed to extracellular dissolution of the mineral through acidic and enzymatic processes.

In our opinion, these cells seem to be specific and could be a direct consequence of the presence of the implant. This hypothesis, suggesting an action of calcium phosphates on the differentiation status of the cell, is supported by in vitro studies showing that cell activity of different cell types is modified consequently to calcium phosphates phagocytosis (Evans et al., 1984a; Evans et al., 1984b; Cheung and McCarty, 1985; Borkow et al., 1987; gregoire et al., 1987; Gregoire et al., 1989; Orly et al., 1989).

These results definitely confirm that hydroxyapatite cannot be regarded as a completely non-resorbable biomaterial. The decreased density of the crystals located at the periphery of the aggregates, the phagocytotic cells described in the present study and the intracellular dissolution of hydroxyapatite biomaterial presented, to our knowledge, for the first time, illustrate the classical sequence of events supposed to occur in the degradation process of calium phosphate biomaterials: an extracellular dissolution step leading to the release of individual crystals or groups of crystals and allowing for phagocytosis and consecutive cell-mediated resorption (de Groot, 1980; Klein et al., 1983; Klein et al., 1984).

The present study emphasizes the complexity of the periodontal environment and suggests the occurrence of a specific response of the periodontium to calcium phosphate implants, involving possible cell differentiation processess.

REFERENCES

Baldock, W.T., Hutchens, L.H., McFall, W.T., Simpson, D.M. — An evaluation of tricalcium phosphate implants in human periodontal osseous defects of two patients. *J. Periodontol., 56:* 1-7, 1985.

Boetto, J., Freeman, E. — Histological evaluation of Durapatite in experimental periodontal defects. *Can. Dent. Assoc. J., 50:* 239-244, 1984.

Bonel, G., Heughebaert, J.C., Heughebaert, M., Lacout, J.L., Lebugle, A. — Apatitic calcium orthophosphates and related compounds for biomaterials preparation. *Ann. NY Acad. Sci.,* 1989 (in press).

Borkowf, A., Cheung, H.S., Mc Carty, D.J. — Endocytosis is required for the mitogenic effect of basic calcium phosphate crystals in fibroblasts. *Calcif. Tissue Int., 40:* 173-176, 1987.

Bourne, G.H. (Ed.) — The Biochemistry and Physiology of Bone, Vol. 1, Academic Press, New York, 1972.

Bowers, G.W., Schallhorn, R.G., Mellonig, J.T. — Histologic evaluation of new attachment in human intrabony defects. A litterature review. *J. Periodontol., 53:* 509-514, 1982.

Bowers, G.M., Vargo, J.W., Levy, B., Emerson, J.R., Bergquist, J.J. — Histologic observations following the placement of tricalcium phosphate implants in human intrabony defects. *J. Periodontol., 57:* 286-287, 1986.

Boyne, P.J., Fremming, B.D. — Periodontal pocket elimination using Durapatite ceramic implants. *Alpha Omegan, 75:* 17-22, 1982.

Carranza, F.A., Kenney, E.B., Lekovic, V., Talamante, E., Valencia, J., Dimitrijevic, B. — Histologic study of healing of human periodontal defects after placement of porous hydroxyapatite implants. *J. Periodontol., 58:* 682-688, 1987.

Cheung, H.S., McCarty, D.J. — Mitogenesis induced by calcium-containing crystals. *Exp. cell Res., 157:* 63-70, 1985.

Cushing, M.J. — Autogenous red marrow grafts: their potential for induction of osteogenesis. J. Periodontol., 40: 492-497, 1969.

De Groot, K. — Bioceramics consisting of calcium phosphate salts. *Biomaterials, 1:* 47-50, 1980.

De Groot, K. (Ed.) — Bioceramics of calcium phosphate, CRC Press Inc, Boca raton, Floride, 1983.

Drobeck, H.P., Rothstein, S.S., Gumaer, K.I., Sherer, A.D., Slighter, R.G. — Histologic observation of soft tissue responses to implanted multifaceted particles and discs of hydroxyapatite. *J. Oral Maxillofac. Surg., 42:* 143-149, 1984.

Evans, R.W., Cheung, H.S., McCarty, D.J. — Cultured canine synovial cells solubilize ^{45}Ca labeled hydroxyapatite crystals. *Arthritis Rheum, 27:* 829-832, 1984.

Evans, R.W., Cheung, H.S., McCarty, D.J. — Cultured human monocytes and fibroblasts solubilize calcium phosphate crystals. *Calcif. Tissue Int., 36:* 645-650, 1984.

Frank, R.M., Gineste, M., Benqué, E.P., Duffort, J.F., Heughebaert, M. — Etude ultrastructurale de l'incision osseuse après implantation d'hydroxyapatite synthétique chez l'Homme. *J. Biol. Buccale, 15:* 125-134, 1987.

Froum, S.J., Kushner, L., Scopp, I.W., Stahl, S.S. — Human clinical and histologic response to Durapatite implants in intraosseous lesions. *J. Periodontol., 53:* 719-725, 1982.

Ganeles, J., Listgarten, M.A., Evian, C.I. — Ultrastructure of Durapatite-periodontal tissue interface in human infrabony defects. *J. Periodontol., 57:* 133-140, 1986.

Greenstein, G., Jaffin, R.A., Hilsen, K.L., Berman, C.L. — Repair of anterior gingival deformity with Durapatite. *J. Periodontol., 56:* 200-203, 1985.

Grégoire, M., Orly, I., Kérébel, B. — In vivo effects of calcium phosphate biomaterials on fibroblastic cell behavior. *Biol. Cell, 59:* 255-260, 1987.

Grégoire, M., Orly, I., Menanteau, J. — The influence of calcium phosphate biomaterials on human bone cell activities. An in vitro approach. *J. Biomed. Mater. Res., 1989* (in press).

Holtrop, M.E., King, G.J. — The ultrastructure of the osteoclast and its functional implications. *Clin. Orthop., 123:* 177-196, 1977.

Kenney, E.B., Lekovic, V., Han, T., Carranza, F.A., Dimitrijevic, B. — The use of porous hydroxyapatite implant in periodontal defects. I: clinical results after 6 months. *J. Periodontol., 56:* 82-88, 1985.

Kenney, E.B., Lekovic, V., Sa Ferreira, J.C., Han, T., Dimitrijevic, B., Carranza, F.A. — Bone formation within porous hydroxyapatite implants in human periodontal defects. *J. Periodontol., 57:* 76-83, 1986.

Klein, C.P.A.T., Driessen, A.A., De Groot, K., van den Hooff, A. — Biodegradation behavior of various calcium phosphate materials in bone tissue. *J. Biomed. Mater. Res., 17:* 769-784, 1983.

Klein, C.P.A.T., Driessen, A.A., De Groot, K. — Relationship between the degradation behaviour of calcium phosphate ceramics and their physical-chemical characteristics and ultrastructural geometry. *Biomaterials, 5:* 157-160, 1984.

Louise, F., Borghetti, A., Simeone, D., Gervesone, V. — Evaluation clinique à 6 mois du comblement de lésons intraosseuses par une hydroxyapatite poreuse. *J. Parodontol., 6:* 203-212, 1987.

Meffert, R.M., Thomas, J.R., Hamilton, K.M., Brownstein, C.N. — Hydroxyapatite as an alloplastic graft in the treatment of human periodontal osseous defects. *J. Periodontol., 56:* 63-73, 1985.

Misiek, D.J., Kent, J.N., Carr, R.F. — Soft tissue responses to hydroxyapatite particles of different shapes. *J. Oral Maxillofac. Surg., 42:* 150-160, 1984.

Moskow, B.S., Lubarr, A. — Histological assessment of human periodontal defect after Durapatite ceramic implant. Report of a case. *J. Periodontol., 54:* 455-462, 1983.

Nery, E.B., Lynch, K.L., Hirthe, W.M., Mueller, K.H. — Bioceramic implants in surgically produced infrabony defects. *J. Periodontol., 46:* 328-347, 1975.

Nery, E.B., Lynch, K.L. — Preliminary clinical studies of bioceramic in periodontal osseous defects. *J. Periodontol., 49:* 523-527, 1978.

Ogilvie, A., Frank, R.M., Benqué, E.P., Gineste, M., Heughebaert, M., Hemmerle, J. — The biocompatibility of hydroxyapatite implanted in the human periodontium. *J. Period. Res., 22:* 270-283, 1987.

Orly, I., Grégoire, M., Menanteau, J., Dard, M. — Effects of synthetic calcium phosphates on the ^{3}H-thymidine incorporation and alkaline phosphatase activity of human fibroblasts in culture. *J. Biomed. Mater. Res.*, 1989 (in press).

Piecuch, J.F., Fedorka, N.J. — Results of soft-tissue surgery over implanted replamineform hydroxyapatite. *J. Oral Maxillofac. Surg., 41:* 801-806, 1983.

Schallhorn, R.G. — Present status of osseous grafting procedures. *J. Periodontol., 48:* 570-576, 1977.

Stahl, S.S., Froum, S.J. — Histological evaluation of human intraosseous healing responses to the placement of tricalcium phosphate ceramic implants. *J. Periodontol., 57:* 211-217, 1986.

Stahl, S.S., Froum, S.J. — Histologic and clinical responses to porous hydroxyapatite implants in human periodontal defects. 3 to 12 months post implantation. *J. Periodontol., 58:* 689-695, 1987.

Yukna, R.A., Mayer, E.T., Brite, D.V. — Longitudinal evaluation of Durapatite ceramic as an alloplastic implant in periodontal osseous defects after 3 years. *J. Periodontol., 55:* 633-637, 1984.